In Vitro and *In Vivo* Availability of Hydrophilized Phenytoin from Capsules

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Abstract □ The effect of phenytoin hydrophilization on the liquid penetration rate into prepared plugs, on the disintegration time, on the *in vitro* release rate, and on *in vivo* absorption in humans was studied. Hydrophilization was performed by intensive mixing of the hydrophobic drug with a small amount of methylcellulose solution. Liquid penetration into the treated plugs was independent of the liquid wetting potency and extremely high compared to the pure drug plugs. Analogous results were obtained for the disintegration time and *in vitro* release rates from capsules loaded with pure and treated drug. A bioavailability study in seven healthy volunteers showed immediate absorption of the treated drug but a 1-hr absorption lag time for the pure drug.

Keyphrases □ Phenytoin, hydrophilized—*in vitro* and *in vivo* availability from capsules □ Bioavailability—hydrophilized phenytoin, capsule formulation, *in vitro* release rates □ Anticonvulsants—phenytoin, effect of hydrophilization, *in vitro* and *in vivo* availability

When a disintegratable tablet or capsule is administered orally to humans, gastric fluid must penetrate the porous mass before disintegration and absorption can occur. Any factor influencing the penetration rate may influence the absorption rate.

The liquid penetration rate into a porous mass is given by the Washburn (1) equation:

$$\frac{dl}{dt} = \frac{m\gamma \cos\theta}{2l\eta} \qquad \text{(Eq. 1)}$$

where l is the penetrated length at time t, m is the hydraulic pore radius, γ is the penetrating liquid surface tension, θ is the contact angle between solid and liquid, and η is the liquid viscosity. The penetration rate is strongly dependent on the solid contact angle.

In earlier studies on pharmaceutical powder wettability (2, 3), reported contact angles varied from 0° for dicalcium phosphate dihydrate up to 125° for chloramphenicol palmitate. Recently (4), the effect on the capsule release rate of surface hydrophilic binder distribution over a hydrophobic drug was studied. The increased hydrophilized hexobarbital release rate was independent of the dissolution medium wetting potency, in contrast to the untreated drug.

The purpose of this investigation was to examine the penetration rate effect on the *in vitro* and *in vivo* availability of poorly soluble, hydrophobic drugs from capsules. Phenytoin was selected as a prototype drug, exhibiting a contact angle of 102°. Phenytoin bioavailability is important because of the closeness of the therapeutic and toxic levels. Marked bioavailability differences between different products have been reported (5–9).

EXPERIMENTAL

Materials—Phenytoin[1] was NF grade. The mean particle size, based on air permeability measurement[2], was 5 μm. Methylcellulose[3], polysorbate[4], β-glucuronidase[5] (purified lyophilized powder, 5×10^6 units/g), 5-(*p*-methylphenyl)-5-phenylhydantoin[6], and 5-(*p*-hydroxyphenyl)-5-phenylhydantoin[6] were used as received. Trimethylanilinium hydroxide[7] was used as a 0.1 *M* solution in methanol, and tetramethylammonium hydroxide[8] was used as a 25% solution in water.

All solvents and other chemicals were analytical grade, except *n*-heptane which was spectrophotometric grade[9]. The methylene chloride used as a mobile phase component in the high-pressure liquid chromatographic (HPLC) procedure was freshly distilled and 50% saturated with water before use.

Methods—*Treatment of Drug*—Methylcellulose dispersion over the phenytoin surface was carried out in a small high-speed mixer[10]. The drug (20 g) was placed in the mixer, 6 ml of a 3% aqueous binder solution was added, and the mixer was run for 6 min at ~9000 rpm. The mixing was interrupted every 0.5 min to mix, with a spatula, material from dead spots in the mixer. The material was dried in a tray drier at 40° for 1 hr and

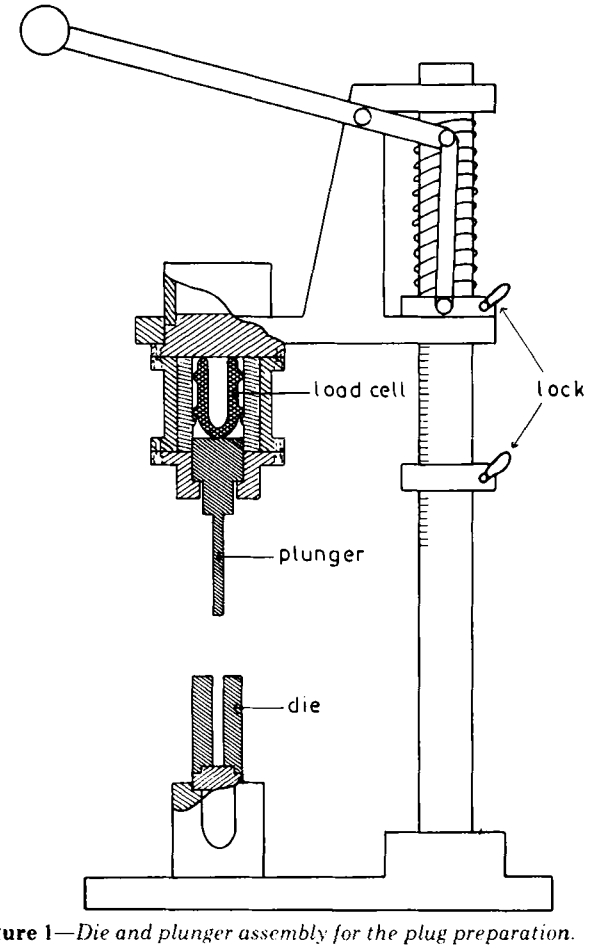

Figure 1—*Die and plunger assembly for the plug preparation.*

[1] Parke-Davis, Mijdrecht, The Netherlands.
[2] Model 95, Fisher Scientific Co., Pittsburgh, Pa.
[3] Celacol M20, British Celanese Ltd., Derby, England.
[4] Tween 80, Atlas Chemie, Essen, West Germany.
[5] Sigma, St. Louis, Mo.
[6] Aldrich Europe, Beerse, Belgium.
[7] B.D.H., Poakstoone, England.
[8] Serva, Heidelberg, West Germany.
[9] Merck AG, Darmstadt, West Germany.
[10] MX 32, Braun AG, Frankfurt/M., West Germany.

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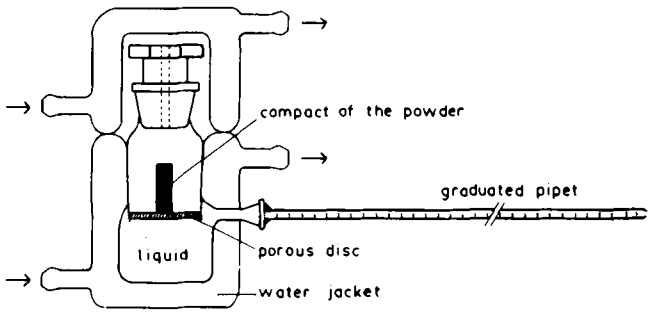

Figure 2—*Thermostated apparatus for measuring unidimensional liquid penetration into the plugs.*

screened through an 80-μm sieve. Treated drug particle-size determination (measured value of 6 μm) and electron micrographs of the untreated and treated powder showed that no milling and hardly any granulation had occurred during preparation.

Wettability—Pure and hydrophilized phenytoin wettability was characterized by contact angle determination as described previously (4).

Plug Preparation—Most commercial capsules are filled by compressing the powder and loading it into the capsule body. To reproduce this process, the pure and treated drugs were compressed with a 120 ± 5 N force to 300-mg plugs (1.8-cm length × 0.6-cm diameter) using a special die and plunger assembly (Fig. 1). The plunger was connected to a load cell for compression force registration.

Capsule Filling—Prepared plugs were manually loaded into No. 1 hard gelatin capsules[11] for the disintegration measurements, the *in vitro* release studies, and the *in vivo* availability studies.

Liquid Penetration—The unidimensional liquid penetration rate into the phenytoin plugs was measured by placing the plug on a modified thermostated apparatus glass filter plate (Fig. 2) (10). The liquid uptake rate was read from the graduated pipet. The penetrations were carried out at 37 ± 0.2° with distilled water or water to which different amounts of polysorbate had been added.

The all-side liquid penetration was measured at room temperature by registration of the phenytoin plug weight increase during penetration. The powder plug was placed in a small glass cylinder with a porous bottom and a gauze cover to prevent floating (Fig. 3). The whole was immersed in the penetrating liquid and connected to an electronic balance[12] with automatic recording. Unidimensional penetration measurements were made four times and averaged. All-side penetrations were so reproducible that they were performed in duplicate only. No significant difference in experimental results was found when the penetrating liquid was presaturated with phenytoin.

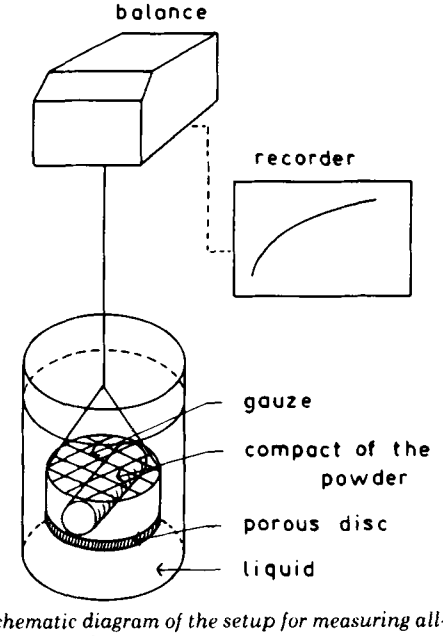

Figure 3—*Schematic diagram of the setup for measuring all-side liquid penetration into the plugs.*

[11] Snap-fit, Capsugel AG, Basel, Switzerland.

[12] PR 1200, Mettler, Greifensee-Zürich, Switzerland.

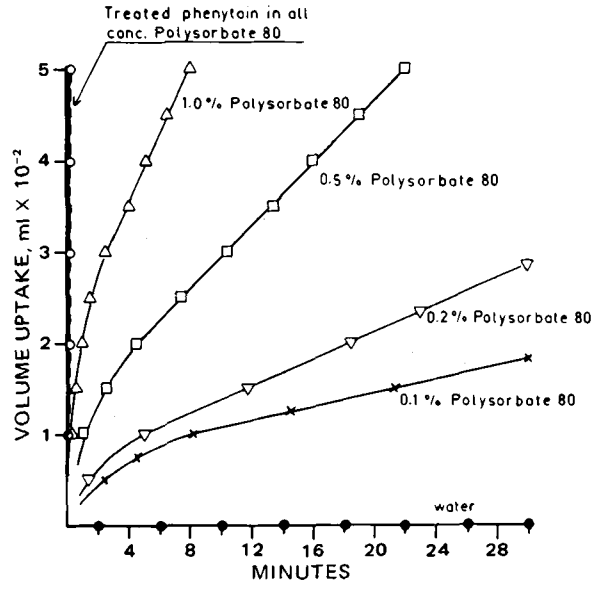

Figure 4—*Unidimensional liquid penetration profiles as a function of the polysorbate concentration. Key: ●, ×, ▽, □, and △, penetration of water and 0.1, 0.2, 0.5, and 1.0% polysorbate into plugs of untreated phenytoin, respectively; and ○, penetration of all concentrations of polysorbate into plugs of treated phenytoin.*

Disintegration—The capsule disintegration time was determined according to the BP 1973. Measurements were carried out without disks at 37 ± 0.5° in water with different polysorbate concentrations. Disintegration measurements were made eight times and averaged.

Dissolution and Solubility—A water-jacketed 1-liter beaker containing the dissolution medium (900 ml) was maintained at 37 ± 0.5°. The dissolution medium consisted of water with different polysorbate concentrations (pH < 5.5). The capsule was held in a stainless steel wire spiral located centrally 2 cm above the beaker bottom. Stirring was with a 5-cm diameter straight four-bladed impeller, rotated at 60 ± 1 rpm 3.5 cm above the capsule (11).

Samples were taken at convenient time intervals through membrane filters (0.8-μm pore diameter) and analyzed by a modified UV spectrophotometric method (12). To a 4.0-ml sample (first diluted, if necessary, with water or the test medium) in a glass-stoppered 25-ml test tube were added 0.5 g of potassium permanganate and 8 ml of 8 *N* NaOH. After

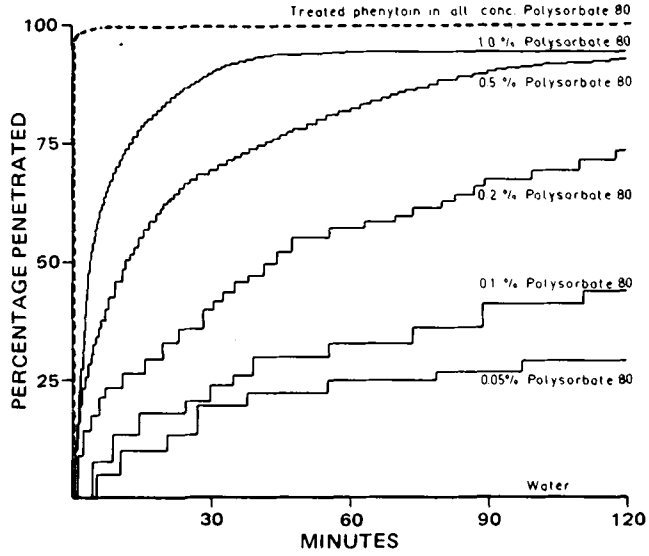

Figure 5—*All-side liquid penetration profiles into plugs of treated and untreated phenytoin as function of the polysorbate concentration. Key: —, untreated phenytoin; and - - -, treated phenytoin.*

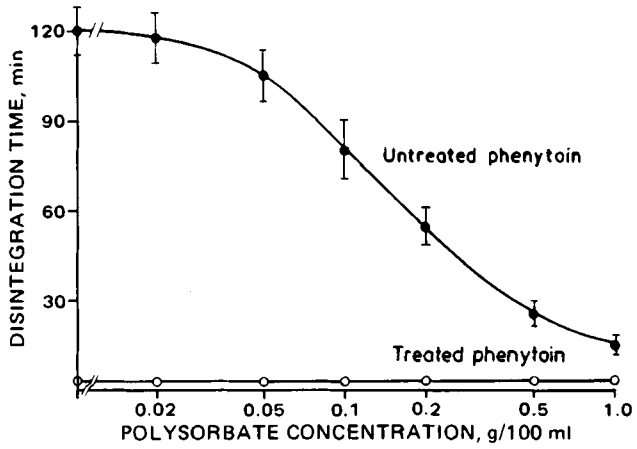

Figure 6—*Untreated and treated phenytoin capsule disintegration time as a function of the polysorbate concentration. Key:* ● *and* ○, *untreated and treated phenytoin, respectively; and* I, *standard deviation.*

being shaken for several minutes, the tubes were immersed in an 80° water bath for 30 min. The samples were allowed to cool to room temperature, and 5.0 ml of *n*-heptane was added. The mixture was shaken for an additional 15 min; after separation of the two layers, the heptane layer absorbance was measured[13] at 247 nm against a suitable blank.

Calibration curves showed that Beer's law was obeyed with slopes of 0.017, 0.026, and 0.057 for water and 0.1 and 0.25% polysorbate, respectively. The correlation coefficients were at least 0.999. Each dissolution profile is the average of three to six determinations. The ranges are indicated in the graphs.

The saturated phenytoin solubility in the different solutions was determined by placing excess solute (about 300 mg) in glass vials filled with each test medium. The vials were closed and rotated at 25 ± 1 rpm in a water bath at 37 ± 0.5° until no increase in the amount dissolved could be measured. Samples were taken at convenient time intervals through membrane filters and analyzed as described for the dissolution study.

For the dissolution and solubility studies, all glass syringes, pipets, and the filter apparatus were prewarmed to prevent drug precipitation.

Absorption—Absorption studies were carried out with nine healthy

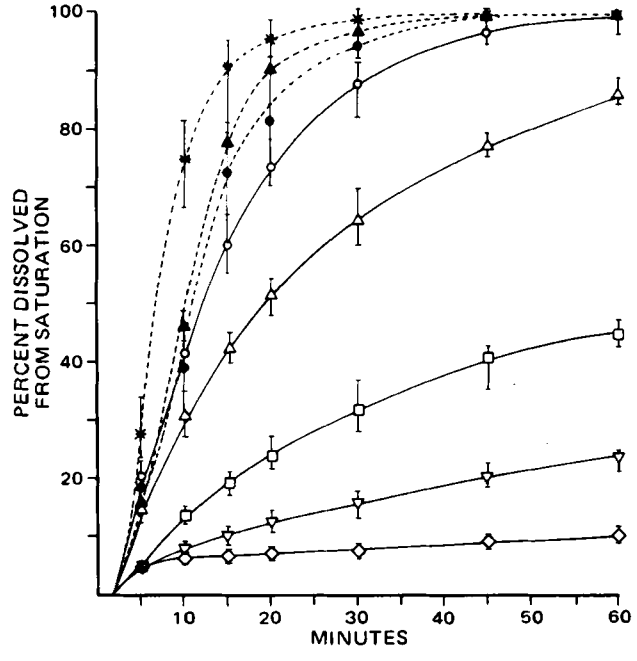

Figure 7—*Treated and untreated phenytoin capsule release profiles as a function of the polysorbate concentration. Open symbols are for untreated phenytoin, and solid symbols are for treated drug. Key:* ◊, *0% polysorbate 80 (γ_{LV}° = 70.1 dynes/cm);* ▽, *0.1% polysorbate 80 (39.9 dynes/cm);* □, *0.2% polysorbate 80 (38.9 dynes/cm);* △, *0.5% polysorbate 80 (37.8 dynes/cm);* ○, *1.0% polysorbate 80 (37.7 dynes/cm);* ✱, *polysorbate solutions with 0–0.2% polysorbate 80;* ▲, *polysorbate solution with 0.5% polysorbate 80;* ●, *polysorbate solutions with 1.0% polysorbate 80; and* I, *intercapsule variation.*

[13] Model 25 UV spectrophotometer, Beckman Instruments Co., Fullerton, Calif.

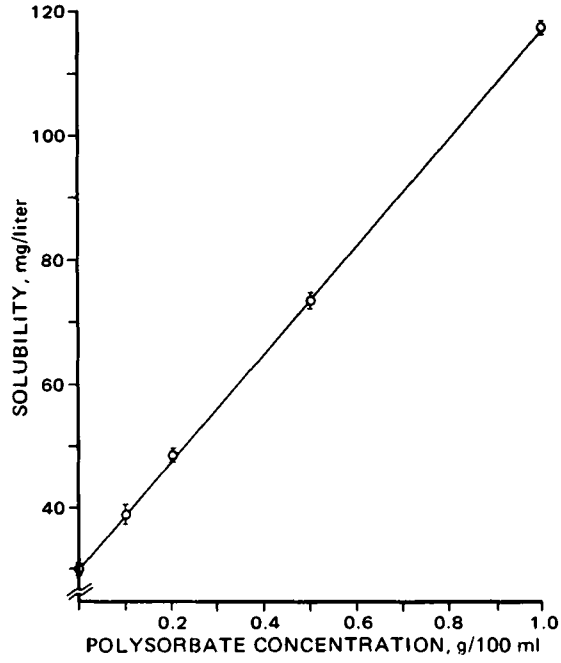

Figure 8—*Phenytoin solubility as a function of the polysorbate concentration (*I = *standard deviation).*

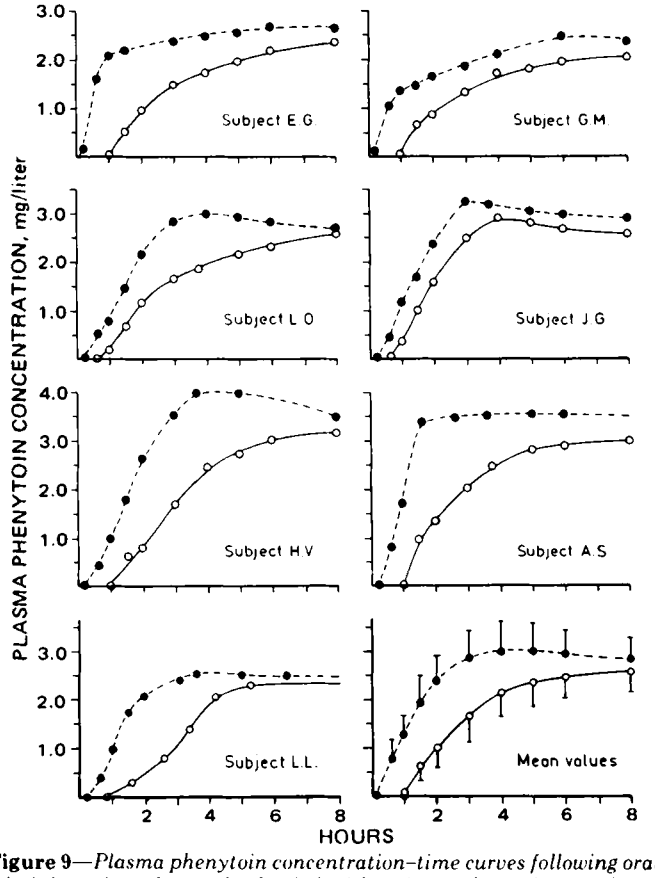

Figure 9—*Plasma phenytoin concentration–time curves following oral administration of capsules loaded with 300-mg plugs of treated and untreated drug. Key:* ● *and* ○, *treated and untreated phenytoin, respectively; and* T, *standard deviation.*

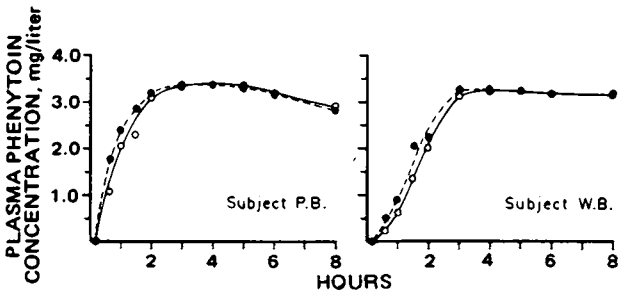

Figure 10—*Plasma phenytoin concentration–time curves following oral administration of a capsule loaded with a plug of 300 mg of treated drug and of a suspension of 300 mg of phenytoin in water. Key: ●, capsule; and ○, suspension.*

volunteers (66–87 kg, 21–28 years of age). Seven subjects received one capsule (300 mg) of pure phenytoin or one capsule of hydrophilized phenytoin. The two administrations were at least 2 weeks apart. The capsules were taken with 200 ml of water after an overnight fast, and no food was allowed for 4 hr after administration. Two subjects received one capsule with 300 mg of hydrophilized phenytoin; 2 weeks later, they received 300 mg of phenytoin dispersed in water.

Blood samples (8 ml) were taken from a forearm vein at 10, 40, 60, and 90 min and 2, 3, 4, 5, 6, 8, 12, 24, 30, and 48 hr. The heparinized blood samples were centrifuged, and the plasma was stored in a refrigerator until analyzed. Urine was collected at (convenient) time intervals over 48 hr. Volumes were recorded, and samples were refrigerated until assayed.

The plasma phenytoin levels were measured by HPLC[14] according to a method developed for carbamazepine (13). The column was 15 cm × 3 mm (i.d.) stainless steel, packed by a balanced-density slurry technique (14) with 5-μm porous particle silicic acid[15]. The solvent system was 5% tetrahydrofuran in methylene chloride. The flow rate was 1.2 ml/min, and the pressure required was ~10 MPa (1500 psi). Nitrazepam was the internal standard. The absorbance[16] was measured at 254 nm. The mean recovery from plasma was 95% with a relative standard deviation of ±2.5% (n = 32). The relative standard deviation of the calibration graph data down to 0.4-μg of phenytoin/ml of plasma did not exceed ±3% (n = 7).

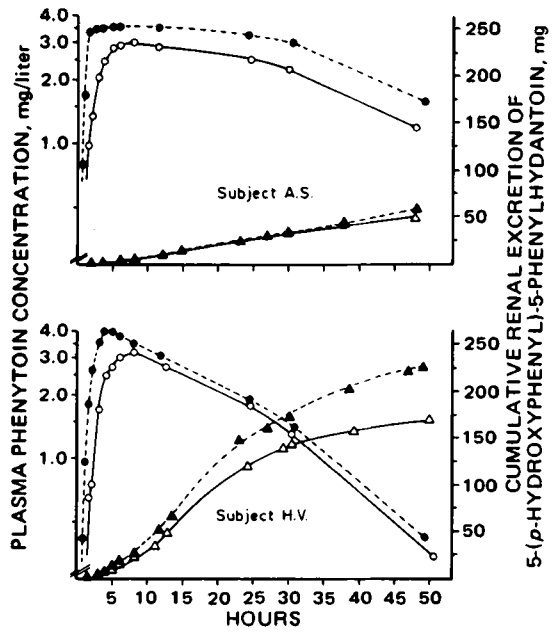

Figure 11—*Plasma phenytoin concentration–time curves and cumulative renal excretion of the total amount of 5-(p-hydroxyphenyl)-5-phenylhydantoin following oral administration of 300-mg treated and untreated phenytoin capsules. Key: ● and ▲, plasma and urine after the treated drug, respectively; and ○ and △, plasma and urine after the untreated drug, respectively.*

5-(*p*-Hydroxyphenyl)-5-phenylhydantoin and its conjugate, the main phenytoin metabolite in humans (15, 16), were assayed in the urine by a modified GLC method (17). To 1.0 ml of urine were added 0.5 ml of 0.066 *M* phosphate buffer (pH 6.9) and 250 units of β-glucuronidase. The samples were incubated for 15 hr at 37°. To the hydrolyzed samples were added 100 μl of an internal standard solution [50 μg/ml of 5-(*p*-methylphenyl)-5-phenylhydantoin in 0.01 *N* NaOH] and 1 ml of 3 *M* phosphate buffer (pH 4.5). After extraction with 8 ml of ether for 15 min and centrifugation, 7 ml of the organic phase was transferred to another test tube. Then 40 μl of 0.1 *M* trimethylanilinium hydroxide dissolved in methanol and 40 μl of 0.54 *M* tetramethylammonium hydroxide dissolved in water were added, and the tube was shaken for 10 min.

After centrifugation, 1 μl of the lower phase was injected into a gas chromatograph[17] equipped with a flame-ionization detector. The coiled glass tubing column, 1.5 m long × 2.2 mm i.d., was packed with 3% OV-17 on 80–100-mesh Chromosorb G.H.P. Operating conditions were: injection port, 300°; column oven, 240°; and detector, 275°. The nitrogen flow rate was 20 ml/min. Hydrogen and compressed air flow rates were adjusted to give maximum response. The relative standard deviation of the calibration graph data down to 15 μg of 5-(*p*-hydroxyphenyl)-5-phenylhydantoin/ml of urine did not exceed ±4% (n = 9).

All plasma and urine samples were analyzed in duplicate.

Liquid Properties—Liquid surface tensions and viscosities were determined with a tensiometer[18] and a capillary tube viscometer[19].

RESULTS AND DISCUSSION

In Fig. 4, the distilled water and polysorbate 80 solution uptakes into pure and hydrophilized phenytoin plugs are graphed against penetration time. As expected, no distilled water penetration into plugs of nontreated drug could be detected because of the strongly hydrophobic nature of the solid and the distilled water surface tension (70.1 dynes/cm at 37°). Addition of 0.1% polysorbate 80 to the penetrating liquid produced a measurable volume uptake. The penetration rate increased with increasing surfactant, decreasing the penetrating liquid surface tension to 37.7 dynes/cm at a 1% concentration.

The wetting of a powder mass by a liquid containing a surfactant is a dynamic process during which surfactant monomers are adsorbed on the substrate. The surfactant micelles are broken down to monomers, which diffuse to the liquid–solid interfaces. During the dynamic penetration process, the micelle breakdown in the capillaries may result in a micelle diffusion from the bulk of the solution into the pores of the mass. In this case, the wetting potency is not limited by the critical micelle concentration [polysorbate 80 = 0.04% (w/v) at 37°]. This is consistent with the theory (18) that micelle diffusion and breakdown will be the rate-limiting steps.

Since the penetration rate into a porous mass is, according to the Washburn equation, strongly dependent on the contact angle of the solid, the extremely hydrophobic phenytoin, exhibiting a contact angle of 102°, was hydrophilized by hydrophilic binder distribution over the solid surface. Methylcellulose treatment decreased the contact angle to 51°. The penetration rate into the hydrophilized phenytoin plugs was strongly increased (Fig. 4). The extremely fast penetration rate was independent of the penetrating liquid wetting potency.

The all-side penetration profiles are represented in Fig. 5. Again no penetration of distilled water into pure phenytoin plugs could be detected. Addition of polysorbate 80 to distilled water resulted in penetrations that increased with increasing surfactant concentrations. The steps in the recorded curves were caused by air bubbles escaping from the porous mass. The hydrophilized phenytoin plugs exhibited instant uptake, which was again independent of the penetrating liquid wetting potency.

The effect of drug hydrophilization on the disintegration of capsules loaded with 300-mg plugs of phenytoin is shown in Fig. 6. The untreated drug capsule disintegration time was 2 hr in distilled water. The disintegration time decreased with increasing surfactant concentration down to 15 min at 1% polysorbate 80. The hydrophilized phenytoin capsules all exhibited, independent of the polysorbate 80 concentration, disintegration times of 3 min, corresponding with the time in which enough of the gelatin had dissolved to open the capsule shell completely.

Analogous results were obtained for the hydrophilization effect on the *in vitro* phenytoin release rate from the different capsules (Fig. 7). The results are expressed as the percentage dissolved from saturation against time. A strong increase in the percentage dissolved for the nontreated

[14] Model M-6000 B, Waters Associates, Milford, Mass.
[15] Partisil, Chrompack, Middelburg, The Netherlands.
[16] Model 440, Waters Associates, Milford, Mass.
[17] Model 5750, Hewlett-Packard, Avondale, Pa.
[18] Du Noüy, 8600 EE, Krüss, Hamburg, West Germany.
[19] Type Ubbelohde, Tamson, Zoetermeer, The Netherlands.

Table I—Area under the Curves for Seven Volunteers after Administration of Capsules Loaded with 300-mg Plugs of Pure or Hydrophilized Phenytoin

Subject	*AUC*, mg min/liter, 0–8 hr		*AUC*, mg min/liter, 0–48 hr	
	Untreated Drug	Treated Drug	Untreated Drug	Treated Drug
A.S.	995	1337	6336	8452
G.M.	673	905	4760	6075
J.G.	991	1192	4184	5324
L.O.	781	1109	4833	5103
E.G.	713	1098	4187	4783
H.V.	943	1447	4821	5661
L.L.	693	1096	—	5194
Mean	827	1169	4853	5900
SD	145	178	787	1328
p^a	<0.001		<0.01	

[a] Evaluated with the Student test for paired observations.

drug was found, along with a slight decrease for the treated drug with increasing surfactant concentrations. The slight decrease in the percentage dissolved from saturation corresponds with an absolute increase in the amount dissolved because of the increased phenytoin solubility with an increasing polysorbate 80 concentration. The latter was due to the strongly solubilizing surfactant (Fig. 8).

Comparative absorption studies were performed in humans. In Fig. 9, all of the individual and the mean plasma curves are given for two capsule preparations loaded with 300 mg of either pure or hydrophilized phenytoin. The plasma levels for all individuals were significantly higher for the treated phenytoin than for the pure drug. Moreover, phenytoin absorption from capsules with untreated drug showed an ~1-hr lag time whereas the hydrophilized drug was absorbed immediately. The area under the plasma level–time curve, evaluated by the trapezoidal rule from 0 to 8 and 0 to 48 hr, showed higher values for the hydrophilized powder than for the pure drug (Table I) ($p < 0.01$).

In two subjects, absorption profiles were compared following oral administration of a 300-mg hydrophilized drug capsule and of a 300-mg phenytoin/200 ml of water suspension (Fig. 10). Nearly identical plasma curves were obtained. The areas under the plasma concentration–time curves from 0 to 8 hr for the capsule and the suspension were 1394 and 1357 mg min/liter for one subject and 1251 and 1244 mg min/liter for the other, respectively. Differences between the areas from 0 to 48 hr after administration were similar.

The total amount of the hydroxylated phenytoin metabolite in the urine over 48 hr is shown in Fig. 11 together with the plasma drug levels in two subjects after pure and hydrophilized drug administration. The results showed remarkable intersubject variation in excretion rates and could not be used to predict relative drug absorption rates from different preparations.

These availability studies are based only on a comparison of hydrophilized drug with pure strongly hydrophobic drug without any adjuvants (*i.e.*, disintegrating agents).

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