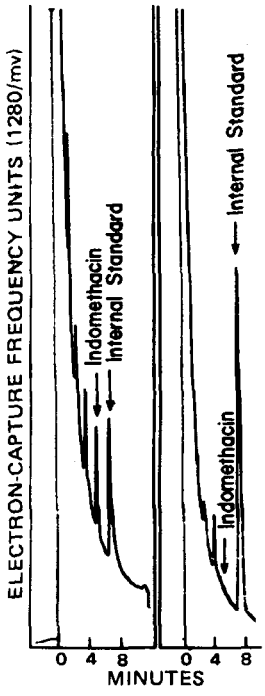

Figure 1—*Gas chromatograms of human plasma. Key: left: plasma (0.1 ml) containing 31 ng of indomethacin and internal standard; and right: control plasma with internal standard.*

domethacin divided by the peak height of the internal standard *versus* the plasma indomethacin concentration gave a computed slope of 42.8/ng of derivatized indomethacin and a coefficient of determination (r^2), a measure of accuracy, of 0.994. Extraction efficiency based on results from 10 samples using ^{14}C-indomethacin was estimated at 92 ± 3%.

Results of indomethacin analysis in a premature infant with patent ductus arteriosus following therapeutic intravenous administration of a 0.2-mg/kg dose of indomethacin sodium trihydrate are shown in Fig. 2. The pharmacokinetic parameters for the intravenous study were evaluated using a modified IGPHARM program (7). The calculated half-life was 15.5 hr, but further study is needed before any definitive statements can be made concerning the indomethacin half-life in the premature infant. Alvan *et al.* (8) demonstrated that the half-life of the β-phase of indomethacin in adults ranged from 2.6 to 11.2 hr. This rapid and sensitive method for indomethacin analysis has been useful in the

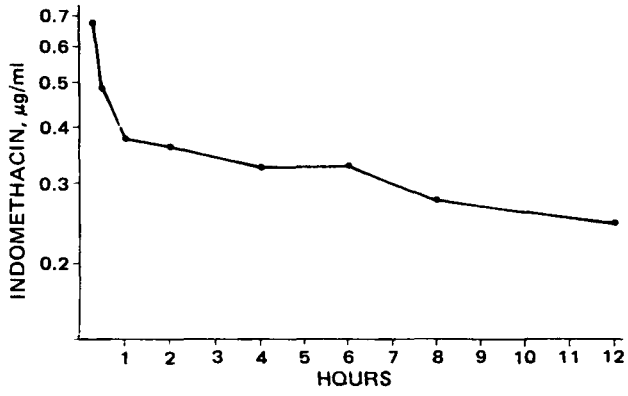

Figure 2—*Plasma concentration–time profile for indomethacin in a premature infant receiving intravenous indomethacin sodium trihydrate ($t_{1/2}$ = 15.5 hr, V_D = 0.37 liter/kg, and Cl_p = 15.3 ml/kg/hr). Sample size ranged from 0.05 to 0.15 ml of plasma.*

therapeutic monitoring of plasma drug levels when the sample volume is necessarily limited.

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Peristaltic Dissolution Apparatus: Prediction of Relative *In Vivo* Performance of Prednisone Tablets in Humans

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Received March 23, 1979, from the *R & D Laboratories, Frank W. Horner Limited, Montreal H3C 2W6, Canada.* Accepted for publication August 29, 1979.

Abstract □ By utilizing a previously established correlation concept, the relative *in vivo* performance in humans of seven unknown prednisone tablets was predicted accurately from peristaltic dissolution rate data. A general discussion is presented on the role of the peristaltic apparatus in selecting a suitable dosage form at the developmental stage.

Keyphrases □ Prednisone—tablets, prediction of relative *in vivo* performance in humans, peristaltic dissolution apparatus □ Dissolution apparatus, peristaltic—prednisone tablets, prediction of relative *in vivo* performance in humans □ *In vitro–in vivo* correlation—prednisone tablets, prediction of relative *in vivo* performance in humans, peristaltic dissolution apparatus

In 1975, a peristaltic dissolution rate apparatus was developed that was capable of predicting the *in vivo* performance of tolbutamide tablets in beagle dogs (1). This work was extended later to include meprobamate tablets (2) and tolbutamide tablets marketed in Canada (3). Despite the excellent *in vitro–in vivo* correlations obtained, a prime concern was the reliance on beagles as an *in vivo* model and the absence of human data in the correlations.

The performance of the apparatus on tablets that had been evaluated in a human bioavailability study was tested in this study. Seven prednisone tablets commercially available in the United States, each containing 5 mg of prednisone, were submitted in coded form by the Food and Drug Administration (FDA). Based on the peristaltic dissolution data, the relative *in vivo* performance of the tablets was predicted accurately.

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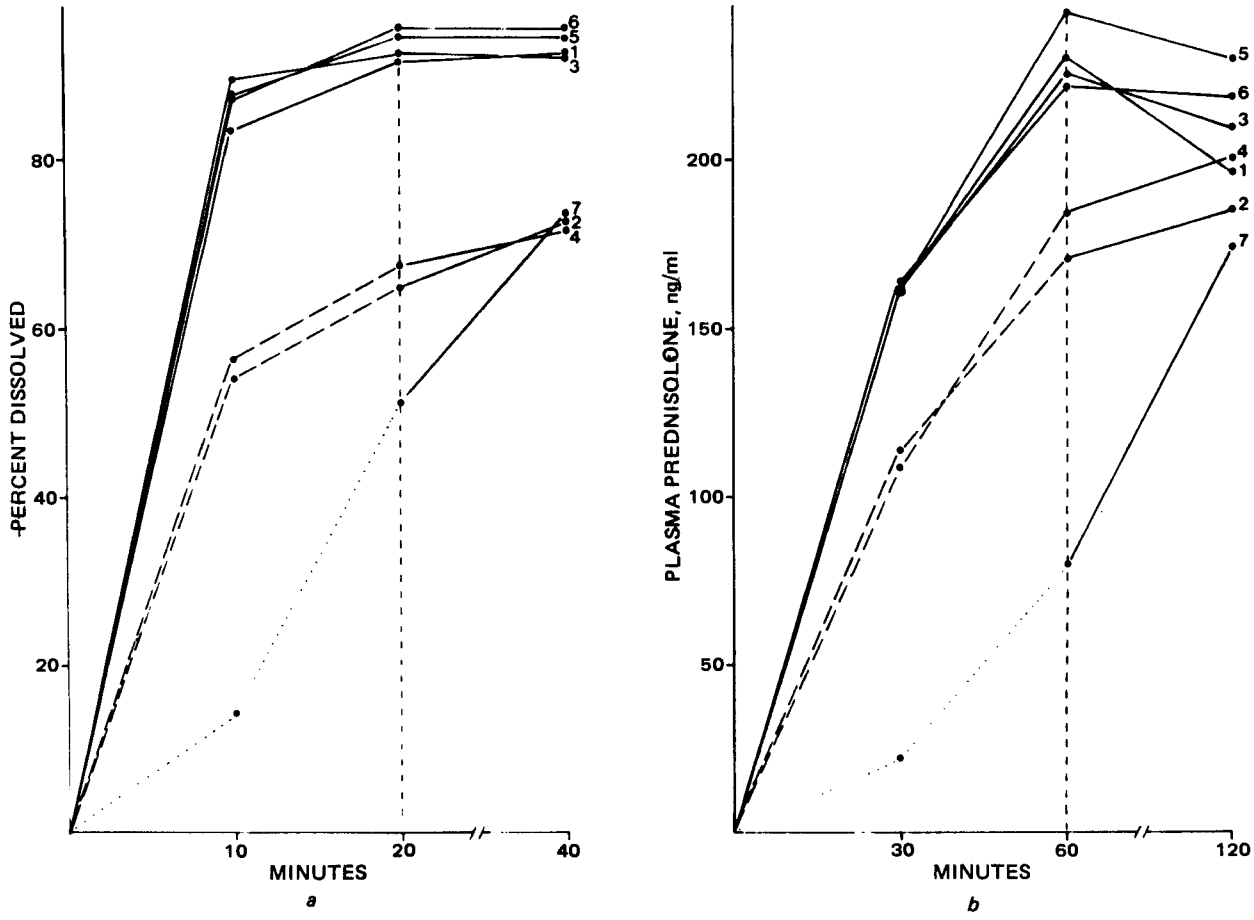

Figure 1—*Peristaltic dissolution plots of prednisone tablets* (a) *and prednisolone concentration–time plots of tablets* (b). *Key: See Table I. Dotted vertical lines at 20 and 60 min represent the maximum time intervals for* in vitro–in vivo *correlation. Relative performances of tablets from 0 to these times are categorized by solid, broken, and dotted lines.*

EXPERIMENTAL

Dissolution Rate Determinations—Dissolution rate profiles (Fig. 1*a*) for the prednisone tablets (5 mg) were obtained from the peristaltic dissolution rate assembly according to the conditions described previously (1). Aliquots (20 ml) of the dissolution medium, pH 7.6 tromethamine buffer, were removed at 10, 20, and 40 min and replenished with fresh buffer. Duplicate filtered aliquots (9 ml) were extracted separately with chloroform (3 × 5 ml), and the combined extracts from each sample were evaporated to dryness under nitrogen on a water bath.

The residues were dissolved in alcohol–chloroform (1:1, 0.5 ml) containing cholesteryl acetate[1] as an internal standard and were assayed for prednisone by GLC. The standard curve was obtained by spiking dissolution medium aliquots with known prednisone[2] quantities and performing the workup as described.

Chromatographic Conditions—The gas–liquid chromatograph[3] was equipped with a flame-ionization detector and a glass column (1.8 m × 6.2 mm o.d.) packed with 3% OV-17 on 80–100-mesh Gas Chrom Q. The column, detector, and injection port temperatures were 265, 295, and 278°, respectively. The carrier gas (nitrogen) flow rate was 48 ml/min. Air and hydrogen flow rates were adjusted for maximum sensitivity. The retention times for prednisone and cholesteryl acetate were 6.1 and 8.4 min, respectively.

RESULTS

In previous *in vitro–in vivo* correlation experiments (1–3) conducted with the peristaltic dissolution rate apparatus and blood drug level studies in beagles during the initial absorption phase, a direct relationship was established between *in vitro* data at t min and *in vivo* data at $3t$ min. By determining the dissolution rate profiles for the tablets during a 40-min period, their relative *in vivo* performance during a corresponding 120-min period was predicted accurately.

In this study, the average percent dissolved values also were obtained during the same *in vitro* period at 10, 20, and 40 min on the seven coded FDA prednisone tablets using the peristaltic dissolution assembly. These values are depicted graphically in Fig. 1*a*. Tablets 1, 3, 5, and 6 demonstrated comparable and excellent dissolution characteristics. Tablets 2 and 4 displayed mediocre dissolution profiles. Tablet 7 provided extremely poor initial performance and showed different intertablet disintegration characteristics, *e.g.*, two tablets in 23 min and one tablet in 32 min.

According to the correlation concept, these dissolution profiles were expected to reflect the relative *in vivo* performance of the tablets during a corresponding 120-min period. Tablets 1, 3, 5, and 6 were expected to exhibit identical drug absorption profiles. Conversely, Tablets 2, 4, and 7 were expected to display delayed drug absorption patterns, with a pronounced difference for Tablet 7.

This information was submitted to the FDA, which subsequently deciphered the code and confirmed that these prednisone tablets were from the same manufacturers and same lot numbers as those utilized in a comparative bioavailability study in human volunteers (4, 5). Figure 1*b* is a plot of the actual plasma prednisolone concentration *versus* time up to 120 min for 12 volunteers administered two 5-mg prednisone tablets. The data revealed that prednisone was absorbed and metabolized rapidly from Tablets 1, 3, 5, and 6 with a peak plasma prednisolone concentration of 222–244 ng/ml at 60 min. Tablets 2, 4, and 7 exhibited delayed drug absorption profiles. An examination of the two profiles in Figs. 1*a* and 1*b* demonstrates the ability of the peristaltic dissolution rate apparatus to simulate effectively the relative *in vivo* performance of the tablets.

Statistical treatment by regression analysis of the percent dissolved values for the tablets at 10 and 20 min *versus* plasma prednisolone concentrations at 30 and 60 min (Table I) revealed an extremely good fit with correlation coefficients of 0.995 and 0.923, respectively.

[1] Cholesteryl acetate, mp 113–116° (lot mp 115–116°) prepared by treating cholesterol with acetyl chloride and acetic acid.
[2] Courtesy of Merck-Frosst Laboratories, Montreal, Canada.
[3] Mikrotek MT 220, Tracor Inc., Austin, Tex.

Table I—Comparison of *In Vitro* and *In Vivo* Data [a]

Tablet	Percent Dissolved [b] 10 min	Percent Dissolved [b] 20 min	Plasma Prednisolone [c], ng/ml 30 min	Plasma Prednisolone [c], ng/ml 60 min
1	83.3	91.5	162	231
2	54.1	64.9	108	186
3	89.6	92.4	162	225
4	56.4	67.4	113	172
5	87.7	94.5	162	244
6	87.2	95.5	164	222
7	14.1	51.2	22	79.7

[a] Manufacturers and lot numbers of the tablets were: 1, Nysco Laboratories, 49571; 2, Barr Laboratories, 4126111; 3, McKesson Laboratories, 3K668; 4, Rexall Drug Co., E11499; 5, Lemmon Pharmacal, 1382; 6, The Upjohn Co., 786AEFI; and 7, Danbury Pharmacal Inc., 4539. The products were submitted by the FDA National Center for Drug Analysis, St. Louis, Mo. [b] Average of three runs. [c] From Ref. 4, Table IV, p. 163, and Table IX, p. 170.

DISCUSSION

The peristaltic dissolution apparatus has provided the authors with complete assurance that a new product launched on the market is bioequivalent to an originator's product or formulated to present optimum drug release characteristics. This assurance has been demonstrated by the excellent *in vitro–in vivo* correlations obtained in beagle dog studies, comparative bioavailability evaluations in humans, and results from clinical studies.

The utility of the peristaltic apparatus in drug product design can be exemplified in the following manner. If the drug substance (*e.g.*, prednisone) has limited water solubility, a drug suspension is screened for dissolution characteristics in dilute hydrochloric acid solutions (0.1–0.001 *N*), distilled water, and pH 7.6 tromethamine buffer. This procedure is followed in the event that the drug exhibits polymorphism or possesses amphoteric characteristics and because of pH variations observed in gastric fluids (6). If the drug is available commercially, a minimum of six lots of the originator's product is subjected to the same dissolution treatment. Any tablet or capsule formulation developed in the laboratories then must comply with the dissolution profiles exhibited by the originator's product. This routine is followed through to production scaleup, and the first six production batches of a new product are monitored carefully by the peristaltic apparatus. Any formulation change including an increase in batch size is investigated for possible changes in drug release characteristics before the product is released for sale.

Additional *in vitro–in vivo* correlations obtained with the peristaltic dissolution apparatus and initial drug absorption profiles in beagles are under investigation.

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High-Performance Liquid Chromatographic Determination of Isoniazid and 1-Isonicotinyl-2-lactosylhydrazine in Isoniazid Tablet Formulations

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Received August 6, 1979, from the *Drug Research Laboratories, Health Protection Branch, Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada.* Accepted for publication August 30, 1979.

Abstract □ A high-performance liquid chromatographic procedure is presented for the simultaneous determination of isoniazid and 1-isonicotinyl-2-lactosylhydrazine (I) in isoniazid tablet formulations. An aliquot of a diluted aqueous tablet extract is introduced onto a microparticulate cyanopropyl bonded-phase column using a valve-loop injector and chromatographed using a mobile phase of acetonitrile–0.01 *M*, pH 3.5 aqueous acetate buffer (5:95). Compound I can be determined at levels as low as 0.5% of the isoniazid label claim. The relative standard deviations are 0.4 and 0.7% for the simultaneous determination of isoniazid and I, respectively. Seven commercial tablet formulations contained 93.8–97.0% of the labeled isoniazid amounts and 0.3–5.8% of I, expressed as equivalent isoniazid relative to the labeled isoniazid level.

Keyphrases □ High-performance liquid chromatography—simultaneous determination of isoniazid and 1-isonicotinyl-2-lactosylhydrazine, isoniazid tablet formulation analysis □ Isoniazid—high-performance liquid chromatography, isoniazid tablet formulation analysis, simultaneous determination with 1-isonicotinyl-2-lactosylhydrazine □ 1-Isonicotinyl-2-lactosylhydrazine—high-performance liquid chromatography, isoniazid tablet analysis, simultaneous determination with isoniazid

1-Isonicotinyl-2-lactosylhydrazine (I) has been reported in isoniazid tablets formulated with lactose (1, 2). The formation of I from the interaction of isoniazid and lactose is marked at high humidity and/or elevated temperatures (3). TLC of various isoniazid tablet formulations available in Canada showed appreciable levels of I. The USP XIX (4) nitrite titration assay for isoniazid responds quantitatively to the isoniazid moiety of I (5); therefore, an alternative procedure was required to monitor isoniazid formulations for I. Several colorimetric procedures (2, 6) are specific for isoniazid in the presence of I but cannot directly measure I.

A chromatographic method would be applicable to the rapid, simultaneous determination of isoniazid and I. TLC was unsuitable because of the difficulty in obtaining quantitative data, and GLC was rejected because of chromatographic difficulties with I. High-performance liquid chromatography (HPLC) has been used for isoniazid analysis in single- (1) and dual- (7) component formulations but has not been applied to the analysis of I except (1) to show that such compounds do not interfere with the isoniazid peak. A forward-phase HPLC system was developed recently for the simultaneous analysis of isoniazid and α- and β-1-glucopyranosyl-2-isonicotinylhydrazine

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