human studies described in this paper. The higher sensitivity and greater convenience of the [^{125}I]-based assay have resulted in its use in current meobentine studies. The plasma profile in the subjects studied indicated that meobentine kinetics are multicompartmental in nature with a long terminal half-life (11–14 hr). A long terminal half-life has also been seen with bethanidine (18) and bretylium (19) in humans. Mean peak meobentine plasma levels in the same group of volunteers essentially doubled from 239 to 451 ng/ml with an increase in dose from 2.5 to 5.0 mg/kg. Mean area under the curve also approximately doubled from 1890 to 3358 ng hr/ml over this dose range. These observations suggest linear kinetics for meobentine in the 2.5–5-mg/kg dose range. Clearance (TBC/F) did not change significantly with increasing dose. The extent of absorption and bioavailability of meobentine are currently under investigation in animals and humans. The related guanidine, bethanidine, has been reported to be well absorbed by humans (18).

Currently, these radioimmunoassay procedures are being applied to detailed studies of the disposition of meobentine sulfate in animals and humans. Studies of the absolute oral bioavailability and pharmacokinetics of meobentine sulfate will be presented elsewhere in the near future.

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Automated Sampling of *In Vitro* Dissolution Medium: Effect of Sampling Probes on Dissolution Rate of Prednisone Tablets

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Abstract □ The effect of sampling probe size and location on the *in vitro* dissolution rate of prednisone tablets was examined. Using USP XX Apparatus 2 with an automated sampling system, dissolution rates were determined using two types of large filter-tipped probes and a small capillary probe. Each probe was tested at three locations within the kettle. The large probes caused hydrodynamic changes which, when compared with results obtained through manual sampling, resulted in significant changes in dissolution rates at each location. These changes were less evident when the capillary probe was used, with an insignificant difference between results of automated and manual sampling when the capillary probe was placed midway between the paddle shaft and the kettle wall.

Keyphrases □ Dissolution rates—effect of sampling probe size and location on dissolution rate of prednisone tablets □ Hydrodynamics—dissolution rates affected by sampling probe size and location, USP paddle method □ Prednisone—tablets in dissolution rate testing with effects of sampling probe size

Automated sampling and analysis of *in vitro* dissolution aliquots can be a useful, timesaving procedure. Several automated sampling systems are commercially available in which aliquots of the dissolution fluid, taken at specified times, travel into sample cups or into an automated analytical system. Results of such a system are not generally considered acceptable, however, unless they agree with those obtained by manual sampling.

A large number of dissolution testing variables have been identified (1). These need to be strictly controlled if reproducible results are to be obtained. Changes in any of these variables can have a significant effect on the dissolution rate of a dosage form. A previous study (2) showed that the dissolution rate is affected by the effects of various dissolution parameters on the hydrodynamics of the dissolution fluid. Another study (3) showed that the hydrodynamic disturbance caused by insertion of a relatively large object, such as a thermometer, into the dissolution fluid may cause a change in dissolution rate, and that the location of such an object can also have a significant effect.

With most automated sampling systems now in use, a relatively large filter-tipped probe is immersed in the dissolution fluid. The above studies imply that a large probe can affect the system hydrodynamics, and therefore the dissolution rate of some dosage forms, thereby causing results which differ from those obtained by manual sampling, and that a smaller probe may have less effect on the

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Table 1—Tape Program for Automated Dissolution Sampling System

Time, min	Peristaltic Valve Position	Function
0	1	Start
29:15	2	Sample
31:00	3	Wash
32:00	4	Standard
33:15	5	Empty Coil 1
34:30	6	Empty Coil 2
35:45	7	Empty Coil 3
37:00	8	Empty Coil 4
38:15	9	Empty Coil 5
39:30	10	Empty Coil 6
40:45	11	Wash
42:00	12	Stop

dissolution rate. A previous study (4) confirmed this implication by showing that manual aliquots taken while a large probe was suspended in the dissolution fluid gave higher results than those obtained when a small capillary probe or no probe was present.

In the work described in this paper, the effect of sampling probe size and probe location within the dissolution kettle were investigated by using an automated sampling system to collect aliquots through probes of several sizes and by comparing these results with those obtained by manual sampling.

EXPERIMENTAL

Principles—Dissolution testing was performed using the USP (5) method on a sample of 10-mg prednisone tablets which had been shown to be sensitive to changes in dissolution testing parameters[1]. Using an automated sampling system, samples were withdrawn at 30 min, sequentially diluted with water, and passed through a spectrophotometer flowcell, with absorbance measured at 242 nm. For comparison, manual samples were taken from separate dissolution runs, filtered, and determined spectrophotometrically.

The dissolution apparatus[2] was USP XX Apparatus 2 (6). Paddle rotation speed was maintained at 50 rpm with a gear plate. Temperature was maintained at 37.0 ± 0.5° using a heater-circulator[3] immersed in the water bath.

The automated sampling and analysis system[4] consisted of a multichannel peristaltic valve; plattered manifold for storage; and sequential analysis of sampled aliquots, tape programmer, peristaltic pump, spectrophotometer (set at 242 nm) with a 15-mm flowcell, and recorder. The apparatus was programmed according to Table I. This program allowed for sampling from each dissolution kettle at 30 min, with each aliquot being stored in a separate holding coil. A portion of standard solution was then introduced to the analytical system followed by sequential introduction of the contents of each sample storage coil. The schematic diagram in Fig. 1 shows the postsampling analytical system.

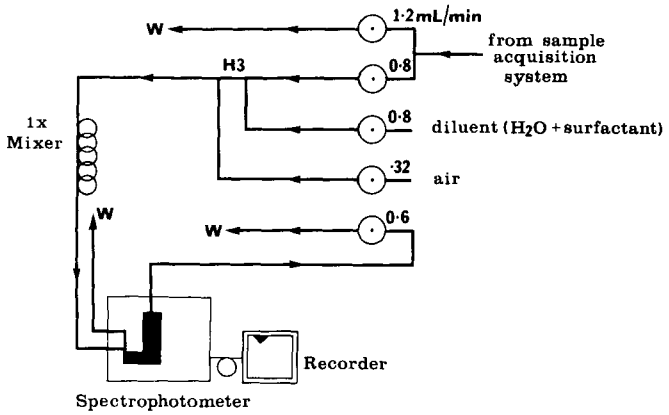

Figure 1—*Flow diagram for postsampling analytical system. All pump tubes are Tygon; all transmission tubing is trifluoroethylene, 1.0-mm i.d.; W indicates flow to waste reservoir.*

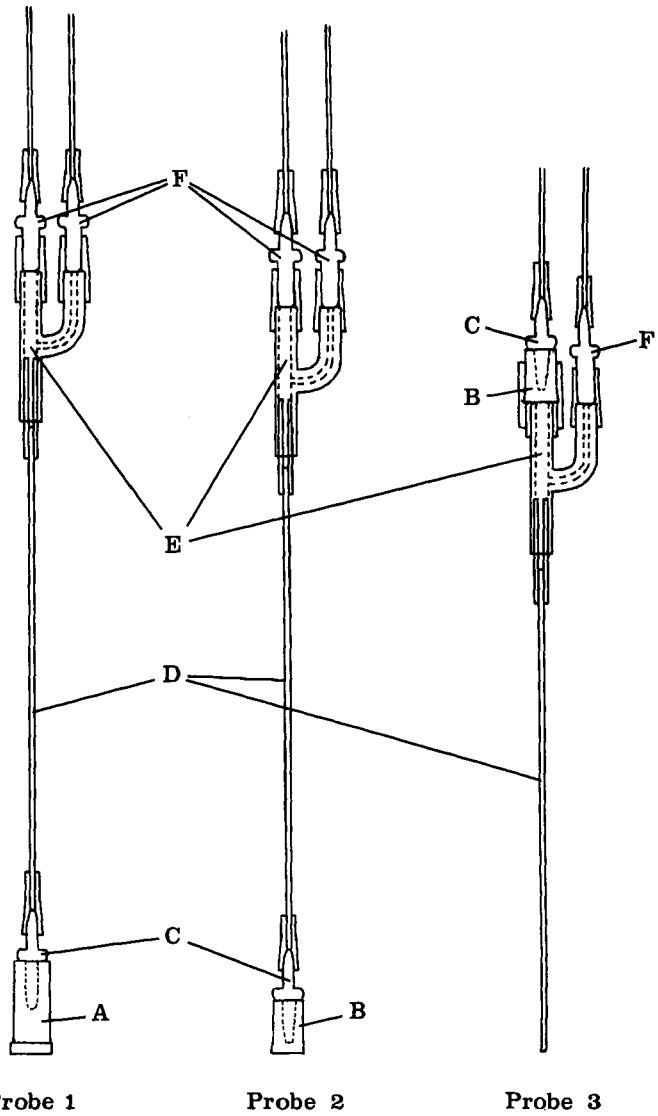

Figure 2—*Three types of probes used in automated sampling. Key: A, filter; B, filter; C, N7 nipple connector; D, glass capillary tube, size 0.8–1.10 × 100 mm; E, D1 "h"-connector; F, N5 nipple connector. Parts are connected to each other with sleeves of rubber tubing.*

The three types of sampling probes used with the automated sampling system are shown in Fig. 2. In Probes 1 and 2, commercially available filters[5,6] were attached to glass stalks connected to the "h"-connectors in the automated sampling system. The filters and stalks were suspended in the dissolution fluid throughout the dissolution run. In Probe 3, the glass stalk connected to the "h"-connector was extended into the dissolution fluid. The filter was attached "downstream" from the connector. Maximum immersed diameters for Probes 1, 2, and 3 were 8.0, 7.2, and 1.5 mm, respectively. Displacements of the immersed portions of the three probes were 1.1, 0.7, and 0.1 ml, respectively.

Manual sampling was performed using 50-ml syringes with 14-gauge needles. Aliquots were filtered through p 0.8-μm porosity membrane filters[7] and analyzed spectrophotometrically.

Reagents and Standards—Distilled water, deaerated by stirring under reduced pressure for 2 hr was used as the dissolution fluid.

[1] Cox, D. C., personal communication.
[2] Easi-Lift model 72-S, Hanson Research Co., Northridge, Calif.
[3] Braun Thermomix model 1441, VWR Scientific, Norwalk, Calif.
[4] Sample Acquisition System for Dissolution Rate Analysis (SASDRA) with Quality Control Manifold, Technicon Instruments Corp., Tarrytown, N.Y.
[5] No. 178-3985-P01 (Filter A), Technicon Instruments Corp., Tarrytown, N.Y.
[6] No. FT-6006 (Filter B), Centaur Chemical Co., Stamford, Conn.
[7] No. AAWP-02500, Millipore Corp., Bedford, Mass.

Table II—Results of Dissolution Testing of Prednisone Tablets, Percent of Label Declaration

Sampling System	Sampling Location A Mean	A SD	A N[a]	B Mean	B SD	B N	C Mean	C SD	C N
Manual	34.5	2.1	18	36.5	3.1	35	36.3	2.3	17
Automated, Probe 1	44.9	4.3	18	43.3	3.4	27	32.7	4.6	24
Automated, Probe 2		NT[b]		43.1	2.4	12		NT	
Automated, Probe 3	38.1	3.1	18	36.7	3.3	52	34.5	3.3	18

[a] Number of aliquots taken. [b] Not tested.

The diluent used in the automated analysis was prepared by adding 1 ml of surfactant[8] to 1 liter of water.

The standard stock solution was prepared by dissolving 25 mg of prednisone USP Reference Standard in 20 ml of ethyl alcohol and diluting to 500 ml with water. A working standard was prepared daily by diluting a 10-ml standard stock solution to 100 ml with water.

Procedure—Before the automated sampling system was used, all lines and coils were filled with their respective solutions, and the system was allowed to equilibrate by running in the "wash" position for ~30 min. To start the dissolution run, the peristaltic valve was set in Position 1, and the dissolution apparatus was turned on. With all paddles rotating and sampling probes in the proper positions within the kettle, tablets were dropped simultaneously into all kettles and the programmer was turned on. After the 30-min sample aliquot collection (with the peristaltic valve in Position 3), the probes were removed from the kettles and placed in a beaker of water until the end of the analysis.

Dissolution testing was performed with samples withdrawn either manually or by automation from one of the three positions shown in Fig. 3. Position A is 0–1 cm from the wall of the vessel, Position B is midway between the kettle wall and the paddle shaft, ±0.5 cm, and Position C is 0–1 cm from the paddle shaft. The vertical location of all three probe positions is midway between the surface of the dissolution fluid and the level of the top of the paddle blade. Position B is the generally accepted position for manual sampling (1).

RESULTS AND DISCUSSION

Accuracy and reproducibility of the automated system were tested by running the system with the probes immersed in standard solution concentration of 7.08 μg/ml, equivalent to 35.4% of the declared tablet content dissolved. Each set of sampling probes was tested in this manner, with two runs of 6 aliquots/run made for each probe. Recoveries were 102.6, 101.7, and 101.4%, with coefficients of variation of 0.408, 0.554, and 0.796 for Probes 1, 2, and 3, respectively. Linearity was tested by aspirating standard solutions of 1.6, 3.2, 4.8, 6.4, 7.8, and 9.6 μg/ml, equivalent to 8.0–48.0% of the declared tablet content dissolved, using each of the three types of sampling probes. The response was linear throughout this range for each type of probe.

Dissolution testing of prednisone tablets was performed, with aliquots taken from each of the three kettle locations. Sampling was done automatically, using the three automated sampling probes, and manually. Results are summarized in Table II.

Statistical comparisons of dissolution results using each of the automated sampling probes with results obtained by manual sampling indicated a significant effect of the size of the probe. When aliquots were taken from the generally recommended Position B, each of the larger probes (Probes 1 and 2) gave significantly higher results than those of manual sampling when the t criterion was used at the 1% level of significance. The difference between the results using the smaller Probe 3 and the manual results was insignificant. In Position A, automated sampling with all probes gave significantly higher results than manual sampling, although the results with the larger probes were much higher than with the smaller. A different hydrodynamic effect was seen, however, when automated sample probes were suspended in Position C. Automated results from this position were lower than those obtained by manual sampling, with the lowering effect being more pronounced with the larger probes.

By subjecting the manual sampling data for the three sampling positions to a two-way analysis of variance at the 2% level of significance, it was shown that there was no significant difference among the three mean percentages dissolved, thereby indicating that no significant horizontal concentration gradient existed within the kettle. The observed changes in dissolution rates obtained by automated sampling were, therefore, caused by interference by the sampling probes with the hydrodynamic flow within the kettle. The degree of interference was directly related to the size of the probe as well as to its location.

The hydrodynamic change caused by insertion of the large probes was confirmed by visual observation. The normal disintegration of the prednisone tablets within the dissolution kettle formed a regular cone-shaped pile of undissolved tablet material, with a small amount of material dispersed throughout the medium. Extension of the large Sampling Probe 1 into the medium at Position B caused an immediate shifting of the conical pile to one side, followed by the dispersal of considerably more tablet material throughout the medium. This dispersal would account for the higher dissolution results obtained. The effect was more pronounced when the probe was extended into Position A. With the large probe in Position C, the conical pile became taller and narrower, with an apparent decrease in the amount of dispersed material accounting for the lower dissolution results. The insertion of the capillary probe into any of the three positions caused little or no visible disturbance of the undissolved tablet material.

CONCLUSIONS

The large sampling probes provided with several commercially available automated dissolution sampling systems were shown to cause significant hydrodynamic disturbances, resulting in changes in the dissolution rate of a sample of prednisone tablets. The disturbances were reduced through the use of a small capillary probe. Probe location within the kettle was also shown to require careful control. While only one type of tablet was used in this study, the changes in dissolution hydrodynamics caused by the sampling probe could be expected to affect the dissolution

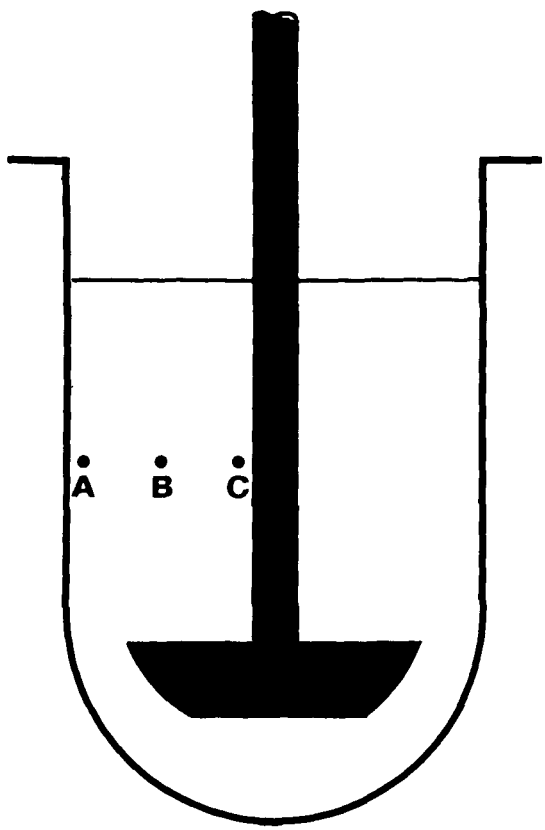

Figure 3—*Three probe locations used in dissolution sampling.*

[8] Brij-35, 30% aqueous solution, Fisher Scientific Co., Fair Lawn, N.J.

rate of other types of dosage forms. It is therefore recommended that the sampling probes used with automated sampling systems be made as small as possible, and that the horizontal sampling position be carefully maintained halfway between the paddle shaft and the kettle wall.

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Sequential Organ First-Pass Effects: Simple Methods for Constructing Compartmental Pharmacokinetic Models from Physiological Models of Drug Disposition by Several Organs

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Received April 22, 1981, from the *Laboratory of Chemical Pharmacology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication September 22, 1981.

Abstract □ The relationships between organ clearances derived from physiological pharmacokinetic models and the first-order rate constants in compartmental pharmacokinetic models are frequently difficult to visualize when drugs are eliminated simultaneously by several organs. Two simple methods for showing these relationships are illustrated in this paper.

Keyphrases □ Pharmacokinetics—construction of compartmental models from physiological models of drug disposition, first-pass effects □ First-pass effects—construction of compartmental pharmacokinetic models from physiological models of drug disposition □ Compartmental models—construction of pharmacokinetic models from physiological models of drug disposition, first-pass effects

Changes in the blood concentrations of drugs after their oral administration are frequently fitted to a pharmacokinetic equation representing a three-compartment model in which it is assumed that all eliminating organs are within the central compartment. In such a model, a first-order rate constant (k_{31}) is frequently used to represent the absorption of a drug from the GI tract into a central compartment and first-order microconstants (k_{12}, k_{21}) are frequently used to represent the passage of the drug between the central and peripheral compartments. The elimination of the drug from the body is represented by a first-order microconstant (k_{10}) emanating solely from the central compartment. This model would be identical to that pictured in Fig. 1 except that k_{13}, k_{20}, and k_{30} would be zero.

Despite the simplicity of this model, it adequately describes the major events in the absorption and disposition of most drugs and, thus, has gained wide acceptance. But it is invalid in many situations, particularly those in which drugs are very rapidly cleared by enzymes in the GI mucosa, liver, and lung (the first-pass organs) and those situations in which the drug passes into the GI tract by reversible diffusion from mucosal blood into the lumen of the GI tract or by biliary excretion. Moreover, the model also fails to describe adequately the pharmacokinetics of metabolites that are rapidly cleared by various organs. In these situations it is necessary to consider physiological models.

Several years ago, Bischoff and Dedrick (1) developed a physiological approach in which Fick's Principle is applied to individual organs. In this approach, each organ comprises three homogeneous compartments: the blood, the interstitial fluid, and an intracellular compartment. Since a differential equation is required to express the rate of change in the amount of drug in these subcompartments of each organ, the number of equations required to describe changes in the disposition of the drug in the body at any given time can be quite large; frequently as many as 15–20 equations are used. It is possible to integrate a set of such equations by means of LaPlace transforms. Unfortunately, the pharmacokinetic constants obtained by integration of the simultaneous equations or from measurements of drug concentrations in blood are virtually impossible to interpret in physiological terms.

The present paper describes a general approach for developing pharmacokinetic models that combine some of the complexities that can occur during rapid simultaneous elimination of drugs by several organs with the simplicity of the linear three-compartment model. The approach is essentially intuitive and, thus, requires very little mathematical ability. It is illustrated by a model in which a drug is rapidly cleared by the GI mucosa, the liver, the lungs, and the kidneys.

THEORY

As in the three-compartment model, it is assumed that after rapid injection into the aorta, a drug is almost instantaneously distributed into a central compartment that includes most of the organs of elimination such as the kidneys, the GI mucosa, the liver, and the lungs. Thus, by the time the arterial concentration of the drug is estimated, the ratios of the intracellular concentrations of the drug in the organs included within the central compartment to the drug concentration in arterial blood have reached constant values. (This is usually estimated indirectly by measuring the drug concentration in systemic venous blood: draining a nonelimination organ such as an arm.) Under these quasi steady-state

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