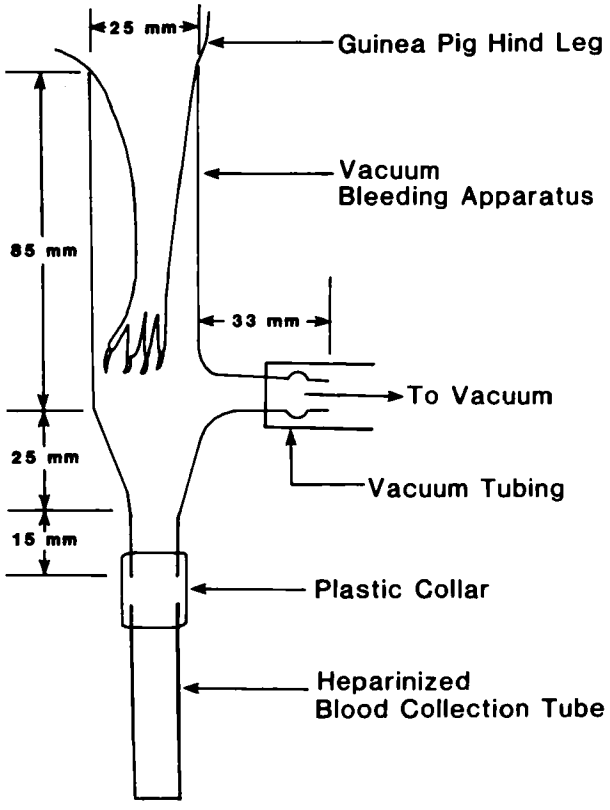

Figure 1—*Schematic diagram of the vacuum bleeding apparatus.*

When a 1- to 2-ml volume of whole blood was collected for each timed sample, the vacuum was turned off and the leg carefully removed from the apparatus. Bleeding usually ceased promptly, but it was sometimes necessary to place a gauze bandage over the toenail until all bleeding had stopped, usually within 1 min. For repeated blood collection from the same animal the nail was cut progressively more proximally at each sampling; the nails on both legs were used. The guinea pigs were not distressed by a sampling of up to 3 ml, and since no anesthetic was used, the animals had normal mobility when returned to their cages. The problem of blood clotting in the collection apparatus was minimized by using silicone grease on the inside glass surfaces.

Previous researchers have sampled guinea pig blood from the ear veins, penis vein, superficial thigh vein, jugular vein, orbital venous plexus and by the methods of heart puncture, indwelling vascular cannulation, cutting the toenail bed, and cutting the lateral saphenous vein or lateral metatarsal vein (4, 5). None of these methods proved completely satisfactory for the purposes of this study, since in most cases, an anesthetic was needed which could confound the observed results by affecting caffeine metabolism and/or plasma ascorbic acid levels (6, 7) if the study involved ascorbic acid supplementation, depletion, *etc.* We believe that the simplified procedure described here can be applied with similar success to studies involving other rodent species, in which anesthesia may be undesirable.

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An Automated Sampling Device for Dissolution Testing

Keyphrases □ Dissolution testing—automated sampling device

To the Editor:

Many automated sampling devices have been designed for commercially available dissolution units[1]. The major criticism of some of these units is that the sampling probes remain in the dissolution medium during the duration of the test, thereby disturbing the hydrodynamics of the solution (1). This can cause dissolution results other than those intrinsic to the dosage form. We have designed a simple sampling device which eliminates this problem while still allowing the convenience of automation. The device is made from a commercially available air-actuated, solenoid-controlled valve[2] and brackets that can be easily made in-house. The design and orientation of the brackets are shown in Figs. 1 and 2. Dimensional and installational information is presented in Fig. 3. Air is supplied to the device at 20 psi. Either house air or an air pump can be used. The unit is connected to a computer-controlled pump-fraction collecter[2] which provides contact closure to the solenoid through its internal-timing sequences which also control the pump-fraction collector's prime, sample, and purge cycles. However, this unit need only be connected to a 110 V contact closure and external air supply to actuate the valve. Once actuated, any suitable multiple-channel pump and collector can provide samples.

As previously indicated, we have chosen a combination pump-fraction collector with computerized timing sequences. A program is entered using a hand-held pad, and at program-designated intervals contact closure is made, the device is actuated, and samples are collected during a three-part 60-sec sequence: for 20 sec the lines are washed with sample to waste; the sample tray is then advanced and the next 20-sec sample is collected; and for 20 sec the pumps reverse to purge the lines. The sample tray then

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[2] Bimba Manufacturing Co., Monee, IL 60449.

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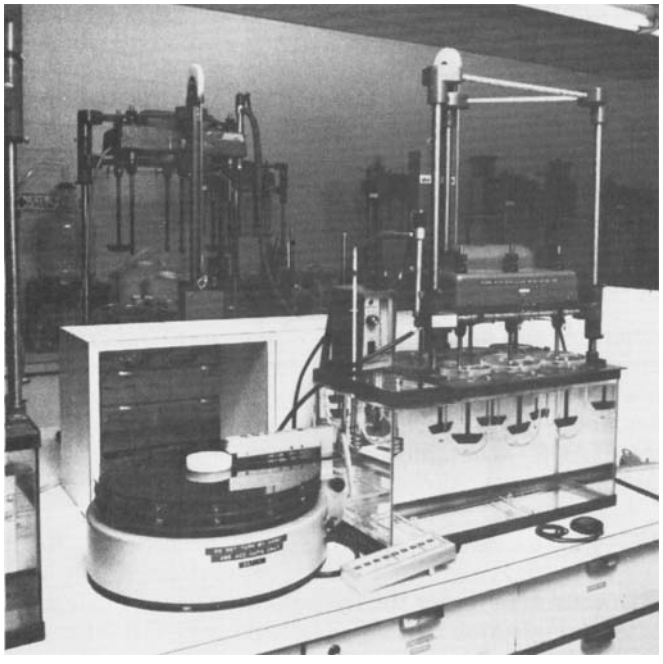

Figure 1—*Automated sampling device, fraction collector and dissolution unit.*

advances in preparation for the next sampling time. During the final 20 sec the probes are not in solution. Sample size can be adjusted by varying pump piston displacement.

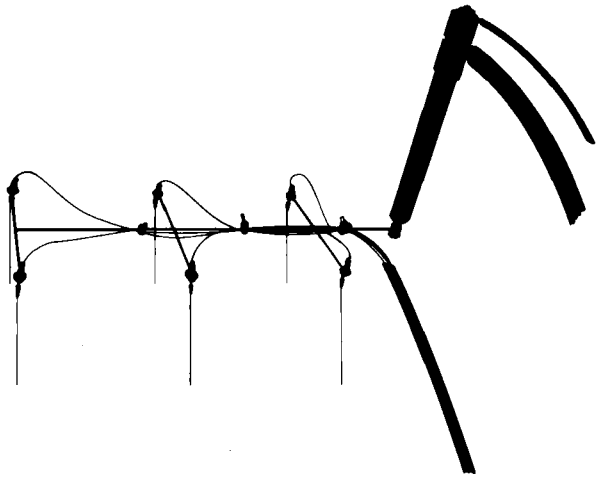

Figure 2—*Automated sampling device.*

During sampling, 50 μl-capillary tube probes are inserted into the medium for only 40 sec. The sampling location of the probes is as recommended in USP XX, and they retract above the surface of the medium after the 40-sec interval.

It has been shown by Savage and Wells that is is advantageous to minimize sample-probe size to reduce the influence on hydrodynamics (1). This design not only uses minimal probe size, but has the additional advantage of limiting the time the sample probes are inserted into the

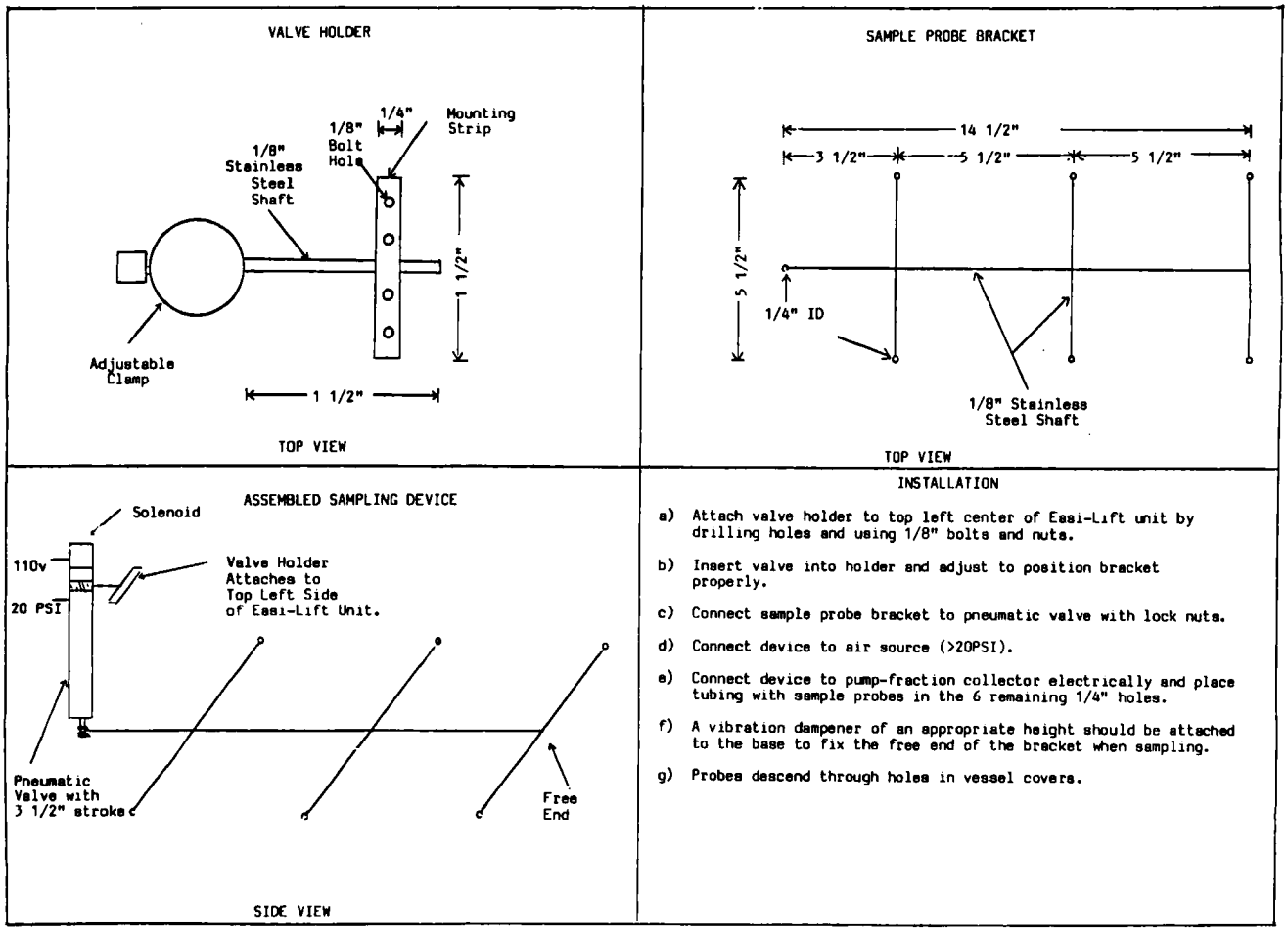

Figure 3—*Dimensional and installational information.*

dissolution medium to 40 sec/sampling time. This combination of reduced probe size and minimal contact with the dissolution medium results in minimal disturbance of the hydrodynamics of the medium.

In summary, this design offers these advantages for adaptation to Hanson Easi-Lift dissolution units:

1. The device does not disturb the hydrodynamics of the dissolution test, and the sampling probes are inserted in the solution only while sampling.
2. There is easy access to the dissolution unit because probes and brackets retract.
3. The sampling device is inexpensive to make and could be linked to a variety of dissolution pump-sample collection devices.

This device has proven to be reliable and essentially carryover-free in our laboratory and is in routine use.

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Computation of Model-Independent Pharmacokinetic Parameters During Multiple Dosing

Keyphrases □ Pharmacokinetics—computation of model-independent parameters, multiple dosing

To the Editor:

Pharmacokinetic analysis by means of traditional compartmental methods is slowly giving way to model-independent or noncompartmental approaches. Computational simplicity and, in some cases, more useful information are among the reasons for this trend. Methods that use the area under the drug concentration *versus* time curve (AUC) and the area under the first moment of drug concentration *versus* time curve (AUMC) are available to determine clearance (CL), mean residence time ($\bar{t}$), and apparent volume of distribution at steady state (V_{ss}) from data obtained after a single dose of drug (1, 2).

Often, the need arises to calculate pharmacokinetic parameters after several doses or at steady state; this is particularly true when patients are being treated with the study drug, and doses may not be manipulated for the purposes of the investigation. With the limited exception of the determination of clearance at steady state, noncompartmental methods have not been considered for this purpose.

Following repeated administration of a fixed dose of a drug at fixed intervals, the AUC during a dosing interval at steady state is equal to the total AUC after the first dose (3). Therefore, drug clearance can be calculated at steady state. On the other hand, AUMC during a dosing interval at steady state ($AUMC_{ss}$) is less than the total AUMC after a single dose. Therefore, $\bar{t}$ and V_{ss} cannot be calculated directly from steady-state data.

The inequivalence of $AUMC_{ss}$ and AUMC (single dose) can be demonstrated by considering multiple intravenous bolus doses of a drug with linear multicompartmental characteristics. Drug concentration (C) after a single dose is given by:

$$C = \sum_{i=1}^{n} A_i \exp(-k_i t) \qquad \text{(Eq. 1)}$$

where A_i and k_i are drug specific constants with units of concentration and reciprocal time, respectively; k_i values are independent of dose; $k_1 > k_2 \ldots > k_n$. The total area under the drug concentration–time curve after a single dose (AUC) is obtained by integrating Eq. 1 with respect to time:

$$\text{AUC} = \int_0^\infty C\,dt = \sum_{i=1}^{n} A_i/k_i \qquad \text{(Eq. 2)}$$

The total area under the first moment *versus* time curve after a single dose (AUMC) is given by the following integral:

$$\text{AUMC} = \int_0^\infty Ct\,dt = \sum_{i=1}^{n} A_i/(k_i)^2 \qquad \text{(Eq. 3)}$$

The analogous equations that apply to a dosing interval at steady state are as follows:

$$C_{ss} = \sum_{i=1}^{n} A_i \exp(-k_i t)/[1 - \exp(-k_i \tau)] \qquad \text{(Eq. 4)}$$

$$\text{AUC}_{ss} = \int_0^\tau C_{ss}dt = \sum_{i=1}^{n} A_i/k_i \qquad \text{(Eq. 5)}$$

$$\text{AUMC}_{ss} = \int_0^\tau C_{ss}t\,dt = \sum_{i=1}^{n} \frac{A_i}{(k_i)^2} \times \frac{[1 - \exp(-k_i\tau)] - k_i\,\tau \exp(-k_i\tau)}{1 - \exp(-k_i\tau)} \qquad \text{(Eq. 6)}$$

where τ is the fixed dosing interval. Note that Eqs. 2 and 5 are equivalent.

However, the relationship between $AUMC_{ss}$ and AUMC is given by the following ratio:

$$\frac{\text{AUMC}_{ss}}{\text{AUMC}} = \sum_{i=1}^{n} \frac{A_i}{(k_i)^2} \times \left[1 - \frac{k_i\,\tau \exp(-k_i\,\tau)}{[1 - \exp(-k_i\,\tau)]}\right] \Big/ \sum_{i=1}^{n} A_i/(k_i)^2 \qquad \text{(Eq. 7)}$$

Clearly, attempting to calculate t or V_{ss} by replacing AUMC with $AUMC_{ss}$ would provide incorrect answers, because $AUMC_{ss} < AUMC$.

We wish to propose an alternate, noncompartmental method to calculate pharmacokinetic parameters during repetitive dosing. This method may be called reverse superposition because a single dose curve is derived from data obtained during the second, third, or nth dosing interval. It is not limited to steady state but does require that subsequent doses be given during the postabsorptive, postdistributive phase of the previous dose. Each data point on the single-dose curve is calculated by means of the following equation:

$$C(t) = C_i(t) - C_i(0) \exp(-k_n t) \qquad \text{(Eq. 8)}$$

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