ground-glass-stoppered flask. This solution is then ready to be chromatographed.

Chromatographic Procedure—The mobile phase was 4% 2-propanol in heptane, the temperature was ambient, and the solvent flow was ~1 ml/min (at an inlet pressure of 1000 psig). The precision photometer detector (254 nm) was set at an attenuation of ×16.

Samples and standards of 3 μl (~15 μg of canrenone) were injected with the flow stopped. A standard was injected for every five sample injections, and the peak heights of canrenone and the internal standard were measured.

Calculations—The concentration of canrenone in milligrams per dose is calculated using:

$$\text{mg/dose canrenone} = F \times \frac{H_1}{H_2} \times \frac{\text{average dose weight (mg)}}{\text{weight sample used (mg)}} \quad \text{(Eq. 1)}$$

$$\text{\% label claim} = \frac{\text{mg/dose found}}{\text{mg/dose theory}} \times 100 \quad \text{(Eq. 2)}$$

where H_1 and H_2 are the peak heights of canrenone and internal standard in the sample, respectively.

The response factor, F, is the ratio of the internal standard peak heights to the canrenone peak height in the standard reference solution multiplied by the weight of canrenone in the standard.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a typical sample containing the internal standard. The major degradation products, II and III, do not interfere with the analysis. Compound III does not elute but is retained on the column. Compound II elutes at ~32 min if present. Canrenone elutes at ~15 min, and the internal standard elutes at ~5 min. Methanol was chosen as the solvent for drug extraction due to the insolubility of common excipients.

The linearity of response has been shown for canrenone pharmaceutical formulations containing 40–60 mg/dose. Table I shows the results of standard addition experiments when the theory was 50 mg/dose.

The precision of the method was determined from 10 replicate determinations of canrenone in 50-mg/dose pharmaceutical formulations performed over 2 days (Table II). Peak height rather than peak area was found to yield better results on this analysis, because the pumping system on the chromatographic apparatus caused a recorder spike which interfered with digital integrator calculations.

This method is also applicable to single-tablet and capsule analysis if the attenuation of the UV detector and the internal standard concentrations are adjusted appropriately.

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Time-Dependent Change in Renal Clearance of Bethanidine in Humans

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Abstract □ Blood levels and urinary excretion rates of bethanidine were determined in three normal human subjects following oral administration of a single dose of the drug. The postabsorptive decline of blood concentration with time was noticeably slower than the corresponding decline in the urinary excretion rate. The discrepancy can be attributed to a continual decrease in the renal clearance of bethanidine throughout the study. Therefore, pharmacokinetic modeling of urinary excretion data alone would lead to erroneous conclusions concerning the persistence of drug in the blood.

Keyphrases □ Bethanidine—time-dependent change in renal clearance and blood levels, humans □ Excretion, urinary—rate of clearance of bethanidine, compared to blood levels, humans □ Antihypertensives—bethanidine, renal clearance compared to blood levels, humans

A previous study (1) on the pharmacokinetics of bethanidine in hypertensive patients found that the renal clearance of unchanged bethanidine fluctuated greatly in the initial 6 hr following a single 25-mg iv dose. The study also revealed that the elimination of bethanidine in humans occurs almost exclusively *via* renal excretion. Hence, a more extensive study on the renal clearance of the drug was undertaken.

EXPERIMENTAL

Three healthy male volunteers, 65–75 kg, were selected. Their

Table I—Recovery of Radioactivity from Urine and Feces following a Single 10-mg Oral Dose of ^{14}C-Bethanidine Hemisulfate to Normal Subjects

Sample	Dose Recovered, %		
	Subject 1	Subject 2	Subject 3
Urine	84.6	82.4	70.7
Feces	13.46	3.39	18.28
Total	98.06	85.79	88.98

medical histories and the results of physical and laboratory examinations were unremarkable. Each subject gave signed, informed consent to participate.

Subjects were instructed to avoid the ingestion of any drug for at least 1 week before and during the study. Following an 8-hr fasting period, each subject received a 10-mg solution of ^{14}C-bethanidine hemisulfate containing approximately 100 μCi of radioactivity. Food was not allowed until 2 hr after drug administration.

The dose administered was approximately 50% of the usual pharmacologically effective dose (0.3 mg/kg). Arterial blood pressure was determined at 0, 2.5, 4.5, 6.5, 8.5, and 12 hr after drug administration with the subject in supine and standing positions. A clear pharmacological effect of the drug was not apparent.

Ten milliliters of blood was drawn in containers[1] containing potassium oxalate at 0.5, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 48 hr after drug administration. The blood samples were immediately refrigerated pending assay. Urine was also collected at the following time intervals after drug administration: 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–36, 36–48, 48–72, and 72–96 hr. Stools were collected for 5–6 days. Both urine and stool samples were frozen until assay.

Aliquots of urine and fecal samples were prepared and assayed for total radioactivity by liquid scintillation counting. Urine and fecal samples were subjected to TLC and reverse isotope analysis as described previously (1). No significant amounts of metabolites (<5% of radioactive dose) were detected. Consequently, all total radioactivity measurements were assumed to represent unmetabolized bethanidine.

RESULTS AND DISCUSSION

The extent of urinary and fecal excretion of radioactivity following a single 10-mg oral dose of ^{14}C-bethanidine hemisulfate in three normal subjects is shown in Table I. The overall recovery of the radioactive dose was essentially complete in Subject 1 but was slightly less than complete in the other two subjects. Urinary excretion accounted for 71–85% of the administered dose.

Figure 1 illustrates both blood levels and urinary excretion rates in Subject 2 following a single 10-mg oral dose. Peak blood levels were observed 2–4 hr after administration, suggesting relatively rapid absorption.

When blood level and urinary excretion rate plots were compared for all three subjects, it became apparent that the postabsorptive decline of blood concentration with time was noticeably slower than the corresponding decline in urinary excretion rate. For example, in Subject 2 (Fig. 1) over the 12–48-hr period the blood concentrations of bethanidine declined from 0.09 to 0.02 μg/ml, a four- to-fivefold reduction. Over the same time interval, the average excretion rates fell by about 100-fold, from approximately 0.3 to 0.004 mg/hr.

The disparity in the rate of decline between blood concentration and urinary excretion rate suggested a lack of constancy in the renal clearance of bethanidine. Therefore, the renal clearance was calculated at various time intervals according to:

$$Cl_R = \frac{[X_u]^{t_1 \to t_2}}{AUC^{t_1 \to t_2}} \qquad \text{(Eq. 1)}$$

where X_u is the amount of drug excreted in the urine during $t_1 \to t_2$, and $AUC^{t_1 \to t_2}$ is the area under the blood curve between t_1 and t_2. The area under the blood curve was determined by the trapezoidal rule.

[1] Vacutainers.

Table II—Change in Renal Clearance of Bethanidine as a Function of Time following a Single 10-mg Oral Dose of ^{14}C-Bethanidine Hemisulfate to Normal Subjects

Time Interval, hr	Clearance[a], ml/min		
	Subject 1	Subject 2	Subject 3
0–2	880	1364	932
2–4	616	740	851
4–6	453	496	603
6–8	364	432	452
8–12	341	366	323
12–24	211	177	167
24–48	245	57.5	91.3

[a] Calculated based on whole blood concentrations.

Results for all three subjects are shown in Table II. The initial clearance values were higher than the normal glomerular filtration rate and of the order of normal renal blood flow, suggesting extensive active tubular secretion of bethanidine. In all three subjects, the renal clearance of bethanidine decreased progressively with time. By 48 hr, clearance values were reduced to about or below that of the normal glomerular filtration rate.

In pharmacokinetics, it is customarily assumed that there is a direct relationship between the urinary excretion rate and the blood or plasma level of intact drug or metabolites. At any instant of time, the relationship is expressed mathematically as:

$$\frac{dX_u}{dt} = Cl_R C_b \qquad \text{(Eq. 2)}$$

where dX_u/dt is the urinary excretion rate, and C_b is the blood concentration. Provided that renal clearance remains constant, any change in blood level will result in a parallel change in the excretion rate of the drug. In the present case, the renal clearance of bethanidine decreased with time. Consequently, the postabsorptive decline of the urinary excretion rate reflects not only the fall in blood concentration but also the diminishing capacity of the renal clearance mechanism, leading to a faster rate of decline in the excretion rate as compared to the blood level decline. A half-life determination from the urinary excretion of bethanidine, therefore, will not be a meaningful index of the persistence of drug in the blood.

The exact cause for the change in renal clearance of bethanidine is not known and requires further investigation. However, the

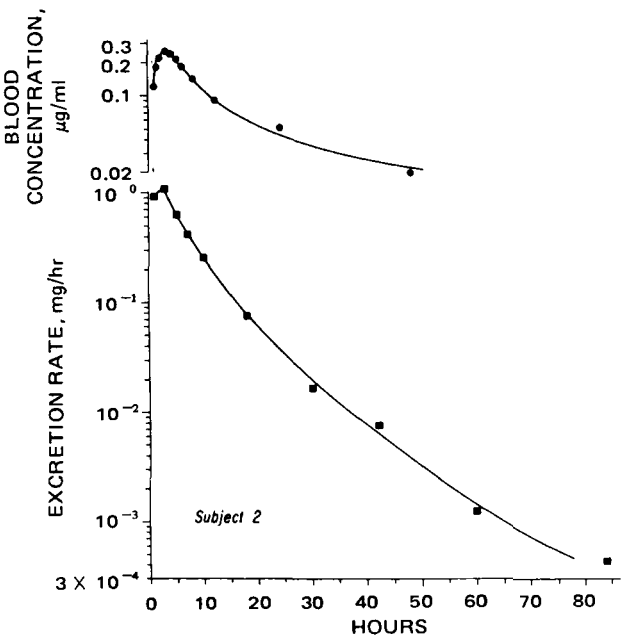

Figure 1—*Blood level (●) and urinary excretion rate (■) data from Subject 2 following administration of a single 10-mg oral dose of ^{14}C-bethanidine hemisulfate.*

renal clearance of bethanidine declined continually with time in the initial absorptive phase during which there were both a rise and a fall in blood concentration. Therefore, the change in renal clearance was not apparently related to the blood concentration of the drug. In addition, the reduction in renal clearance occurred in the absence of any noticeable hypotensive response.

In conclusion, this report clearly demonstrates the desirability of simultaneous measurements of drug levels in both blood and urine when studying the pharmacokinetics of drugs. If concomitant plasma or blood measurements are not available and if the constancy of renal clearance cannot be assured, information derived from urinary excretion data should be interpreted with caution.

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Oxygen Solubilization by Lung Surfactant

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Abstract □ To illustrate the concept of solubilization as a possible mode of gas transport in biological systems, dog lung surfactants in varying concentrations were tested for their ability to solubilize oxygen. The degree of gas solubilization was determined by GC, using a modified tonometer as an absorption chamber. The concentration of surfactant was found to be an essential factor for gas solubilization. Surfactant concentration above the CMC yielded anomalously high gas absorption. Solubilization of the gas is thought to occur by a partitioning effect into the interior of surfactant micelles.

Keyphrases □ Surfactants, lung—oxygen solubilization at various surfactant concentrations □ Solubilization, oxygen—by various concentrations of dog lung surfactant

Throughout this study, the theory of gas uptake was developed from a reasonably well-established concept, *i.e.*, that of solubilization. Since solubilization is frequently applied to pharmaceutical systems, it is natural for this pharmaceutical background to allow correlation of many biological occurrences through similar mechanisms.

Solubilization is defined as the "spontaneous dissolution of normally water-insoluble substances by an aqueous solution of surfactant." The surfactant molecule is amphiphilic, possessing both a nonpolar (hydrophobic) portion and a polar (hydrophilic) portion which exist in solution as individual molecules in very dilute concentrations. With an increase in surfactant concentration above the critical micelle concentration (CMC), all surface-active agents have the ability to form micelles.

A micelle can be defined as an aggregate of surface-active agents acting as a unit. The basic physical characteristics of surface-active agents, which are present during and after the formation of micelles, are that they lower surface tension in dispersion, produce a wetting effect, are strongly adsorbed to hydrophobic surfaces, and can solubilize normally insoluble substances.

In this study, varying concentrations of dog lung surfactant were analyzed for oxygen-absorbing or solubilizing characteristics to illustrate the concept of solubilization as a possible mode of gas transport in biological systems, as proposed by Ecanow *et al.* (1–3). A great deal of information is available regarding the nature and composition of mammalian alveoli based on histochemical and extraction studies (4–11). Analyses of lung extracts have indicated that lecithin (phosphatidyl choline) depicts the characteristic surface-active properties of lung surfactant and that dipalmityl lecithin is the essential lung surfactant (12, 13).

EXPERIMENTAL

Gas solubilization was analyzed by GC. A modified tonometer, constructed of glass and fitted with rubber septums for the introduction and extraction of gas samples, was used as an absorption chamber (14, 15).

Gas syringes were used for transfer of the gas from the preparation tonometer to that containing the gas uptake sample. These syringes were fitted with 22-gauge, 6.35-cm (2.5-in.) needles. Gastight syringes[1] (100 and 250 μl), fitted with Teflon hub needles [24 gauge, 3.8 cm (1.5 in.)], were used for removal of gas analysis samples from the second tonometer and for injection of the samples into the gas chromatograph.

A gas chromatograph[2] equipped with a thermal conductivity detector was utilized to follow gas uptake. Helium, used as the carrier gas, was maintained at a flow rate of 40 ml/min. Operating temperatures were as follows: injection, 172°; column, 135°; internal detector, 137°; duct, 204°; outlet, 252°; and external oven, 232°. Stainless steel columns[3] used for analysis of gas uptake were 1.8 m × 0.64 cm (6 ft × 0.25 in.), packed with molecular sieves (5A).

Gas analysis samples were removed at intervals of approximately 1 min for 20 min; this time length had been determined as sufficient for data evaluation. The studies were conducted at ambient temperature and atmospheric pressure.

The lung surfactant solution was first warmed in a water bath at

[1] Hamilton.
[2] WCLID 1670, Warner-Chilcott Laboratories, Richmond, Calif.
[3] HCl Scientific Inc., Rockford, Ill.