both assays (Table II). The accuracy of the methods was further validated by comparing results from the assay of captopril and its disulfide metabolites by the GC–EC method with results from the GC–MS assay (15) in plasma from dogs that had been given oral doses of captopril (2.5 mg/kg). The generally excellent agreement obtained for the two methods is shown in Table III.

The GC–EC assay described appears suitable for determining blood concentrations of unchanged captopril and plasma concentrations of captopril and its disulfide metabolites in samples from dogs and humans. In the range of blood concentrations (captopril) from 20 to 200 ng/mL and plasma concentrations (captopril and its disulfide metabolites) from 50 to 1000 ng/mL, the linearity, reproducibility, precision, and accuracy of the assays were generally excellent. The lower limits for quantitation of captopril and its disulfide metabolites by the GC–EC method are comparable to the existing GC–MS (15) and liquid chromatography–electrochemical detector methods (16, 17). The thin-layer radiochromatographic method (18) is more sensitive when high specific activity [^{14}C]captopril is used. The GC–EC method is 4- to 10-fold more sensitive than the other quantitative methods (12, 14) previously reported.

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Cascade Impactor Method for the Droplet Size Characterization of a Metered-Dose Nasal Spray

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Abstract □ A relatively rapid and simple method was developed to characterize the droplet size of a metered-dose nasal spray. The study primarily concerned the measurement of the relative proportion of small to large droplets. A small droplet could potentially reach bronchi or alveoli, depending on its size, and was therefore undesirable for the topical corticosteroid therapy of rhinal disease. The nasal spray was a solution of flunisolide, a topically active anti-inflammatory corticosteroid, administered by a manually operated, metered-dose pump spray system. The method utilized a cascade impactor fitted with a glass chamber; the cascade impactor collected and sized droplets into six fractions 0.5–16 μm in diameter, while the glass chamber collected droplets >16 μm in diameter as another fraction. Results showed that the majority of the spray droplets deposited in the glass chamber. Less than 0.5% by weight of the spray dose was delivered in droplets <8 μm aerodynamic diameter. These results are in good agreement with the droplet size distribution obtained by laser holography. The cascade impactor method showed that the number of undesirable small droplets produced by the flunisolide nasal spray unit was negligible. The method can be used with other aerosols where there is a similar concern for the inhalation of small particles.

Keyphrases □ Cascade impactor method—droplet-size characterization, metered-dose nasal spray □ Nasal spray—metered-dose, cascade impactor method for the droplet-size characterization □ Droplet-size characterization—cascade impactor method, metered-dose nasal spray

The particle or droplet size of an aerosol spray dosage form is important for both efficacy and toxicity. Inhalation aerosols intended for local activity in the lung must have the majority (by weight) of the particles in the size range of 2–6 μm in diameter to reach the terminal bronchi and alveoli (1). Particles <0.5 μm may fail to remain in this area (2), while particles >10 μm in diameter are mostly deposited in the upper respiratory tract (3).

In contrast, a nasal spray such as the metered-dose flunisolide nasal solution requires a majority of droplets having diameters >10 μm to localize delivery in the nose and to avoid possible undesired effects resulting from the material reaching the lung. It is therefore important, from an efficacy standpoint, to ensure that the nasal spray unit primarily produces droplets >10 μm in diameter. However, droplets <10 μm in diameter inevitably exist in small quantity in a spray. The ability of these small droplets to advance in the trachea–bronchi system depends on their

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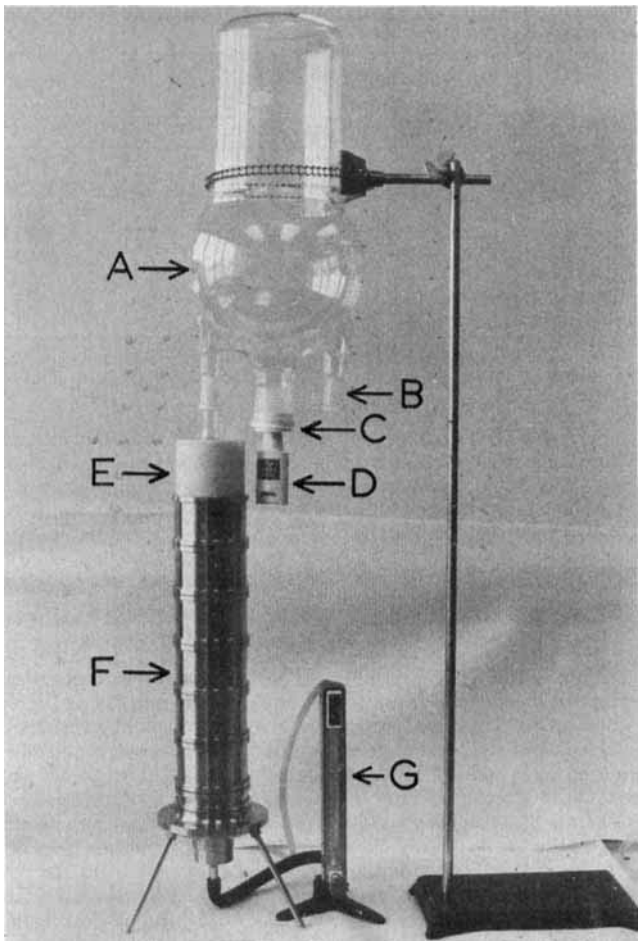

Figure 1—*Experimental setup for characterization of the droplet size of flunisolide nasal spray. Key: (A) glass chamber; (B) air inlet; (C) polytetrafluoroethylene stopper with a hole to fit the nasal spray unit; (D) flunisolide nasal spray unit; (E) polytetrafluoroethylene adaptor which has a conical cavity; (F) cascade impactor; (G) flow meter.*

sizes. Therefore, it is equally important, from a safety standpoint, to determine the amount and the size distribution of droplets <10 μm in diameter.

BACKGROUND

Numerous methods for sizing aerosol particles have been developed in the past (4–6), the most common of which are direct microscopy (7), light scattering (8), laser holography (9), and the impaction method (10–12). In the microscopic method, particles collected on a slide are counted and sized simultaneously. The method is simple, but tedious without the aid of an automatic image analyzer. Furthermore, in the case of liquid aerosols (as opposed to solid aerosols) the observed sizes need to be corrected for the spreading of the droplets. Light-scattering methods are based on the phenomenon that particles suspended in air are capable of scattering incident light. The method determines the particle size directly in the aerosol cloud. Laser holography employs a pulsed laser of short pulse duration to illuminate moving particles and consequently obtain the stop-action image of the droplets. The image is usually video-taped and replayed in slow motion for manual or automatic sizing. This method gives relatively accurate particle sizes and distributions, since the reconstructed hologram is essentially an undistorted image of

Table I—Total Recovery and Droplet Size Distribution of the Flunisolide Nasal Spray by Cascade Impactor

Spray Unit	Flunisolide Recovered, %			
	Stage 1	Stages 2–6	Glass Chamber	Total Recovery[a]
No. 1	1.20 (102.1)[b]	0.49 (104.7)	98.30 (101.4)	99.99
No. 2	1.75 (100.5)	0.55 (102.7)	97.70 (100.8)	99.50
No. 3	0.90 (102.1)	0.34 (106.0)	98.76 (101.4)	102.10
No. 4	0.33 (103.0)	0.19 (105.0)	99.48 (100.5)	99.50
No. 5	0.40 (102.0)	0.24 (105.0)	99.37 (99.0)	101.60
No. 6	0.38 (102.0)	0.20 (104.0)	99.42 (97.0)	102.10
Mean ± S.D.	0.83 ± 0.57	0.34 ± 0.15	98.84 ± 0.72	100.80 ± 1.28

[a] Percentage based on the weight difference of the spray unit before and after the experiment; converted to micrograms of flunisolide using density = 1.04 and concentration = 0.025% (w/v). [b] Number in parentheses is the recovery of the internal standard added into the washing solvent to quantitate the evaporation during washing.

particles in the aerosol cloud. However, the equipment is costly.

A cascade impactor, on the other hand, is relatively inexpensive. Various stages of the cascade impactor are capable of collecting and classifying particles ranging from 0.5–16 μm in diameter; a glass chamber can be employed to collect droplets >16 μm in diameter. The use of a cascade impactor fitted with a suitable glass chamber could therefore adequately provide the aforementioned critical droplet size information for the nasal spray product, namely the percentage of droplets >16 μm in diameter and the size distribution of the remaining small droplets. Although a 10-μm cutoff point is more desirable, the 16-μm cutoff point is dictated by the design of the cascade impactor. The six stages of the cascade impactor collect and size the droplets into 16-, 8-, 4-, 2-, 1-, and 0.5-μm diameter fractions. Nevertheless, since the primary concern about the particle size of a nasal spray is the relative proportion of small to large particles, the cascade impactor method is well suited for the purpose. This report describes the design of a glass chamber and its use with a cascade impactor for the droplet size characterization of a metered-dose nasal spray.

EXPERIMENTAL

Materials—The flunisolide nasal solution consisted of flunisolide[1] (0.25 mg/mL) in an aqueous vehicle containing propylene glycol, polyethylene glycol 3350, citric acid, sodium citrate, and benzalkonium chloride.

Assembly and Operation of the Nasal Spray—The nasal spray system consisted of four basic elements: a plastic bottle, a cap, a pump, and a shroud (plus its dust cap). Flunisolide nasal solution was packaged in the plastic bottle and capped, with the pump and shroud presented unassembled. Prior to the experiment, the original cap was removed from the bottle and discarded, and the pump/shroud system was assembled onto the bottle. During the experiment, the system was operated by rapid, firm finger and thumb pressure on the shroud and the base of the bottle, which forced a metered amount of drug solution up through the actuator to exit as a fine spray at the orifice. Metering was accomplished by the length of stroke of the pump piston, which forced the drug solution through the orifice. The meter chamber and the sealing action of the steel

[1] Flunisolide, (6α,11β,16α)-6-fluoro-11,21-dihydroxy-16,17-[(1-methylethylidene)bis(oxy)]-pregna-1,4-diene-3,20-dione; Institute of Organic Chemistry, Syntex Research, Palo Alto, Calif.

Table II—Recoveries of Flunisolide from Various Components of the Cascade Impactor and the Glass Chamber for Flunisolide Delivered by a Nebulizer

Stage	50% Cutoff Diameter[a], μm	Flunisolide Recovered[b], % Exp. 1	Exp. 2	Exp. 3
1	16.0	0.95	0.73	0.83
2	8.0	2.14	1.23	1.49
3	4.0	11.95	9.90	7.85
4	2.0	36.20	35.70	33.20
5	1.0	36.20	37.20	39.90
6	0.5	12.60	15.20	16.80
Glass chamber	—	0.00	0.00	0.00
Total	—	100.00	100.00	100.00

[a] Diameter of spherical particles of unit density for which 50% will impact on a given slide and 50% will pass around to a succeeding stage. [b] Recoveries of flunisolide from 20 sprays of the nebulizer.

ball on release of the actuator provided a fixed volume of drug solution for dosing. Each actuation of the pump system administered ~100 μL of the solution.

Assembly and Operation of the Cascade Impactor—The single-orifice metal cascade impactor[2] (10) was constructed of a succession of six jets, each followed by a glass impaction slide. It was based on the principle that particles in a moving air stream impacted on a slide placed in their path, if their momentum was sufficient to overcome the drag exerted by the air stream as it moved around the slide. As each jet was smaller than the preceding one, the velocity of the air stream, and therefore that of the dispersed particles, was increased as the aerosol advanced through the impactor. Consequently, small particles eventually acquired enough momentum to impact on a slide, and a complete particle sizing of the aerosol was achieved.

The air flow rate was maintained at 12.5 L/min in the system by applying a suitable vacuum at the impactor outlet and was monitored by a flow meter[3]. At this flow rate, the 50% cutoff diameters for each of the six jet stages were precalibrated[4] to be 16, 8, 4, 2, 1, and 0.5 μm. The 50% cutoff diameter is defined as the diameter of spherical particles of unit density for which 50% will impact on a given slide and 50% will pass around to a succeeding stage.

The impaction glass slide was attached to each of the six jets and the jets were assembled prior to the experiment. No entrapment was used for the impaction glass slide because flunisolide droplets remained liquid after spraying and, thereby, self-entrapping.

Glass Chamber—The design of the glass chamber for this study was similar to that of Sciarra *et al* (12). The glass chamber was made of a 5-L three-necked round-bottom flask with the bottom removed, and the remaining part of the flask joined to a 4-L beaker. The chamber was placed upside down on top of the cascade impactor with a ground glass joint and a polytetrafluoroethylene[5] adapter connecting a side-neck of the flask to the top stage of the impactor as depicted in Fig. 1. The other side-neck of the flask was left open for the air inlet, while the central neck of the flask was attached to the spray unit. Introduction of the spray mist into the chamber was accomplished by a vertical upward spraying at the central neck. A polytetrafluoroethylene[5] stopper with an opening to fit the head of the spray unit was employed to prevent the loss of the mist from the central neck.

Droplet Size Characterization Study—The glass chamber and cascade impactor were connected as shown in Fig. 1. After establishing an airflow of 12.5 L/min, the flunisolide nasal solution was sprayed into the glass chamber a total of 20 times at 10-s intervals. The glass chamber and its parts were removed and washed with the HPLC mobile phase. The impaction glass slide of stages 1–6 were detached and washed separately with the HPLC mobile phase. Washings from stages 2–6 were combined in certain experimental runs as indicated in the tabulated results. The amounts of flunisolide in the washings were then determined by HPLC.

Liquid Chromatographic Assay for Flunisolide—A high-performance liquid chromatograph[6] equipped with a variable-wavelength UV detector[7] and a 100-μL fixed-loop injector[8] was used for the quantitation of flunisolide. The wavelength chosen for detection was 240 nm. A commercially available microparticulate reverse-phase column[9] was operated at ambient temperature. A mixture of acetonitrile–water–acetic acid (35:64:1, v/v/v) was used as the mobile phase. Fluocinonide[10] was used as an internal standard. None of the excipients other than flunisolide and the internal standard appeared on the chromatogram.

Table III—Droplet Size Distribution of Flunisolide Nasal Spray by a Cascade Inpactor

Stage	50% Cutoff Diameter[a], μm	Flunisolide Recovered, % Exp. 1	Exp. 2[b]
1	16.0	1.30	1.14
2	8.0	0.38	0.20
3	4.0	0.29	0.13
4	2.0	0.12	0.04
5	1.0	0.07	0.01
6	0.5	0.00	0.00
Glass chamber	—	97.82	98.74

[a] Diameter of spherical particles of unit density for which 50% will impact on a given slide and 50% will pass around to a succeeding stage. [b] The same spray unit was used.

RESULTS AND DISCUSSION

Design of the Glass Chamber—The design of the glass chamber is critical for sizing aerosol sprays. Care must be taken to ensure that: (*a*) the aerosol cloud is carried through the cascade impactor by the imposed air flow and not by the imparted pumping force, in the case of a manually operated spray unit, or by the force of the propellant, in the case of a propellant-driven spray unit; (*b*) there is no dripping of the condensed liquid from the chamber wall into the cascade impactor and onto the first slide; (*c*) there is no retention of the small droplets, which are to be sized by the cascade impactor, by the glass chamber.

Sciarra *et al.* (12) studied several designs of glass chambers for sizing an aerosol hair spray. They claimed that a 1- or 2-L round-bottom flask with the bottom removed to fit the top stage of a cascade impactor, and the neck enlarged and extended at an angle 105° to the body was most suitable. However, in their design, the aerosol was sprayed into the chamber along with the imposed air flow at the neck which extended at an angle of 105° to the body of the cascade impactor. It is questionable that their design best fulfilled the aforementioned caution *a*. Furthermore, their design was not tested for fulfillment of caution *c*. Violation of caution *c* would result in a lower weight percentage of the impactor-detectable small droplets, which might lead to a misconception of product safety.

The glass chamber for the present study, to overcome the aforementioned shortcomings, was designed in such a manner that the mist produced by the spray unit would lose the momentum imparted by the pump operation and be carried into the cascade impactor by the imposed airflow. The large volume (9 L) of the chamber and the long distance (45 cm) between the tip of the spray unit and the top of the chamber helped to reduce the probability of droplets hitting the glass wall. The glass joint connecting the chamber and the cascade impactor aided in preventing the condensed liquid from dripping into the cascade impactor.

Total Recovery—The total recovery of the flunisolide sprayed into the system was checked by comparing the weights of the materials recovered from the various components of the system and the weight differences of the spray unit before and after the experiment, as shown in Table I. Of the six trials conducted, the total recoveries ranged from 99.50 to 102.10% with a mean value of 100.78%. The satisfactory total recovery indicated that there was no loss of the droplets through the open air inlet. Also listed in Table I are the recoveries (the numbers in parentheses) of the internal standard which was added to the washing solvent to determine the possible evaporation during washing. This procedure is necessary because only 1 mL of the HPLC mobile phase was used to wash an impaction slide plate of 3.8 cm in diameter. The increase in concentration

[2] Model CI-S-6; Sciotec Corp., Columbus, Ohio. It was originally designed by Mitchell and Pilcher of Battelle Memorial Institute, Columbus, Ohio.
[3] Precision flowmeter; Lab Crest Scientific, Warminster, Pa.
[4] Factory-precalibrated, at 12.5 L/min flow rate, using dibutyl phthalate droplets.
[5] Teflon, Du Pont.
[6] Altex model 110A; Altex, Berkeley, Calif.
[7] Schoeffel model 770; Schoeffel Instrument Corp., Westwood, N.J.
[8] Valco universal inlet; Altex.
[9] Spectra-Physics prepacked Spherisorb ODS 10 μm column; Spectra-Physics, Santa Clara, Calif.
[10] Fluocinonide, (6α,11β,16α(-21-(acetyloxy)-6,9-difluoro-11-hydroxy-16,17-[(1-methyl ethylidene)bis(oxy)]-pregna-1,4-diene-3,20-dione. Institute of Organic Chemistry, Syntex Research, Palo Alto, Calif.

Table IV—Effect of Emptying on the Droplet Size of Flunisolide Nasal Spray

Subject	Flunisolide Recovered, %							
	Stage 1		Stages 2–6		Glass Chamber		Total Recovery[a]	
	Day		Day		Day		Day	
	0	19[b]	0	19[b]	0	19[b]	0	19[b]
7	0.33	0.94	0.19	0.37	99.48	98.69	99.5	100.2
12	0.38	0.22	0.20	0.01	99.42	99.77	102.1	99.3
14	0.40	0.80	0.24	0.27	99.37	99.94	101.6	101.0

[a] Percentage based on weight difference of the bottle before and after the spray, converted to micrograms of flunisolide using density = 1.04 and concentration = 0.025% (w/v). [b] Nineteen days after the unit was used according to directions of four sprays, twice daily.

Table V—Droplet Size Distribution of Flunisolide Nasal Spray by Laser Holography

Diameter, μm	Flunisolide, %			
	Spray Unit No. 1		Spray Unit No. 2	
	2.54 cm[a]	7.62 cm[a]	2.54 cm[a]	7.62 cm[a]
0.5–1.0	0.000	0.001	0.000	0.000
1.0–5.0	0.000	0.051	0.001	0.000
5.0–10.0	0.003	0.435	0.008	0.003
10.0–20.0	0.053	11.917	0.173	0.079
20.0–30.0	0.191	11.379	0.514	0.105
30.0–40.0	0.294	19.870	1.071	0.075
40.0–50.0	0.417	10.055	1.665	0.187
50.0–60.0	0.729	14.686	1.216	0.097
60.0–70.0	0.628	6.060	1.840	0.241
70.0–80.0	0.563	0.000	3.083	0.741
80.0–90.0	0.468	0.000	2.618	0.000
90.0–100.0	0.980	0.000	3.133	0.251
100.0–110.0	0.662	25.547	0.705	0.000
110.0–120.0	1.159	0.000	1.853	0.000
120.0–130.0	0.744	0.000	1.190	0.000
130.0–140.0	0.938	0.000	2.997	0.000
140.0–150.0	1.162	0.000	3.714	0.000
150.0–160.0	2.129	0.000	4.536	0.000
160.0–170.0	3.425	0.000	2.736	0.000
170.0–180.0	1.021	0.000	0.000	0.000
180.0–190.0	1.207	0.000	0.000	0.000
190.0–200.0	0.000	0.000	0.000	0.000
>200.0	83.227	0.000	66.947	98.221
Total	100.0	100.0	100.0	100.0

[a] The sample was taken at either 2.54 or 7.62 cm downstream from the tip of the unit.

as a result of the evaporation was found to be only a few percent (Table I).

Condensation on the Chamber Wall—As discussed in the section on the design of the glass chamber, the condensation of droplets on the chamber wall could retain some small droplets which should have entered the cascade impactor. The relative proportion of small to large droplets determined by this device could potentially be biased if this phenomenon is not accounted for. The degree of condensation of the small droplets was therefore examined using a nebulizer[11] which generated primarily 1–2-μm size droplets[12]. The procedure was the same as that for the nasal spray. The nebulizer was equipped with a squeeze bulb. Each complete squeeze delivered approximately one one-hundredth of the amount delivered by one spray of the nasal unit. Table II lists the flunisolide recovered from various stages of the cascade impactor and the sampling chamber for three experiments with the nebulizer. The distribution patterns agreed well with one another. The majority of the droplets had diameters between 1 and 2 μm (Table II) which was consistent with the data from the manufacturer's technical report. As can be seen in Table II, the flunisolide retained by the glass chamber was essentially undetectable for a total of 20 squeezes. Evidently, the deposition of small droplets on the glass chamber is negligible.

Droplet Size Characteristics of the Nasal Spray—Results of the droplet size characterization are shown in Tables I and III. As can be seen in Table I, 98.84 ± 0.72% (mean ± *SD*) (by weight) of the spray was found in the glass chamber. The glass chamber was shown (Table II) not to retain droplets <16 μm in aerodynamic diameter, as discussed in the previous section. It is therefore believed that the nasal spray unit primarily produces droplets of >16-μm aerodynamic diameter.

The data in Table I were produced with six randomly selected spray units. The unit-to-unit variation was reasonably small, as shown by the standard deviation. The amounts of flunisolide found in stage 1 (16 μm) and in stages 2–6 combined (8–0.5 μm) were 0.83 and 0.34%, respectively (Table I). Details of the size distribution of this small droplet portion of the spray are shown in Table III. Only two experiments on one spray unit were performed because their purpose was to illustrate the size distribution obtained by the cascade impactor, not to show the within-unit variability. A clear trend can be seen, *i.e.*, the smaller the droplet size, the smaller their weight percentage.

Table IV shows the reproducibility among operators and the effect of emptying of the spray unit on the droplet size. The study was a part of a use test (13) in which 17 units were sprayed by 17 operators according to label instructions until the units ceased to deliver. Only three units were analyzed for the droplet size, among other parameters studied (13), because the assay reduced the availability of the nasal solution for other testings. It can be seen that the relative proportion of small to large droplets is reasonably consistent among the operators and between a full unit and an almost empty unit for each operator.

Table V lists results of the droplet size distribution[13] obtained independently by laser holography[14]. Examining Tables I, III, IV, and V, one can see that the relative proportion of small to large droplets obtained by the cascade impactor agrees well with that obtained by laser holography. The laser holographic results (Table V) reveal that the weight percentage of the small droplets between 0.5 and 10.0 μm in diameter is 0.13 ± 0.24% (mean ± *SD*). The weight percentage of the small droplets collected on stages 2–6 of the cascade impactor (i.e., droplets 0.5–8.0 μm in diameter) is 0.34 ± 0.15% (Table I). Evidently, there is no statistical difference between the two results.

CONCLUSION

Since concern for the droplet size of a nasal spray is primarily to ensure that the weight percentage of droplets <10 μm in diameter (and thereby potentially deliverable to the lower respiratory tract) produced by the spray unit is negligble, the cascade impactor method was demonstrated to be quite satisfactory for this purpose. The cascade impactor method developed here is a simple, economical, and reliable method for the droplet size characterization of a metered-dose flunisolide nasal spray. The method can be used for other aerosol products where there is a similar concern for the inhalation of small particles.

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Pharmacokinetics of Diazepam and Nordiazepam in the Cat

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Abstract □ The cat has been used extensively as an experimental model for studying the pharmacology of compounds that exhibit CNS activity including diazepam and nordiazepam. However, since little is known about the distribution and elimination of diazepam in this species, the pharmacokinetics of diazepam and nordiazepam were studied in the cat following intravenous doses of 5, 10, and 20 mg/kg of diazepam and 5 and 10 mg/kg of nordiazepam. The disappearance of diazepam and nordiazepam from blood was fitted with classical equations. Theoretical and trapezoidal areas under the curve (AUC_{th} and AUC_{tr}) were calculated. The volumes of distribution (Vd_{β}) were calculated as model-independent parameters for diazepam and nordiazepam. Intrinsic hepatic clearance, extraction ratio, and tissue binding parameters were also calculated for diazepam. From the observed data, it is apparent that the blood concentrations and the resulting areas under the curves are proportional to the dose of diazepam administered and that the pharmacokinetics of diazepam were linear over the dose range studied. In addition, nordiazepam formed after diazepam administration appeared to be proportional to the dose of diazepam administered. The terminal elimination rate constant of nordiazepam remained constant over the dose range studied. It appears that both diazepam and nordiazepam are highly bound to tissue. The total body clearance of diazepam (4.72 ± 2.45 mL/min/kg) is approximately six times that of nordiazepam (0.85 ± 0.25 mL/min/kg). Approximately 50% of an administered dose of diazepam was biotransformed to nordiazepam in the cat.

Keyphrases □ Diazepam—pharmacokinetics in the cat, nordiazepam □ Nordiazepam—pharmacokinetics in the cat, diazepam □ Pharmacokinetics—diazepam and nordiazepam in the cat after intravenous doses

The benzodiazepines are centrally active compounds that reduce anxiety, produce sedation and sleep, have anticonvulsant effects, and may cause muscle relaxation. In addition, the benzodiazepine diazepam is also used in the treatment of alcohol abusers during potentially life-threatening withdrawal episodes (1). Diazepam has a long effective duration of action, probably due to slow elimination of parent compound and biotransformation to active metabolic products including nordiazepam (2). Many of the pharmacological properties of the benzodiazepines seen in humans are present in the cat (3–5). Diazepam has been shown to be effective in the cat in the control of seizures in experimental epilepsy (6) and with the sudden abstinence of alcohol after chronic use (5). Although the cat has been used extensively to study the effects of diazepam, little is known about the pharmacokinetics of diazepam and nordiazepam in this species. Morselli *et al.* (7) described the distribution of diazepam and its major metabolites in plasma and in several areas of the brain of the cat. A direct correlation was established between blood flow to various tissues of the brain and the diazepam found in these tissues. However, the accumulation of nordiazepam, the major blood metabolite, in the brain appeared to be inversely related to blood flow. Unfortunately, this data did not lend itself to an overall pharmacokinetic profile of diazepam or nordiazepam in this species.

The present study in the domestic short-hair cat was performed to (*a*) establish the pharmacokinetics of diazepam and nordiazepam following 5-, 10-, and 20-mg/kg iv doses of diazepam and (*b*) to compare the pharmacokinetic profile of nordiazepam after nordiazepam administration to its profile observed after the administration of diazepam in this species. These data will establish the fundamental pharmacokinetic profile of diazepam and nordiazepam in the cat.

EXPERIMENTAL

Animal Model—Domestic short-haired female cats (2.5–3.0 kg) were used for all experiments. Hematocrit values were determined before surgery and drug administration and were found to be within the normal range (8). Cats were anesthetized with 0.33–0.44 mL of a solution containing 100 mg/mL of ketamine and 7.5 mg/mL of promazine[1]. An incision was made in either the right or left leg exposing a branch of the femoral vein. A butterfly infusion set[2] with the needle removed, leaving the tubing and Luer fitting intact, was used as a cannula. The catheter was inserted into the vein, exteriorized, and the incision was closed with 000 surgical silk[3]. The catheter was cleared daily and kept patent with a sodium heparin flush. Diazepam or nordiazepam (10 mg/mL) was dissolved in dimethylacetamide[4] immediately prior to administration.

Study Design—Unanesthetized fasted cats, with a femoral vein catheter previously implanted to facilitate blood sampling, were administered 5-, 10-, and 20-mg/kg doses of diazepam or 10 mg/kg of nordiazepam intravenously as a short infusion (20 s) *via* a peripheral vein. Two weeks were allowed for recuperation between doses.

Sampling and Analysis—Blood specimens (0.5 mL) were obtained from the femoral vein catheter. The first specimen was obtained 2.5 min following intravenous administration and subsequent specimens were obtained for as long as 96 h. The blood specimens were collected in tubes containing dried heparin[5] and kept at −20°C until analyzed. The samples were analyzed simultaneously for diazepam and nordiazepam using the electron capture GC method of Weinfeld *et al.* (9) with the following modifications. Whole blood (0.1 mL) was extracted into 1 mL of benzene after adjusting the pH to 9.0 with a 1 M H_3BO_3–KCl–Na_2CO_3 buffer. The percent standard deviation for the intraassay variability averaged 3.6% for diazepam and 3.4% for nordiazepam over the range of 50–2000 ng/mL

[1] Ketaset Plus; Bristol Labs, Syracuse, N.Y.
[2] Model No. 44492; Abbott Laboratories, Chicago, Ill.
[3] Ethicon, New Brunswick, N.J.
[4] Model No. 4972, Eastman Kodak, Rochester, N.Y.
[5] Model No. 3206, Becton, Dickenson, Rutherford, N.J.

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