Novel Device for Quantitatively Collecting Small Volumes of Urine from Laboratory Rats

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Abstract □ A reusable urinary collection device suitable for quantitative collection of uncontaminated urinary samples from rats without using a metabolism cage has been designed. The device can be attached to the pelvic skin within 5 min with minimum handling and discomfort to the unanesthetized rat using an adhesive. The suitability of this device was investigated by collecting and analyzing urine over a 48-h period following the intraperitoneal injection of [^{14}C]inulin. A two-way crossover study was done with samples from one of the two experiments being collected while the animal was housed in a commercially available metabolism cage equipped to separate urine and feces. The percent of the dose excreted, food and water consumption, and the urinary output were not statistically different for rats housed in the metabolism cages or with the collection device attached. Histopathological examination of the rats revealed no pathology after a 24-h period except minor skin inflammation which occurred both in controls and animals to which the device was attached. These studies demonstrate the comparability of the new urine collection device to a commercially available metabolism cage in the quantitative collection of small (0–7 mL) urine samples with less contamination of the samples from the environment.

Keyphrases □ Urine collection—quantitative, rats, reusable device, comparison with metabolism cages

The preclinical evaluation of a drug requires that the acute and chronic toxicity, metabolic pattern, and bioavailability be evaluated in animal species. Two of the primary species utilized in preclinical screening are rats and mice. These species have proven useful because of their low cost, minimal holding space, relatively short life span, and defined genetic lineage. Several recent reports have presented data related to the bioavailability of drugs in rats and mice comparing gavage with administration in the diet (1, 2).

Despite the reported results, a major concern in these and other studies that require analysis of urinary and fecal data is the possible contact and resultant contamination of these excreta by each other or by the environment. A new commercially available cage[1] has made progress in this area, but it still cannot completely eliminate contamination of urine by either animal fur-containing food particles or contact between urine and feces, especially if the animal has diarrhea.

There are currently several modified metabolism units which have been presented in the literature (3, 4). The general approach to the construction of these units has been to place the animal in a cage with a stainless steel floor and then, utilizing either another screen of smaller mesh or a funnel with various designs, to effect the separation and collection of urine and feces. However, flat-bottom and polycarbonate metabolism cages have proven unsatisfactory because of food contamination of urine and contamination of samples for GC analysis by leached plastics following repeated washes of the polycarbonate cage with benzene (3). The modified unit, which is quite similar to those commercially available, still utilizes the same basic principles and in addition has metal in direct contact with the urine prior to collection (3).

A urine-collecting device (patent pending) has been developed in our laboratory that (*a*) prevents contact and assures 100% separation of urine and feces, (*b*) gives a quantitative collection of small (0–7 mL) urine samples comparable with those obtained with a commercially available metabolism cage, (*c*) is devoid of metal in its design, and (*d*) is resistant to organic solvents. The application of this device to the quantitative collection of urine from rats is described in this report.

EXPERIMENTAL

Materials—[Carboxy-^{14}C]inulin[2] had a specific activity of 2.02 μCi/mg. Phosphate-buffered saline contained 92 mM sodium chloride, 23 mM dibasic sodium phosphate, and 11 mM monobasic sodium phosphate. A commercially available metabolism cage[3] was used for comparison to the urine collection device[4].

Urine-Collecting Device Design—A perspective side photograph of a rat with the collection device attached to the pelvic region is presented in Fig. 1. The collection device apparatus is shown schematically in Fig. 2, with portions represented in phantom.

The mounting plate has a projecting funnel which receives the penis of the rat (or urethral region for female animals), with the plate being adhered to the pelvic skin by a quick-drying methylcyanoacrylate adhesive[5]. The mounting plate is made from either acetal resin[6] or acetal resin coated with polymethyl methacrylate[7] with the container being constructed of either material. The urine collector and funnel are separate units joined by screw threads disposed around a cylindrical projection or annulus at the mouth of the funnel. Screw threads couple with screw threads in an opening through the top wall of the container, with the container being essentially a chamber that is vented in the top wall.

To empty the container, a drain is positioned in the end wall. The drain is threaded and plugged with a nylon screw. Urine can be sampled with a syringe or by merely removing the container from the funnel. A metal set screw, which is not in contact with the collecting chamber, is utilized as a detent to keep the longitudinal axes of the container and mounting plate aligned. The set screw is advanced to set in an indentation formed in the bottom surface of the funnel mounting plate. When the screw is advanced to set tightly in the indentation, the container rotates with difficulty about the axis of the funnel. Consequently, the container remains in place and thus eliminates urine loss and the amount of gnawing which the rat can do to the mounting plate.

Attachment of the Urine Collecting Device—The hair was removed from the region around the penis using small animal clippers[8] fitted with a size 40 blade, without the use of soap since surfactants interfere with subsequent bonding of the funnel to the skin. In addition, the abdomen of female animals was shaven using a disposal razor (again without soap). The skin was then cleaned with isopropyl alcohol, and the methyl cyanoacrylate glue was applied as a thin film to the mounting plate to effectively bond it to the skin. Attachment was accomplished by having the rat restrained by hand while the mounting plate was positioned. (During the attachment the animal can be restrained by hand with careful alignment of the penis or urethral opening (female rats) with the funnel opening; contact between the respective urethral opening and the adhesive should be avoided.) Bonding was rapid (1–5 min). Curing occurs best at a relative humidity of 58–68%. Once the plate was firmly attached, the container was screwed onto the funnel.

Although still in place at 24 h, the edges of the mounting plate may not be as securely bonded to the skin due to normal turnover of epidermal cells and the animals tendency to gnaw at this surface, but the device can be rebonded

[1] Nalgene Co., Rochester, N.Y.

[2] Amersham Corp., Arlington Heights, Ill.
[3] Econo; Maryland Plastics Co., Federalsburg, Md.
[4] Jackson and Weems, Assoc., P.O. Box 4407, Alexandria, VA 22303.
[5] Permabond.
[6] Delrin; E. I. DuPont de Nemours & Co.
[7] Plexiglas;
[8] Oster; Scientific Products, Columbia, Md.

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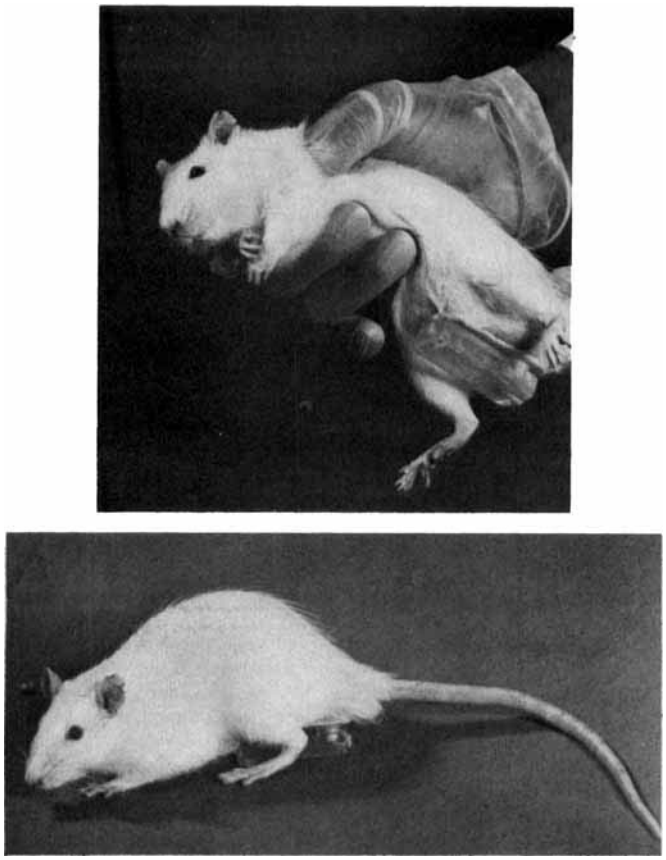

Figure 1—*Photographs (lateral and ventral views) of a rat which has had the urine-collection device attached to the pelvic skin for a 48-h period.*

readily by application of the adhesive to the mounting plate in any exposed area. Although not done in these studies, the animal can be lightly anesthetized with ether and the funnel sutured around the edges after attachment with the glue[9]. Either acetone or nail polish remover can be applied to the skin to remove the device at the termination of the study if only glue is used for attachment. The collection device can also be gently pulled from the abdomen with some minor discomfort to the animal.

Experimental Design—The comparison of the urinary excretion of [^{14}C]inulin administered in phosphate-buffered saline (pH 7.4) to 12 male Dub (SDD) rats[10] was investigated using the metabolism cage and the urine collection device. The animals were divided into three groups (50–200 g; 200–400 g; >400 g) with four animals in each group. A comparative excretion study was also done in eight female rats (four/group) with weights ranging between 50–200 g and 200–400 g. One microcurie of [^{14}C]inulin was administered intraperitoneally (0.5 mL) using a 1-mL plastic syringe fitted with a 27-gauge needle. Animals were housed in a metabolism cage (after being prepared similar to device-carrying rats) or in a regular stainless steel cage while the urinary collection device was attached. Food pellets[11] and distilled water were provided *ad libitum*. Urine was sampled by restraining the rat by hand, removing the drain screw, and collecting the samples using a syringe.

All samples were analyzed by liquid scintillation counting. The collection period for all studies was 48 h with animals randomly receiving either treatment. Following a 7-d washout period, the rats received the other treatment, *i.e.*, those animals that had been housed in metabolism cages had the urine collection device attached while animals that had urine collected with the urine device were housed in metabolism cages for urine collection. Food and water consumption, urinary output, and percent inulin recovered in urine were determined for each collection method at 48 h.

Histopathology—In a separate study using nine male rats (150–550 g) and nine female rats (100–400 g), the attachment area was examined for evidence of pathology. The rats were equally divided into three groups, with the rats in control groups I and II having their pelvic region shaven or shaven with glue (identical lot) applied, respectively. Animals in treatment group III were

[9] Unpublished results.
[10] Flow Laboratories, Dublin, Va.
[11] Lab Chow; Ralston Purina, Richmond, Ind.

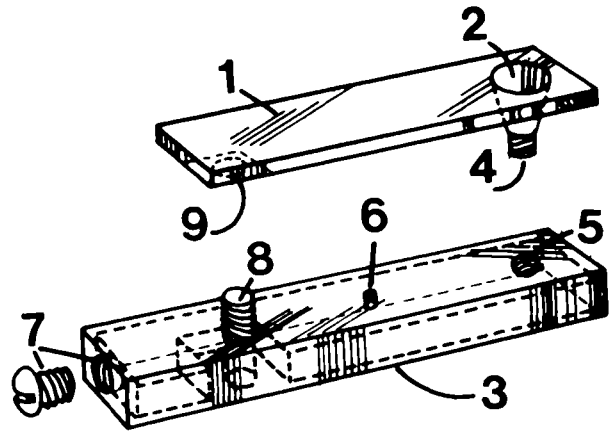

Figure 2—*Representation of the urine-collection device showing the major components. Key: (1) mounting plate; (2) removable funnel; (3) urine container; (4, 5) screwthreads; (6) vent; (7) drain; (8) set screw; (9) indentation.*

shaven with the urine collection device attached for a 24-h period. After 24 h the animals in groups I and II were anesthetized with ether and necropsied. Sections were taken of the skin of the lower abdomen adjacent to the penis, the urinary bladder, and one testicle for male animals. For female animals, the skin adjacent to the urethral opening, vagina, uterus, ovaries, and urinary bladder were evaluated.

Rats from treatment group III were similarly examined following removal of the urine in the urine collection device. Sections of the aforementioned tissues were fixed in 10% formaldehyde, embedded, sectioned, and stained with hematoxylin and eosin for histopathological examination.

RESULTS

Urinary Excretion of Inulin—Data for the urinary collection of [^{14}C]inulin over the 48-h period is presented in Table I. Food and water consumption, urinary volume, and percent inulin recovered were not significantly different for the two methods of urine collection. Urine samples collected with the device were free from contamination, while those collected while the animal was housed in the cage contained elements from the environment such as food, hair, and feces.

Histopathology—*Male Rats*—Intradermal abscesses were observed in the skin from six rats. Four of these animals were controls: one was from control group II, shaven plus adhesive, while the other three were from control group I, in which the animals were only shaven. The abscesses were usually superficial, although a rat from control group II exhibited more extensive abscesses extending into the superficial dermis with a mild infiltration of polymorphonuclear leucocytes into the superficial fat. Such infiltration was also seen in one of the rats carrying the collection device and in one control animal from group II. Inflammation was always localized, never extending beyond the boundaries of the shaved area of skin.

A slight infiltrate of eosinophilic leucocytes was seen in the subcutaneous tissue of the penis for two rats in control group II and one animal in treatment group III. The urinary bladders all appeared normal, except in two of the animals in control group I where there was a small amount of blood in each bladder. The testicles in all animals were unremarkable. Two of the animals that had the collection device attached showed dilated preputial glands, and in one of these animals a plug was found that appeared to be desquamated squamous cells from a preputial gland. These results are summarized in Table II.

Female Rats—No pathological findings were noted in the ovaries or the urinary bladder. The chief findings were those in the skin. Like the male rats, there were epidermal abscesses noted in one rat which was only shaven and in all of those shaven with glue applied and the cup-bearing rats. Inflammation increased, extending into the dermis, for both rats to which glue had been applied and the cup-bearing rats. Also two rats from each of these groups showed abscesses in the dermis. Inflammation continued into the fat for these two groups. Sections of the vaginas showed a few polymorphonuclear leucocytes in the mucosa of all rats. Two rats, both shaven with glue applied, appeared to have long-standing vaginal infections with pus. In the second and third groups (shaven with glue and cup-bearing), there were a few more polymorphonuclear leucocytes in the wall of the vagina as compared with those only shaven.

Eosinophils were found in the wall of all vaginas, with two rats having more

Table I—Food and Water Consumption, Urine Output, and Percent [^{14}C]Inulin Recovered Following Collection of Urine from Rats using the Urine-Collection Device (UCD) or Metabolism Cages [a]

	50–200 g				200–400 g				>400 g [d]	
	Male [b]		Female [c]		Male [c]		Female [c]		Male [c]	
	Cage	UCD	Cage	UCD	Cage	UCD	Cage	UCD	Cage	UCD
Food consumption (g)	40.8 ± 1.1	40.9 ± 1.3	38.7 ± 5.1	36.7 ± 5.3	26.0 ± 8.7	22.7 ± 7.2	35.1 ± 3.2	31.9 ± 8.0	30.6 ± 9.4	27.6 ± 5.1
Water consumption (mL) [e]	79.0 ± 12.7	73.5 ± 9.2	72.7 ± 7.8	70.0 ± 1.09	35.7 ± 9.8	46.7 ± 3.1	71.0 ± 9.2	67.2 ± 12.7	79.0 ± 2.7	90.2 ± 18.4
Urine output (mL)	27.5 ± 3.5	16.0 ± 5.6	25.5 ± 3.8	21.2 ± 2.1	20.5 ± 7.8	15.3 ± 3.2	23.4 ± 1.8	20.0 ± 2.0	29.3 ± 17.5	21.5 ± 7.9
[^{14}C]Inulin recovered, % of dose	89.1 ± 5.9	81.0 ± 1.4	89.3 ± 4.1	91.7 ± 2.14	89.6 ± 9.1	91.2 ± 3.9	87.3 ± 3.7	90.3 ± 2.8	78.8 ± 5.0	85.9 ± 3.4

[a] 48-h collection; all values are mean ± *SD*, $n = 4$. [b] Not statistically different, paired *t* test ($p > 0.20$) for all parameters except urine output and inulin recovery, which were not different at the ($p > 0.10$) level. [c] Not statistically different, paired *t* test ($p > 0.20$). [d] Dub (SDD) female rats do not weigh >400 g. [e] Some of the *SD* values are unusually large because of water loss from the inverted bottle.

eosinophils than the others. One of these was a rat which had been shaven with glue applied and another was a cup-bearing rat. Otherwise, the eosinophilic distribution seemed about equal. Examination of uteri showed eosinophils in the submucosa and muscularis of the uteri of all rats with very little difference between the three groups. A summary of these pathological findings is presented in Table II.

DISCUSSION

The accurate collection and evaluation of data from experimental animals utilized in toxicity feeding, pharmacokinetic, and metabolic studies is essential in the preclinical screening of biologically active drug products. Current methods of collecting urinary samples are somewhat compromised because of the tendency of these samples to become contaminated by the environment. The method presented in this report provides a means of accomplishing this objective with overall rat response similar to that of those housed in a standard metabolism cage. Based on the results of the study, it would appear that the device is well tolerated by laboratory rodents and did not appear to alter their food and water consumption nor their excretion of inulin. The urinary collection device is suitable for male and female rats as evidenced by the results from the current study.

Table II—Number of Rats in which Pathological Abnormalities were Found *versus* the number of Rats Examined

Pathological Observation	Treatment: Shaven Only	Treatment: Shaven Plus Adhesive	Urine Collecting Device Attached
Intraepidermal abscess(es), cup site	4/6	4/6	5/6
Abscess(es) in superficial dermis, cup site	2/6	2/6	5/6
Acute inflammation of dermis, diffuse	4/6	4/6	4/6
Acute inflammation of penile skin	1/3	1/3	2/3
Acute vaginitis	3/3	3/3	3/3
Eosinophilia in skin, cup site	0/6	2/6	1/6

Preparing the abdominal region for device attachment appears to be a limitation, since hair removal of this area with clippers resulted in intradermal abscesses of the abdominal and, in some cases, penile skin. The presence of abcesses in both control and treated groups (male and female) indicates that the preparation of the area was the primary cause, not device attachment. The presence of the mild infiltrate of eosinophilic leucocytes suggested a slight hypersensitivity to the adhesive, which is consistent with the manufacturer's report of a mild irritant effect to the skin from the adhesive. Dilation of the preputial glands (male and female rats) was the only histological observation that was exclusive to device-carrying animals. Therefore, one would conclude that the acute pathology associated with the device is indeed minor.

Because of the limited capacity of this self-contained unit, it will not allow quantitative collection over 24 h without intermittent collections. Nevertheless, judicious utilization of the urine collection device in conjunction with other established methods of rodent urine collection could prove a valuable tool. It would be especially applicable in validating urinary excretion data obtained from rodents by conventional methods during toxicological feeding, pharmacokinetic, or metabolic studies without removing the animals from their normal environment.

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