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A NEW TECHNIQUE FOR COLLECTING FECAL-URATE SAMPLES FROM NESTLING AMERICAN KESTRELS

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Abstract.—A new technique for collecting fecal-urate samples from nestling American Kestrels (*Falco sparverius*) was developed and tested while conducting field research on the detection and effects of agrochemical exposure. The technique was later modified and successfully used to collect samples from hand-reared nestling kestrels in a controlled exposure experiment. Sample collection in both studies incorporated the use of a "diaper" fastened to nestlings in different manners. The original design consisted of making a diaper out of a corner section of a plastic sandwich bag. The modified design secured a small balloon over a much smaller surface area by means of elastic string suspenders. Sampling was successful in 74% of all field attempts (n = 111) and in 97% of collections in the controlled dosing study (n = 32). This technique simplifies collection of fecal-urate samples from nestling kestrels for use in a variety of biomonitoring studies. The technique may also be adaptable to other species of birds.

DESCRIPCIÓN DE UNA NUEVA TÉCNICA PARA RECOLECTAR MUESTRAS FECALES DE POLLOS DE *FALCO SPARVERIUS*

Sinopsis.—Durante un estudio de campo sobre exposición potencial de cernícalos americanos (*Falco sparverius*) a agroquímicos, se desarrolló una nueva técnica para recolectar muestras fecales de polluelos de esta especie; posteriormente, esta técnica se modificó y utilizó exitosamente para recolectar muestras de cernícalos criados en cautiverio en un estudio con dosificaciones controladas. La recolección de las muestras en ambos estudios incorporó la utilización de un "pañal" sujetado a los polluelos de distintas formas. El diseño original consistía en fabricar un "pañal" a partir de la esquina de una bolsa de plástico a la que se hacían un par de orificios para las extremidades del ave. El modelo modificado sujetaba un pequeño globo sobre una superficie mucho más pequeña mediante el uso de unos tirantes de hilo elástico. La recolección fue exitosa en un 74% de los intentos en el campo (n =

111) y en un 97% de las recolectas en el estudio de dosificación controlada (n = 32). El uso de esta técnica presenta un gran potencial para la facilitación de la recolección de muestras fecales de polluelos de cernícalo americano para su uso en una gran variedad de estudios de biomonitoreo.

The collection of fecal-urate samples from wild birds is a useful tool for many aspects of biomonitoring. Researchers have used fecal material to estimate numbers of blackbirds and starlings in roosts (Stewart 1973), sex monomorphic raptor species by ascertaining steroid levels excreted by the kidney (Bercovitz et al. 1978, Czekala and Lasley 1977, Fry 1983), and identify agrochemical exposure to both raptor (Buck 1992, Hooper et al. 1989) and passerine species (Hooper et al. 1990). In this paper, we discuss a new technique for collecting fecal-urate samples from nestling birds.

METHODS

Fecal-urate collection from wild nestlings.—As part of a larger field study conducted in 1991 near the Edith Angel Research Center (The Institute of Wildlife and Environmental Toxicology (TIWET), Clemson University) in Chariton, Iowa (40°57′N, 93°18′W), we collected fecal-urate samples from American Kestrel (*Falco sparverius*) nestlings to quantify exposure to an organophosphate (OP) agrochemical used extensively in the prevention of corn rootworm (Hoff 1992). All trapping and banding of kestrels was conducted under permit. Many of the techniques used successfully in past studies to collect fecal-urate samples were not suitable for our research. Specifically, our experimental design required non-invasive sampling of known individuals. Furthermore, samples were difficult to obtain without the stimulus from egestion of a newly acquired food item. We therefore developed a new technique to collect samples from individual nestlings within the nestbox during primary feeding times in both the mornings and evenings. Essentially, the technique consisted of securing a "diaper" on the nestling, leaving it in place during the desired collection period, and later collecting the sample.

We collected fecal-urate samples from nestlings in the field by making a diaper out of a corner section of a plastic sandwich bag (Fig. 1A). Holes for the legs were cut in the bag, which was then drawn up the bird's legs and over its posterior. We secured the bag to the nestling by rolling the loose ends of the section between our thumb and index finger to make it snug against the rump and to create strands for tieing a knot. The configuration was then checked to assure that a reservoir protruded from the cloaca to provide space for fecal-urate accumulation. We then taped the loose strands and knot to the dorsal surface of the rump on the diaper (Fig. 1B).

Sample collections were scheduled for nestlings aged 2, 6, 10, 15, 20 and 24 d. We put diapers on the nestlings just prior to sunrise for collection during feeding events in the morning and 2 h before sunset for collection during evening feeding events. They remained attached to the nestlings for no longer than 2 h. Upon our return to the nestbox, we

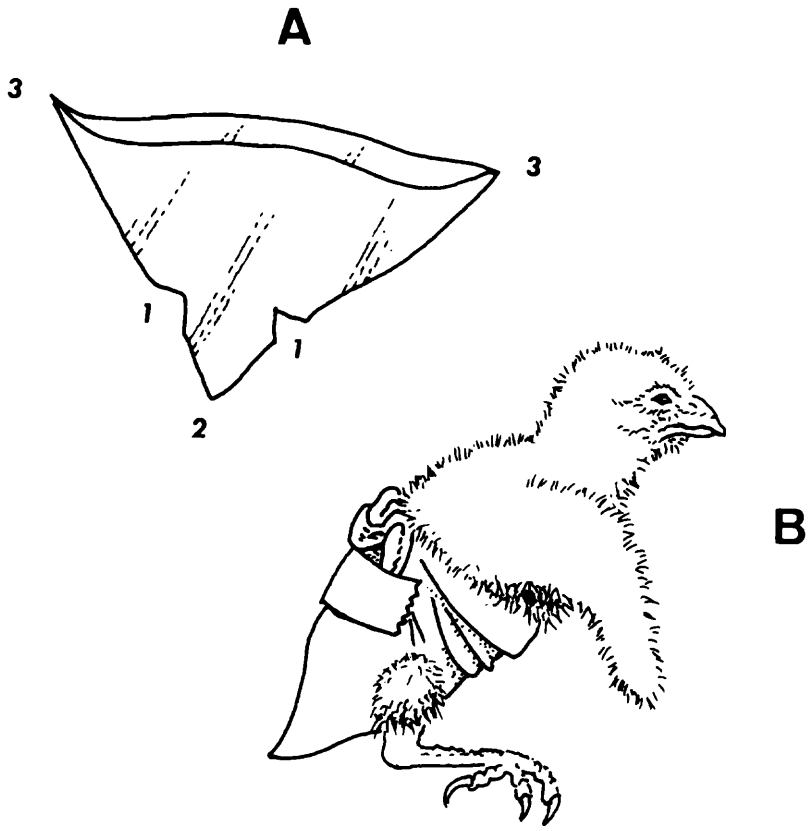

FIGURE 1. Plastic baggie diaper for collection of fecal-urate samples in American Kestrels. A) Diaper showing (1) leg holes, (2) fecal-urate reservoir, and (3) ends to be rolled and tied. B) View of an American Kestrel nestling wearing the plastic baggie diaper.

removed the diapers by using scissors to cut from the leg holes ventrally to the top edge. Baggies containing fecal-urate material were carefully folded and sealed in a plastic collection bag.

Fecal-urate collection from hand-reared nestlings.—After completing a successful field season employing this technique, we conducted a controlled exposure experiment during the summer of 1993 using hand-reared birds obtained from a captive kestrel colony at the Avian Science and Conservation Centre, Macdonald Campus of McGill University in Ste. Anne de Bellevue, Quebec, to evaluate more fully the excretory toxicokinetic profile of OP insecticides in nestling kestrels and to correlate the data to those obtained from wild nestlings. Part of this study required collecting the entire volume of fecal-urates excreted over increasing intervals (up to 24 h) following oral administration of the pesticide or vehicle control.

The same individual was often sampled over several consecutive collection periods.

The technique initially used to collect fecal-urate samples was that described above for use on wild kestrel nestlings raised in nestboxes. It became apparent to us, however, that under the separate set of sample collection requirements in the toxicokinetic study, i.e., longer collection intervals, pesticide-dosed individuals, and increased frequency of repeated sampling of the same individual, the diaper design devised for use in field collection of samples was not entirely suitable. Consequently, we modified the diaper design to improve sampling efficiency.

The modified design for use on hand-reared nestlings consisted of securing a small balloon (approximately 9.5 × 4.5 mm) over a very limited area surrounding the cloaca by means of elastic string suspenders. The suspenders were secured by flexible cross pieces positioned in both the front and the back of the bird and resembled German lederhosen. To assemble the diaper, we first cut the open end of the balloon into a four-pointed star shape at the point where it began to widen, and made a hole using a sharp scissors in each of the points for the string attachments (Fig. 2). Two pieces of 0.5 mm elastic string, cut to approximately 12 mm in length, were knotted securely to each of the two holes in the back of the balloon. The back and front cross pieces were then threaded between the two strings, leaving a space for the nestling's head. We constructed the cross pieces from masking tape, doubled in thickness by sticking two pieces together, cut into small rectangles approximately 5 × 15 mm. We rounded the sharp corners of the rectangles using a pair of scissors and made two small holes 2 mm apart in each end parallel to the short side and approximately 2 mm from the edge. The holes were large enough to allow the elastic to fit through, but not large enough to allow the cross piece to slide freely. Finally, we threaded the loose end of each string through each of the two holes in the front of the balloon and left them untied.

We put the diaper on by lowering the bird feet first through the space at the top, between the suspenders and the cross pieces, and guiding the legs to the outside of the balloon on each side through the loops of elastic string. The suspenders were then loosely draped over the bird's shoulders, the balloon positioned correctly over the cloaca, and the slack on the elastic strings taken up by pulling on the loose ends in front. We adjusted the back cross piece high enough on the bird's back so that it prevented the suspenders from slipping off the shoulders (Fig. 3A). The front cross piece was moved down low enough so that it did not interfere with the crop (Fig. 3B). After performing all the final adjustments, we secured the elastic strings in front so that they rested snugly, but not tightly, on the bird's shoulders, tied them off with a knot, and cut off the extra length.

We assembled different sized diapers ahead of time by obtaining various sized balloons (9.5 × 4 or 5 mm) and by altering the size of the star-shaped hole cut into them. Assorted lengths of cross pieces were also

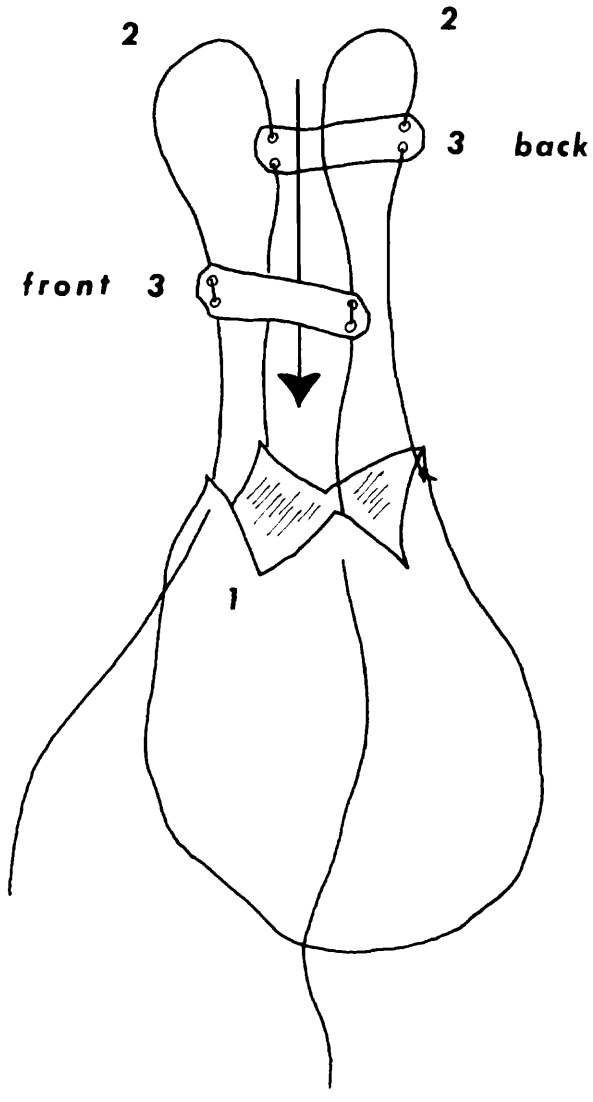

FIGURE 2. Balloon diaper for collection of fecal-urate samples in American Kestrels showing (1) four-pointed, star-shaped cut, (2) elastic string suspenders, and (3) front and back flexible cross pieces. Arrow indicates the direction the nestling is lowered into the diaper.

prepared to match to the balloons for individual sizing in nestlings of different ages and masses.

Samples from 4–7-d old American Kestrel nestlings were collected over periods of 1, 2, 12 or 24 h. During the 24-h collection periods, diapers

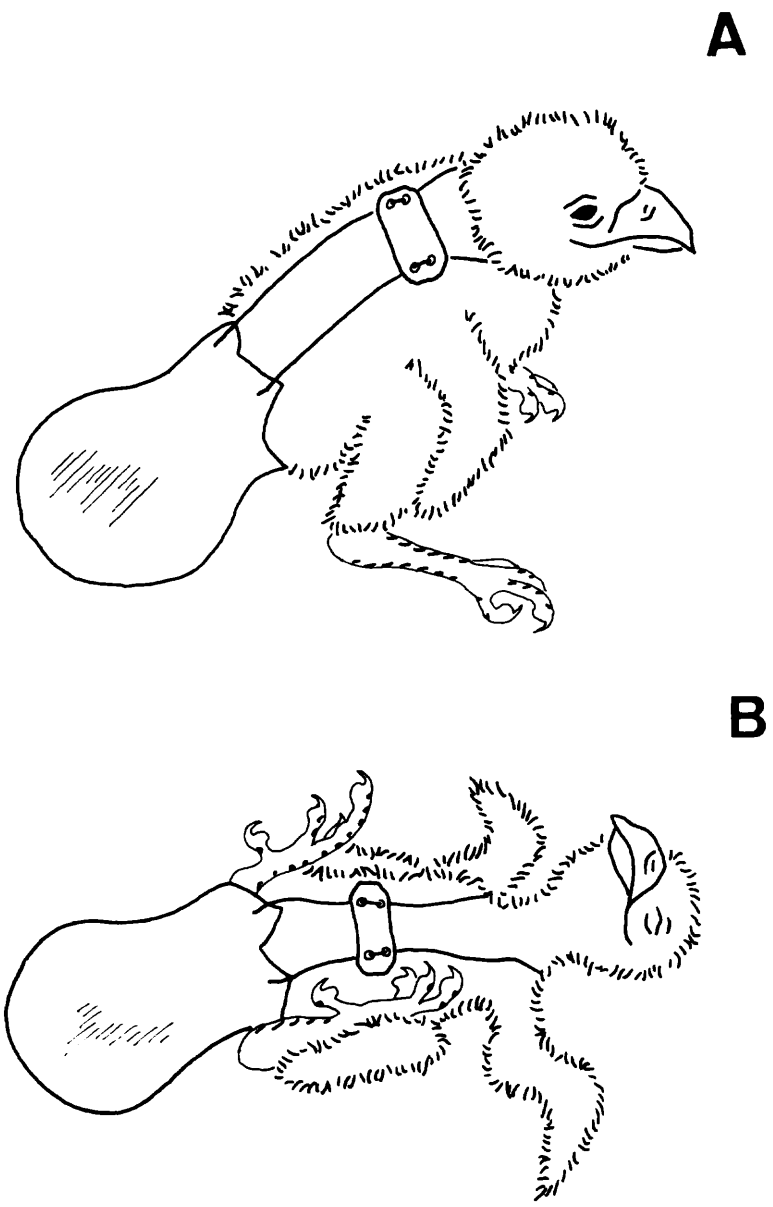

FIGURE 3. Views of an American Kestrel nestling wearing the balloon diaper for collection of fecal-urate samples. A The back cross piece is adjusted high enough to prevent the suspenders from slipping off the shoulders. B The front cross piece is positioned low enough so that it does not interfere with the crop.

were removed for approximately 20 min every 4 h while nestlings were hand fed (0800, 1200, 1600 and 2000 hours). Fecal-urate samples were collected with a disposable pipette, and the diaper was placed back on the nestling and readjusted if necessary. Between feedings, nestlings were housed in brooders in groups of up to six birds in 20-cm diameter pie tins lined with wood shavings and fitted with 23-cm cylindrical cardboard sides.

RESULTS

Fecal-urate collection from wild nestlings.—Using the plastic baggie diaper, 82 fecal-urate samples were successfully collected in 111 attempts (74%). As tail feathers matured and lengthened, the technique became increasingly ineffective. It was only marginally successful (47%) when kestrels were 15 d old, and not at all effective by the time birds were 20 d old. Larger diapers were needed for growing birds on subsequent nestbox visits to insure appropriate space for fecal-urate accumulation. Unsuccessful collection attempts were largely due to lack of defecation events. In three unsuccessful attempts, however, the diapers were not attached tightly enough and fell off, whereas another resulted when the attending adult female tore away portions of the baggie without removing it from the body. Masses for nestlings sampled in the field ranged from a mean ± SD of 24.3 ± 6 g (2 d old) to 122 ± 12 g (15 d old). The plastic baggie diaper weighed approximately 0.35 g, representing 0.3–1.4% of total body mass.

Fecal-urate collection from hand-reared nestlings.—We evaluated the performance of the balloon diaper on 40% of the sample collections in the 12- and 24-h collection periods of the toxicokinetic study. This evaluation included several individuals who were repeatedly sampled, and represented 33% of all nestlings. Unfortunately, it was not until collection was underway on the majority of 4-d-old birds dosed in the study that we first realized the difficulties associated with the use of plastic baggie diapers during the longer collection intervals. Condensation which formed under plastic baggie diapers on nestlings in brooders appeared to contribute to skin irritation, feather wetting, and a decrease in body temperature. We also observed fecal-urates leak from around the leg openings of plastic baggie diapers. Nestlings fitted with the balloon diaper ranged in mass from 18.8 to 63.9 g. The balloon diaper weighed approximately 0.75 g, representing between 1.1 and 4.0% of body mass.

In 31 of 32 cases in which fecal-urate samples were collected with the balloon diaper, sampling was successful. The star shape of the balloon formed a snug seal against the nestling in almost all cases. In the only unsuccessful collection attempt, the balloon diaper leaked during a 12-h (overnight) period and the majority of urates in the sample were lost. None of the balloon diapers, however, fell off during sampling.

The quantity of sample retrieved using balloon diapers was greater than plastic baggie diapers, where the sample spread out over a much larger surface area. A possible treatment effect cannot be entirely ruled out

though, because more fecal-urate samples from dosed nestlings were collected with balloon diapers and the majority from controls were collected with plastic baggie diapers. Diarrhea, a general symptom of OP intoxication, may have contributed to increased sample volumes.

DISCUSSION

A benefit of employing the fecal-urate collection technique on hand-reared birds was that nestling behavior in response to wearing diapers could be easily monitored at all times. We observed no restriction of movement in nestlings wearing either type of diaper while housed on wood shavings, nor did either type appear to interfere with their normal behavior in the brooder. Although there was some degree of stress inevitably associated with wearing both types of diapers, neither design appeared to cause any more stress to the nestling than did the other. In hand-reared nestlings, apparent distress calls were given by some individuals regardless of the type of diaper they were wearing. We could not determine if distress calls were in response to lack of sibling contact, the effects of the pesticide, skin irritation as a result of repeated sampling during extended periods of wear, or simply the presence of the diaper.

Both diaper designs caused some degree of skin irritation (i.e., a reddening of the covered area) especially during longer collection periods and repeated, high frequency sampling of the same individual. Skin irritation was considerably minimized with the balloon diaper because of the small surface area it covered. Constant contact with the fecal-urates was also generally averted because the shape and depth of the balloon tended to hold the sample away from the nestling. This reduced contact also substantially reduced the potential for dermal reexposure to any parent pesticide and or metabolites present in fecal-urate samples of dosed nestlings. The use of balloon diapers also limited the problem of condensation, which formed under plastic baggie diapers on nestlings in the brooders. Prolonged exposure to condensation may have contributed to decreased body temperatures in nestlings, which we observed even when nestlings were in close contact with siblings. Contact with both condensation and fecal-urates under the plastic baggie diapers not only wet and discolored the nestlings' feathers and skin, but also appeared to retard feather development as compared to nestlings wearing balloon diapers. This effect was made worse by the longer collection intervals and increased frequency of repeated sampling of the same individual not previously tested with the original design.

The plastic baggie design was not entirely suitable for sampling over the extended periods of the toxicokinetic study. Preparation of the balloon design was considerably longer, however, and correct fitting was slightly more tedious. We do not know whether the balloon design would work well on older, more mature nestlings with full tail feathers. Further testing with parent-reared birds would determine whether parental interference is likely to occur with the balloon diaper.

In conclusion, the use of this technique simplifies collection of fecal-

urate samples from nestling kestrels for use in biomonitoring and identifying toxicokinetic profiles of excretion of environmental contaminants in a variety of studies. Research involving toxicology, nutrition, energetics, hormones and metabolism may especially benefit. The technique may also be adaptable to other species of birds, although how adults would react in species where nestling feces are eliminated in a sac following parental stimulus remains to be determined. Both designs described here for collecting fecal-urate samples are inexpensive, do not involve extensive preparation time, allow for easy and efficient collection of samples, permit the investigator to collect samples selectively from individual nestlings, and allow birds to remain in the nest, thereby being much less invasive than other techniques.

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