Removing the rubbish: frogs eliminate foreign objects from the body cavity through the bladder

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During the course of a telemetry study on three species of Australian frogs (Litoria caerulea, Litoria dahlii and Cyclorana australis), we found that many of the surgically implanted transmitters had migrated into the bladder. We subsequently implanted small beads into L. caerulea and they were expelled from the body in 10–23 days. Beads implanted into cane toads (Rhinella marina) to document the process were either expelled or were enveloped into the bladder. This appears to be a unique pathway for expulsion of foreign objects from the body, and suggests that caution should be employed in telemetry studies when interpreting the separation of some animals from their transmitters as a mortality event.

Keywords: anuran amphibians; trans-bladder expulsion; radio telemetry; Hylidae; Bufonidae

1. INTRODUCTION

Spines, splinters or other foreign objects are dangerous to animals if they are in the body cavity, but surgical implants are designed to be inert and are usually implanted with the assumption that they will remain indefinitely. However, some fishes, mammals and reptiles can expel foreign objects (e.g. radio transmitters) through the intestine [1–3]. Our research uncovered a novel pathway used by frogs to sequester and void objects from the body in 10–23 days. Beads implanted into cane toads (Rhinella marina) and five cane toads (Litoria caerulea) and five cane toads (Rhinella marina). We then monitored the frog cages for reappearance of the beads in the urine or faeces. We also documented the sequence of absorption by implanting beads into 31 additional cane toads and dissecting animals on successive days after implantation.

Surgeries were identical for implantation of beads and radio transmitters. Frogs were anaesthetized with MS222 (0.1% for Litoria dahlii, Cyclorana australis and R. marina, 0.15% for L. caerulea), buffered with sodium bicarbonate. Beads and transmitters were sterilized in 95% perchloric acid and implanted laterally, on the left side of the body, approximately midway between the front and hind legs. Plastic beads were 7.8 mm long and 4 mm diameter (significantly smaller than transmitters: 22.9 × 14.4 × 8.5 mm or 20.2 × 11.1 × 7.4 mm (average)). The incision was closed with 4.0 polyglycolic acid absorbable suture (Safil green or violet, Aesculap AG & CO KG, Tuttlingen, Germany). Once the frogs awoke from the anaesthetic, they were kept individually in plastic containers with free access to water. Frogs were fed crickets and Tenebrio larvae every 2–3 days, beginning 24 h after surgery. Cages were checked daily for beads. We periodically euthanized and dissected one to four of the 31 toads per day, from 2 to 51 days post-surgery.

3. RESULTS

All five L. caerulea expelled their beads within an average of 19 days (range 15–23 days). After 45 days, only one of the initial five cane toads had expelled a bead (on day 15), so we dissected the remaining four. All four toads had beads floating freely within the bladder.

Beads in the experiment to document their absorption were surrounded by tissue originating from the bladder in as few as 2 days, although some beads were found without any adhesion of tissue up to 7 days after surgery. In many cases, the beads moved within the peritoneal cavity, but this did not seem to affect the envelopment and subsequent expulsion. In all cases, the beads were first surrounded by a thin, transparent tissue layer that progressively grew from the bladder to surround the bead (figure 1c). After the bead was surrounded by this thin tissue, a thicker and obviously vascularized tissue, presumably composed mainly of granular cells similar to those in the bladder epithelium [4,5], grew around the bead until it was fully enveloped (figure 1d–f). Once in the bladder, the beads apparently floated freely in the urine, and were expelled if they happened to be near the duct connecting the bladder to the cloaca when the bladder was voided. We assume that this is because voiding fluids from the bladder rarely involves completely emptying the bladder, so the bead can remain unvoided even after many urinations.

4. DISCUSSION

In our experiments, both L. caerulea and R. marina expelled beads that had been implanted into the abdominal cavity. Beads were first sequestered into the bladder, and then were expelled completely from the body. Other vertebrates can expel foreign objects in the body cavity, including some fishes [6–9], camels [10], humans [11–13], snakes [3,14] and relatively large compared with the frog. These observations led us to hypothesize that frogs may have a unique mechanism for eliminating foreign objects from the peritoneal cavity: they are moved into the bladder and then expelled during urination.

2. MATERIAL AND METHODS

To test the hypothesis that frogs could remove foreign objects from their peritoneal cavity, we surgically implanted small beads into the body cavities of five Litoria caerulea and five cane toads (Rhinella marina). We then monitored the frog cages for reappearance of the beads in the urine or faeces. We also documented the sequence of absorption by implanting beads into 31 additional cane toads and dissecting animals on successive days after implantation.

Surgeries were identical for implantation of beads and radio transmitters. Frogs were anaesthetized with MS222 (0.1% for Litoria dahlii, Cyclorana australis and R. marina, 0.15% for L. caerulea), buffered with sodium bicarbonate. Beads and transmitters were sterilized in 95% perchloric acid and implanted laterally, on the left side of the body, approximately midway between the front and hind legs. Plastic beads were 7.8 mm long and 4 mm diameter (significantly smaller than transmitters: 22.9 × 14.4 × 8.5 mm or 20.2 × 11.1 × 7.4 mm (average)). The incision was closed with 4.0 polyglycolic acid absorbable suture (Safil green or violet, Aesculap AG & CO KG, Tuttlingen, Germany). Once the frogs awoke from the anaesthetic, they were kept individually in plastic containers with free access to water. Frogs were fed crickets and Tenebrio larvae every 2–3 days, beginning 24 h after surgery. Cages were checked daily for beads. We periodically euthanized and dissected one to four of the 31 toads per day, from 2 to 51 days post-surgery.
crocodiles (N. Whitaker 2010, personal communication). However, unlike frogs, those species expel implanted objects either through the skin (fish), or through the intestine by trans-intestinal expulsion, not through the bladder. Thus, expulsion through the bladder is a newly discovered pathway by which animals can remove foreign objects from the body.

The mechanism by which the foreign objects moved into the bladder of amphibians appears to be different from that used to move transmitters into the intestines of other species. In channel catfish, surgical implants were first encapsulated by fibrous tissue, following a typical foreign body reaction [15], and then they were pushed through the intestinal wall into the intestinal lumen by myofibroblast contraction [1,9]. In humans, surgical sponges left in the body can erode into the intestine and be expelled [2,12,13,16]; presumably through a mechanism similar to that described for fish. This seems to be an unusual combination of two typical reactions to foreign objects in the body: first, an aseptic fibrinous response to isolate and encapsulate the object, followed by erosion of the object into a hollow organ [2]. There have also been reports of penile prostheses appearing in the bladder of humans [17,18]; however, these appear to be cases of erosion into the bladder or surgical misplacement, rather than a pathway for expulsion.

In our study, beads implanted into frogs were first surrounded by a thin layer of tissue originating from, and attached to, the bladder. Secondarily, muscular and highly vascularized tissue surrounded the foreign objects. Finally, the objects were moved into the bladder lumen and expelled. The type of tissue that frogs used to envelop beads did not appear to be the same fibrous capsule that has been reported in fishes and snakes, rather it appeared to be membranous. Over time, the membranous tissue developed obvious vascularization, and eventually the tissue became muscular and visually indistinguishable from the rest of the bladder tissues. The mechanism of this engulfment may be similar to one common to cell tissue cultures. Bladder granular cells may behave similarly to A6 epithelial cells from *Xenopus* kidneys, which grow to confluence when they contact porous and smooth surfaces [19]. This type of cell proliferation could explain the engulfment of foreign objects by tissue apparently similar to the bladder tissue.

Frogs frequently move by leaping and landing on their ventral surface rather than on their feet. This predisposes frogs to having foreign objects (e.g. sticks or spines) pierce their thin skins and enter the body cavity. Furthermore, frogs often swallow insects whole without biting or killing them. Thus, the insects, or sharp parts of insect exoskeletons, could burrow their way through the walls of the digestive system into the peritoneal cavity, as seen in some lizards [20]. It is reasonable, therefore, to think that removing foreign objects through the bladder is an adaptive ability. Frog bladders can contain huge volumes of urine, even exceeding the body mass of the frog in some species [21,22], and occupy a large proportion of the body cavity. Thus, foreign objects are more likely to contact the bladder than any other organ. In other vertebrates, much of the body cavity is filled with intestinal tissue. Thus, it may be common for non-mammalian vertebrates to use organs to remove objects, and the unique pathway of frogs may simply reflect how organs fill the peritoneal space.

For the frogs in our studies, foreign objects were sequestered into the bladder and expelled from the body using a novel mechanism. This ability, as well

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*Figure 1. Sequence of envelopment of an implanted, 7.8 mm bead by the bladder. Beads surgically implanted into the body cavity of cane toads (*a,b*) are progressively enveloped by tissue from the bladder. First, a thin layer of tissue that originates from the bladder surrounds the bead (*c*). Next, the bead is surrounded by thicker, more heavily vascularized tissue (*d,e*). Eventually, the bead is fully incorporated into the bladder lumen and expelled with the urine (*f*).*
as trans-intestinal expulsion, could lead researchers to draw misleading conclusions about mortality of telemetered individuals assumed to disappear owing to predation. Such incorrect conclusions could be dangerous by leading to inaccurate estimates of population dynamics for population viability models of endangered species.

We have seen that four species of anurans either sequestered surgically implanted objects into the bladder, as was the case for radio transmitters in *L. caerulea* (this study), *L. dahlia* and *C. australis* (C. R. Tracy & K. A. Christian 2006, unpublished data), or they completely expelled implanted objects that were small enough to pass through the pelvic girdle, such as the experimental beads in *L. caerulea* and *R. marina*. Because all of the species that we studied were able to sequestrate and/or remove objects via the bladder, despite being from different families, this novel ability may be widespread among amphibians.

This study was under permit from the Charles Darwin University Animal Ethics Committee (A02028) and the Parks and Wildlife Commission of the Northern Territory (no. 22734, no. 28636).

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