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Gastrointestinal transit in the common brushtail possum measured by gamma scintigraphy

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Abstract

This paper reports an example of the application of pharmaceutical technology to wildlife management, specifically the design of an oral delivery system for the common brushtail possum in New Zealand. Designing an oral delivery system requires a knowledge of the time taken for particulates to reach target sites within the gastrointestinal tract (GIT). The transit time for fluid and indigestible particles of two different size ranges was determined in the common brushtail possum (*Trichosurus vulpecula*). Technetium-labelled (^{99m}Tc) anion exchange resin particles (75–125 or 500–700 μm diameter) or solution (^{99m}Tc-labelled diethylenetriamine pentaacetic acid, ^{99m}Tc-DTPA) was administered orally. At predetermined times after dosing (3, 6, 12, 24 or 32 h), the distribution of radioactivity throughout excised gastrointestinal tracts was determined by gamma scintigraphy. The transit profile was similar for the three formulations investigated. Unlike other closely related hindgut fermenting marsupials, there was no evidence to support the presence of a colonic separating mechanism in the common brushtail possum. Gastrointestinal transit was independent of body mass, gender and time of day that the dose is given. To target the hindgut for oral delivery of protein and peptide biocontrol agents, the formulation would need to protect the bioactive for approximately 12 h prior to release.

Keywords: Transit time; Brushtail possum; Gamma scintigraphy; Gastrointestinal tract; Peptide delivery

1. Introduction

Gamma scintigraphy has been used extensively in studies of gastrointestinal transit of pharmaceutical dosage forms in subjects ranging from humans, dogs, cats and pigs (Christensen et al., 1985; Davis et al., 2001; Wilding et al., 2001). Gamma scintigraphy has

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the advantage that it can be used to monitor real-time transit, enabling the visualization and quantification of the movement of fluid and particulates through different parts of the gastrointestinal tract (GIT). Whilst the technique is usually adopted as a non-invasive procedure, measuring the activity of fluid and particulates in an excised GIT provides greater accuracy as it eliminates the problem of overlying tissues contributing to incorrect estimates of activity in the region of interest.

The common brushtail possum (*Trichosurus vulpecula*) is a marsupial herbivore native to Australia that has reached pest status in New Zealand following deliberate introductions in the 1800s. One strategy under investigation to reduce the number of common brushtail possums in New Zealand is to identify compounds that will reduce fertility (Cowan, 2000). Although specific biocontrol agents for the brushtail possum are yet to be developed, it is likely that they will be peptide or protein molecules. Any biocontrol agent identified will need to be administered to animals in the wild and in the case of the brushtail possum, this will involve delivery to millions of free-ranging, feral possums widely distributed across New Zealand, often in remote and inaccessible areas. The controlled release of an encapsulated bioactive, delivered in non-toxic baits, is a potential solution for delivery to brushtail possums.

Previous studies have investigated the transit time of fluid and particulates in the common brushtail possum fed different types of diets (semi-purified pellets or eucalypt leaves). Markers of transit used were methylene blue; plastic chips and heavy metals including chromic oxide (Gilmore, 1970); ^{51}Cr–EDTA and ^{103}Ru–P (Wellard and Hume, 1981; Foley and Hume, 1987); Co–EDTA, Ytterbium (Yb) and chromium (Cr) mordanted cell wall components of hay (Sakaguchi and Hume, 1990). A limitation with these studies is that mouth to anus transit time was calculated and the concentration of markers during their passage through sections of the GIT was not determined. In addition, there is evidence that when a heavy metal is used as a marker it may not stay bound to one particular particle and that small particles may be preferentially bound (in ref. Foley and Hume, 1987).

The brushtail possum is a hindgut fermenter with a relatively simple stomach and an enlarged caecum and proximal colon (Foley et al., 1989). The hindgut of the brushtail possum has been identified as a suitable site for release of peptide or protein bioactives because the activity of proteolytic enzymes is significantly lower in the hindgut compared to the small intestine (Wen et al., 2002). To successfully design a controlled-release system to deliver a biocontrol agent to the hindgut of the brushtail possum, it will be necessary to have information on transit and residence times of particulates in the GIT. The aim of this study was to use gamma scintigraphy to quantify the transit of radiolabelled particles of different sizes and of fluid, through the regions of the GIT of the common brushtail possum at set times after oral administration and prior to elimination in the faeces. The influence of feeding and activity on transit time was also investigated by dosing the animals at different times during the day.

2. Materials and methods

2.1. Materials

Amberlite IRA-400 (Cl^-) anion-exchange resin (500–700 μm) and Amberlite IRA-420C anion-exchange resin (75–125 μm) were purchased from BDH Chemicals Ltd. (Poole, England) and Sigma (St. Louis, MO, USA), respectively. Technetium 99m sodium pertechnetate solution and Tc-DTPA was obtained from the Department of Nuclear Medicine, Dunedin Hospital (Dunedin, New Zealand). Animals were anaesthetized with halothane (Flurothane) purchased from ICI New Zealand Ltd. (Lower Hutt, New Zealand) and euthanased using barbiturate (Euthal^{TM}) purchased from Delta Veterinary Laboratories Pty Ltd. (Hornsby, NSW, Australia).

2.2. Radiolabelling transit markers

Indigestible resin particles of two size ranges (75–125 and 500–700 μm diameter) were radiolabelled with the gamma-emitting radioisotope technetium (^{99m}Tc; half-life 6 h) following the method of Theodorakis (1980). The choice of particle size was to enable comparison with previous studies. In brief, 3.0 g resin was stirred in a beaker with 30 ml distilled water and a solution of ^{99m}Tc-sodium pertechnetate ($Na^{99m}TcO_4^1$; 90–110 MBq) in 2 ml saline was added. The mixture was stirred for 10 min and the

labelled resin recovered by filtration, washed and then re-suspended in distilled water. The fluid formulation used was a non-absorbable, aqueous solution of ^{99m}Tc-labelled diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA; 90–110 MBq) in saline.

2.3. Stability of radiolabelled resins

Fresh digesta were collected from the stomach, small intestine, caecum and proximal colon (two samples per region) from each of three brushtail possums. Radiolabelled resin particles (500–700 μm diameter) were incubated with either digesta (5 g in 5 ml distilled water), 0.1M HCl or 0.1M phosphate buffer (pH 7) for 32 h at 37 °C. Resin was separated from the gut contents by centrifugation using a 50% sucrose solution at 4000 × *g* for 1 h. The radioactivity associated with the resin recovered from the gut contents was measured in duplicate using a 11/2^{H} NaI gamma counter (Wiper^{TM} Well Counter).

2.4. Animal husbandry

Adult brushtail possums (24 male and 48 female) were wild-caught from different localities in the Dunedin region of New Zealand. The body mass of animals ranged from 1.46 to 3.69 kg. After capture, animals were housed in environmentally-enriched pens (5–12 possums/pen) under conditions of natural daylight and temperature. Animals were fed a mixed diet of fresh fruit and cereal-based pellets containing approximately 8% fibre (Opossum pellets, Western Animal Nutrition, Rangiora, New Zealand). Water was available ad libitum and fresh branches of *Pinus radiata* were included in the pens as a source of browse (McLeod et al., 1997). The procedures described had prior approval from the University of Otago Animal Ethics Committee and conforms to the University of Otago Code of Ethical Conduct for the Manipulation of Animals, 1987.

2.5. Administration of transit markers

The experimental design used was four animals for each of the three formulations at five time points (3, 6, 12, 24 and 32 h) after receiving the radiolabelled marker ($n = 60$). Animals were assigned to treatment groups with no particular order.

Each brushtail possum was lightly anaesthetized with halothane by inhalation (0.3–0.4 l/min). Radiolabelled particles (∼0.4 g in 2 ml distilled water) or solution (2 ml) was administered to the anaesthetized possum via a flexible tube (external diameter 4 mm) inserted into the oesophagus. The dose was followed by a further 2 ml of water to flush the oesophagus. The animals recovered from anaesthesia within 5 min of receiving the dose and were then held in individual wire mesh cages (W490 mm × H360 mm × L540 mm) with access to possum pellets, fruit and water ad libitum. Faeces and urine (if produced) were collected over the interval between dosing and euthanasia and measured for radioactivity using the gamma camera. Animals were given the radiolabelled markers at approximately the same time of day (morning). To investigate diurnal differences in GIT transit in this nocturnal mammal, an additional 12 possums were dosed in the evening and sacrificed the following morning, 12 h after dosing ($n = 4$ per formulation).

At the predetermined time after receiving the radiolabelled particles or solution (3, 6, 12, 24 or 32 h), the animal was lightly anaesthetized with halothane by inhalation (0.3–0.4 l/min) and euthanased by intracardiac injection of barbiturate (4–8 ml). Immediately after death, a midline incision was made on the ventral surface of the possum to expose the abdominal cavity. The gastrointestinal tract was removed from the oesophagus (1 cm anterior to the stomach) to the rectum. Excess mesentery and fat was removed and each section of the tract (stomach, small intestine, caecum and colon) was ligated to prevent mixing of digesta. The dissected GIT was stored in a sealed plastic bag on ice until measured by gamma scintigraphy (maximum of 2 h after euthanasia). To investigate systemic absorption of the radioisotope, the kidneys, spleen, liver and whole carcass (minus GIT) were also measured for radioactivity in some animals.

2.6. Scintigraphy

To enable accurate assignment of radioisotope distribution to specific regions of the GIT, each tract was arranged on a tray following a standard template (see Fig. 1(a)). A large field Technicare Sigma 438 gamma camera with a 0.5 in. NaI crystal and a low energy, parallel, multi-hole collimator was used to obtain static images of each GIT (automatic

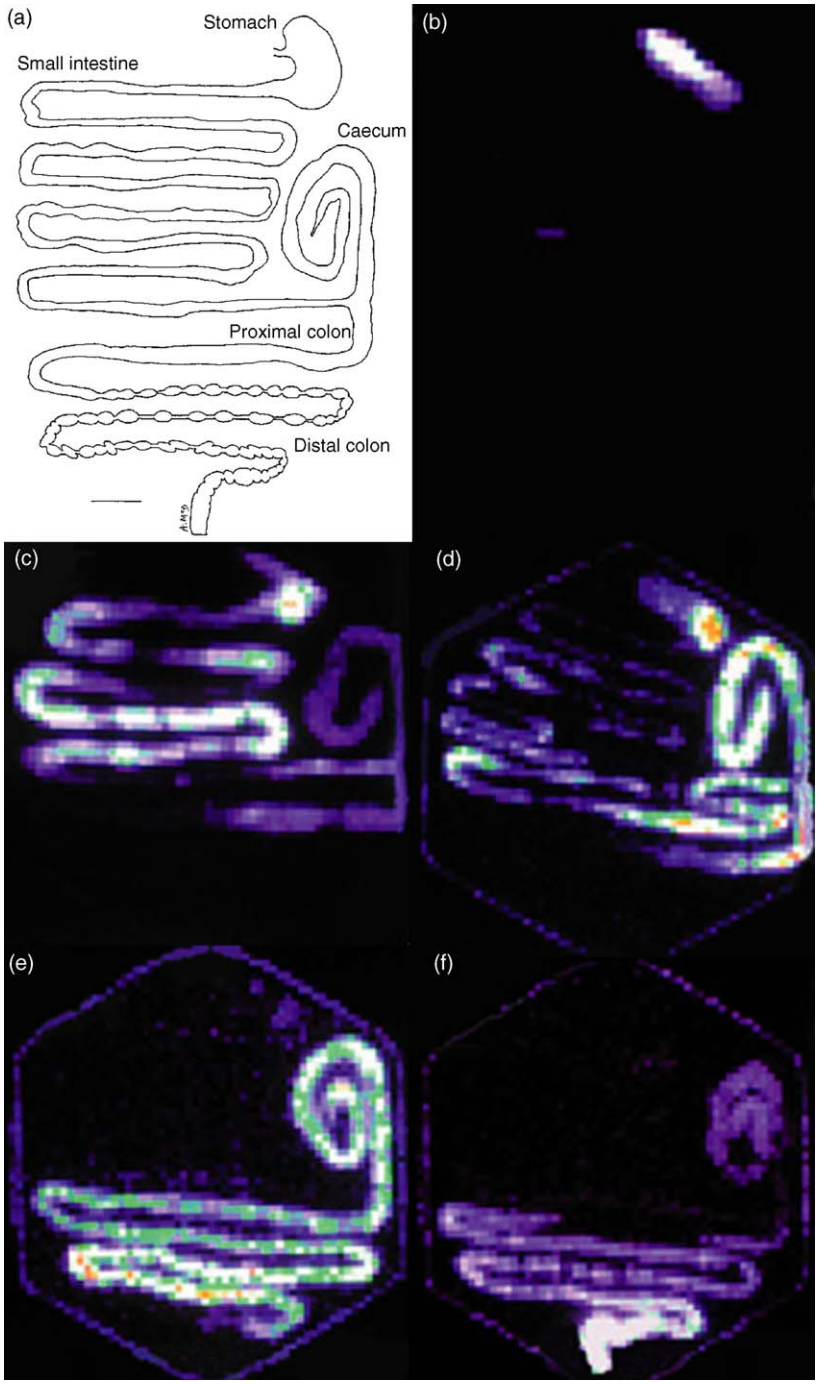

Fig. 1. (a) Line drawing of the gastrointestinal tract of the common brushtail possum (*Trichosurus vulpecula*). Scale bar = 5 cm. The orientation of the GIT is the same in the scintiscans that follow. The scintiscans were taken at 3, 6, 12, 24 and 32 h (b–f, respectively) after oral dosing with small (75–125 μm) radiolabelled exchange resin.

acquisition time of 300 s). Gamma camera images were digitised in a 64 × 64 matrix and analysed using Siemens MicroDELTA Clinic software by manually delineating each region of interest (ROI) around the stomach, small intestine, caecum and colon and obtaining a count of radioactivity for each region.

The distribution of activity, expressed as a percentage of the total radioactivity in each GIT, was determined for each region of the gut at each time point.

2.7. Statistical analyses

The dose of radioactivity administered to the animals varied due to differences in the time of day that the technetium became available and to its short half-life (6 h). Therefore, to overcome this variability, raw gamma counts were log transformed (+1 to account for zeros in the data) prior to analysis. Data were analysed by restricted maximum likelihood (REML, Patterson and Thompson, 1971). Analyses were performed using GenStat Release 6.2 (GenStat Committee, 2003).

3. Results and discussion

After 32 h incubation of the radiolabelled resin with possum gut contents, the lowest amount of in vitro binding recorded was 77% and this occurred after incubation in caecal contents (Table 1). The radioactivity associated with the resin after incubation in either 0.1M HCl or 0.1M phosphate buffer (pH 7) was greater than 99%. Thus, unlike heavy metal chelate markers such as Cr–EDTA used by previous investigators, the integrity of binding between technetium and resin complex is substantially maintained in the presence of gut contents from the common brushtail possum.

Table 1
Radiolabel (%) remaining bound to anionic exchange resin following 32 h incubation in different media ($n = 3$)

Incubating media	Mean activity (%) ± S.D.
Digesta	
Stomach	81.5 ± 3.5
Small intestine	97.5 ± 0.7
Caecum	77.0 ± 3.5
Proximal colon	81.7 ± 4.0
HCl	99.7 ± 0.2
Phosphate buffer	99.9 ± 0.04

The typical progression of radiolabelled particles through the GIT of the common brushtail possum is illustrated in scintiscans taken at 3, 6, 12, 24 and 32 h post-dosing (Fig. 1). The animals included in this study were wild-caught and varied in age and body weight. This represents the variation present in the wild, target population in New Zealand. Transit time was variable between individual animals, but was independent of gender ($P = 0.184$) and body mass ($P = 0.640$), at least over the range 1.42–3.69 kg.

The profiles of transit through each section of the GIT in the common brushtail possum were similar for all three formulations (Fig. 2). However, some trends were observed. Radiolabelled fluid had the fastest transit time through the stomach; 3 h after receiving the radiolabelled fluid in the morning, 67% of the activity remained in the stomach and 31% was detected in the small intestine (Fig. 2(a and b)). In contrast, 3 h after dosing with radiolabelled particles, on average 88% (small particles) and 95% (large particles) of the activity remained in the stomach (Fig. 2(a)). The radiolabelled fluid also reached the hindgut earlier than either the small or the large resin particles and this occurred approximately 6 h after oral dosing (Fig. 2(c and d)). Very little of either of the particulate formulations were observed in the caecum at this time point.

Sakaguchi and Hume (1990) reported a rapid mean transit time of 5.8 h for Co–EDTA (fluid) and plant cell wall constituents as particulates (<75 μm labelled with ytterbium and >300 μm labelled with chromium), with no differences in transit time being noted between the different-sized markers. This transit is much faster than recorded in the present study and we suggest that the differences in transit times between these earlier reports and the present study reflect differences in diet quality. In the study by Sakaguchi and Hume (1990), animals were fed leaves from Eucalyptus species that are high fibre and low in nitrogen content. A digestive strategy of the brushtail possum is to rapidly remove larger hard-to-digest bulk in the diet so that the animal is able to maintain a sufficient feed intake rate to meet its energy demands (Hume and Warner, 1980). Brushtail possums in New Zealand have the opportunity to select a higher quality diet than their counterparts in their natural habitat in Australia (Nugent et al., 2000). Consequently, rapid removal of indigestible fractions

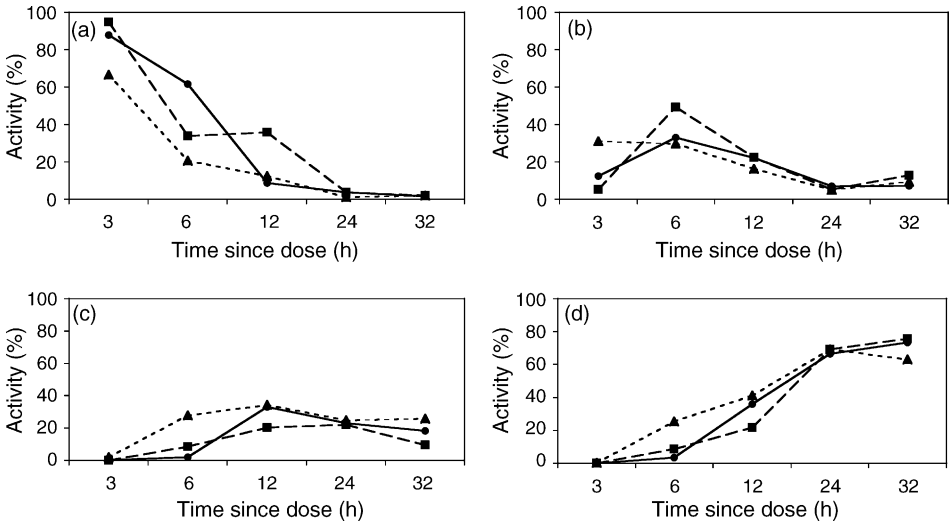

Fig. 2. Transit of radiolabelled markers through the gastrointestinal tract of the common brushtail possum 3, 6, 12, 24 and 32 h after morning dosing. Markers were small (75–125 μm) (●) and large (500–700 μm) (■) resin particles and fluid (▲). Data represents activity detected in the (a) stomach (b) small intestine (c) caecum (d) colon, as a percentage of total activity in the whole gastrointestinal tract. Data points are the average of four replicates.

of the diet may be less important, which is reflected in the slower transit time recorded here.

Selective retention of small particulate digesta in the caecum is another digestive adaptation that marsupial herbivores related to the common brushtail possum, such as the common ringtail possum (*Pseudocheirus peregrinus*), adopt to deal with their fibrous diet (Chilcott and Hume, 1985). By retaining the smaller and more digestible fraction of their diet, they are also able to retain microorganisms in the caecum for fermentation. Sakaguchi and Hume (1990) and Wellard and Hume (1981) were unable to show any evidence of selective retention in the caecum of brushtail possums and concluded that it does not occur in this species. In addition, Wellard and Hume (1981) removed the caecum of the common brushtail possum and reported an increase in the retention time of radiolabelled fluid and particles. From this result, it can be concluded that the caecum does not contribute to the process of selective retention in the common brushtail possum. Data from the present study supports this finding, as we found no difference in the transit of the different sized formulations in this species.

Maximum concentration of the radioactive label in the caecum was recorded between 12 and 24 h after dosing for all formulations used in the present study (Fig. 2(c)). Therefore, any dosage formulation will need to protect the bioactive during passage through the stomach and small intestine for approximately 12 h before degrading if the caecum is to be the target site for delivery. This gamma scintigraphy study did not allow us to determine the retention time of each formulation within specific regions of the GIT; however, we observed an extended residence in the hindgut. For all formulations, radioactivity was still detected in the caecum 32 h after dosing. It was not possible to measure activity beyond 32 h post-dose in this study due to the short half-life of technetium. In previous studies, mean retention times of 46+ h for small particles, 36.3 ± 13.1 h for fluid and 39.6 ± 13.0 h for fine particles have been recorded (Foley and Hume, 1987; Sakaguchi and Hume, 1990).

Feeding and activity level of the animal may affect the gastrointestinal transit of oral dosage forms. The common brushtail possum is nocturnal and the animals in this study were maintained on ambient day/night regimes. The feeding behaviour of the common brushtail possum in the wild is to emerge 2–3 h after sunset to feed and return to their nest several hours before sunrise. Feeding duration is 1–2 h in 2–3 sessions throughout the night (MacLennan, 1984). Thus, the animals dosed in the morning in the present study would have

been given the dose of radiolabelled formulation when the stomach contained food. After dosing, possums were inactive and not eating. Conversely, animals dosed in the evening would have been given the dose on an emptier stomach than those in the morning dose group and they would have been active and consuming food within a few hours after receiving the radiolabelled dose. The experiment in this study where the distribution of radioactivity was compared 12 h after animals were dosed at either 06:00 a.m. or 18:00 p.m. investigated diurnal differences in gastrointestinal transit. Observations made on the fullness of the stomach at the time of dissection concur with those described above. Surprisingly, transit through the GIT of the common brushtail possum was not different between animals dosed in the evening or the morning (Fig. 3). This suggests that by 12 h after administration of the oral dose, GI transit of formulations is relatively constant, at least in this captive population where feed resources are unlimited. It remains to be determined whether a longer period of fasting prior to the dose would influence transit times. In addition, gender ($n = 10$ males; $n = 14$ females) and body mass (range 1.97–3.27 kg) of the animal was found to not significantly effect transit times ($P > 0.05$).

It should be noted that anaesthesia is necessary to administer an oral dose to the common brushtail possum because of the potential they have to cause injury. The effect of anaesthetics on gastrointestinal transit varies with animal species as well as the type and duration of agent used (Asai et al., 1998). Opioid drugs have been shown to delay gastric transit in some animals (Nimmo, 1989). The effect of halothane on gastrointestinal transit in the common brushtail possum is not known. Nevertheless, the halothane anaesthesia used in the present study was brief ($\leq$2 min) and the possums recovered within a few minutes of receiving the dose, so it is unlikely that the anaesthesia would have affected the transit appreciably.

In summary, for site-specific delivery of protein or peptide biocontrol agents to the hindgut of the common brushtail possum, the bioactive will need to be protected during passage through the stomach and small intestine for approximately 12 h. After reaching the caecum, it would appear that formulations might reside in the caecum for up to 32 h. The lack of correlation between body mass of the animal, gender and time of day that the dose is given is desirable in this situation because the formulation will be ultimately applied to a wild population.

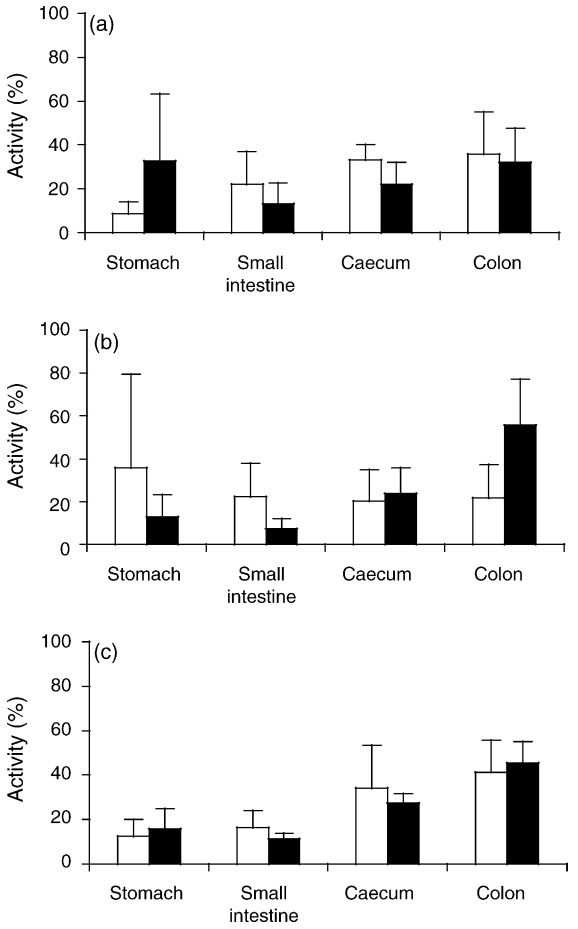

Fig. 3. Comparison of gastrointestinal transit times in the common brushtail possum 12 h after being dosed in the morning (open columns) or the evening (filled columns). Animals were dosed with either (a) small (75–125 μm) or (b) large (500–700 μm) radiolabelled resin particles or (c) fluid. Graphs are the percentage of total radioactivity detected in the stomach, small intestine, caecum and colon. Columns are the mean of four replicates (±S.D.).

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References

Asai, T., Mapleson, W.W., Power, I., 1998. Effects of nalbuphine, pentazocine and U50488H on gastric emptying and gastrointestinal transit in the rat. Br. J. Anaesth. 80, 814–819.

Chilcott, M.J., Hume, I.D., 1985. Coprophagy and selective retention of fluid digesta: their role in the nutrition of the common ringtail possum, *Pseudocheirus peregrinus*. Aust. J. Zool. 33, 1–15.

Christensen, F.N., Davis, S.S., Hardy, J.G., Taylor, M.J., Whalley, D.R., Wilson, C.G., 1985. The use of gamma scintigraphy to follow the gastrointestinal transit of pharmaceutical formulations. J. Pharm. Pharmacol. 37, 91–95.

Cowan, P.E., 2000. Biological control of possums: prospects for the future. In: Montague, T. (Ed.), The Brushtail Possum. Biology, Impact and Management of an Introduced Marsupial. Maanaki Whenua Press, Lincoln, New Zealand, pp. 262–279.

Davis, S.S., Illum, L., Hinchcliffe, M., 2001. Gastrointestinal transit of dosage forms in the pig. J. Pharm. Pharmacol. 53, 33–39.

Foley, W.J., Hume, I.D., 1987. Passage of digesta markers in two species of arboreal folivorous marsupials—the greater glider (*Petauroides volans*) and the brushtail possum (*Trichosurus vulpecula*). Physiol. Zool. 60, 103–113.

Foley, W.J., Hume, I.D., Cork, S.J., 1989. Fermentation in the hindgut of the greater glider (*Petauroides volans*) and the brushtail possum (*Trichosurus vulpecula*)-2 arboreal folivores. Physiol. Zool. 62, 1126–1143.

Gilmore, D.P., 1970. The rate of passage of food in the brushtailed possum, *Trichosurus vulpecula*. Aust. J. Biol. Sci. 23, 215–218.

Hume, I.D., Warner, A.C.I., 1980. Evolution of microbial digestion in mammals. In: Ruckebusch, Y., Thivend, P. (Eds.), Digestive Physiology and Metabolism in Ruminants. MTP Press, Lancaster, pp. 665–684.

MacLennan, D.G., 1984. The feeding behaviour and activity patterns of the brushtail possum, *Trichosurus vulpecula*, in an open eucalypt woodland in south-eastern Queensland. In: Smith, A.P., Hume, I.D. (Eds.), Possums and Gliders. Surrey Beatty and Sons Pty Ltd., Chipping Norton, NSW, Australia, pp. 155–161.

McLeod, B.J., Thompson, E.G., Crawford, J.L., Shackell, G.H., 1997. Successful group housing of wild-caught brushtail possums (*Trichosurus vulpecula*). Anim. Welfare 6, 67–76.

Nimmo, W.S., 1989. Gastric emptying and anaesthesia. Can. J. Anaesth. 36, S45–S47.

Nugent, G., Sweetapple, P.J., Coleman, J., Suisted, P., 2000. Possum feeding patterns: dietary tactics of a reluctant folivore. In: Montague, T.L. (Ed.), The Brushtail Possum. Biology, Impact and Management of an Introduced Marsupial. Manaaki Whenua Press, Lincoln, New Zealand, pp. 10–19.

Patterson, H.D., Thompson, R., 1971. Recovery of inter-block information when block sizes are unequal. Biometrika 58, 545–554.

Sakaguchi, E., Hume, I.D., 1990. Digesta retention and fiber digestion in brushtail possums, ringtail possums and rabbits. Comp. Biochem. Phys. A 96, 351–354.

Theodorakis, M.C., 1980. External scintigraphy in measuring rate of gastric emptying in beagles. Am. J. Physiol. 239, G39–G43.

Wellard, G.A., Hume, I.D., 1981. Digestion and digesta passage in the brushtail possum, *Trichosurus vulpecula* (Kerr). Aust. J. Zool. 29, 157–166.

Wen, J.Y., Ledger, R., McLeod, B.J., Davies, N.M., Butt, A.G., Tucker, I.G., 2002. Protein and peptide degradation in the intestine of the common brushtail possum (*Trichosurus vulpecula*). J. Comp. Physiol. B 172, 553–559.

Wilding, I.R., Coupe, A.J., Davis, S.S., 2001. The role of gamma-scintigraphy in oral drug delivery. Adv. Drug Deliv. Rev. 46, 103–124.