RELATIVE PASSAGE RATES OF LIPID AND AQUEOUS DIGESTA IN THE FORMATION OF STOMACH OILS

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ABSTRACT.—We used tritium-labeled glycerol triether as a nonabsorbable lipid-phase marker and carbon-14 labeled polyethylene glycol as a nonabsorbable aqueous-phase marker to examine gastrointestinal transit of a homogenized fish meal fed to 4-week-old chicks of Antarctic Giant-Petrels (*Macronectes giganteus*) and Gentoo Penguins (*Pygoscelis papua*). Both aqueous-phase and lipid-phase markers passed through the gastrointestinal tract without being metabolized. Label recoveries from the two species were statistically indistinguishable. Mean retention time was significantly longer for lipid-phase components than for aqueous-phase components in both species. In the petrel, mean retention time for lipid-phase and for aqueous-phase was significantly longer than in the penguin. Interspecific differences in retention were largely the result of differing rates of gastric emptying. Both markers emptied rapidly from the proventriculus and gizzard of the penguins, while in giant-petrels the lipid-phase was retained for extended periods in the stomach. Differential transit of lipid and aqueous phases coupled with the lower rate of gastric emptying in giant-petrel chicks provides a physiological basis for accumulation of dietary lipids in the proventriculus. The large, distensible proventriculus and the ventral position of the pyloric valve relative to the gizzard and proventriculus are morphological traits which enhance the formation and retention of stomach oils. *Received 31 May 1988, accepted 19 December 1988.*

OF all avian internal organs, the range of morphological variation in the stomach is the greatest. This reflects both widely differing dietary habits and the importance of the stomach in processing solid food items (Ziswiler and Farner 1972). The avian stomach consists of two chambers which are externally distinguishable in most species (McLelland 1979). The glandular proventriculus is continuous with the esophagus and secretes gastric juice. The gizzard or ventriculus is caudal to the proventriculus and functions as the site of gastric proteolysis and, in many species, of mechanical digestion (Duke 1986b).

In procellariiforms (petrels, fulmars, shearwaters, and albatrosses), the most pelagic avian order, the division between the proventriculus and gizzard is readily apparent. Forbes (1882) described a distensible proventriculus that consisted of a large sac with a fundus, a small gizzard twisted so that the pylorus faces back instead of forward, and an ascending loop of the duodenum before the descending and ascending limbs. The proventriculus may be so large when distended with food that it extends caudally in the body cavity well beyond the gizzard (Matthews 1949, Duke et al. 1989). In other birds the much smaller proventriculus is cranial to the gizzard.

With the exception of diving petrels (Pelecanoididae), all members of the Procellariiformes store lipids ("stomach oils") in the enlarged proventriculus. The origin of stomach oils was previously thought to be secretory (Matthews 1949, Lewis 1966), but evidence is now compelling for a dietary origin (Cheah and Hansen 1970, Warham et al. 1976, Clarke and Prince 1976, Imber 1976). The function of stomach oils has been viewed primarily as an energy and water reserve and secondarily as a defense against predators (Warham 1977, Jacob 1982). Previous work (Roby et al. 1986a, Place and Roby 1986, Duke et al. 1989) indicated that petrels retain lipids in the proventriculus longer than seabirds without stomach oils. This suggests that, for seabirds that store stomach oils, gastrointestinal passage rates for lipid digesta are considerably lower than those for aqueous digesta. However, there are no published reports of relative passage rates of lipid and aqueous digesta in birds; hence, there is no basis for comparison with those species that store stomach oils.

Nonabsorbable markers are widely used to monitor movement of a specific phase along the gastrointestinal tract. They are also used to estimate nutrient absorption from or secretion into a marker specified phase. It is important that the marker have properties similar to components of the phase under study (Wiggins and Dawson 1961; Carlson and Bayley 1972a, b). Polyethylene glycol (MW 4000) is a widely accepted aqueous-phase marker (Wingate et al. 1972) and glycerol triether, a neutral lipid-phase marker, is used extensively in studies with mammals (Morgan and Hofmann 1970; Carlson and Bayley 1972a, b; Meyer et al. 1986), but has not been used with birds. The triethers will mark only the neutral lipid phase; polar lipids, such as phospholipids and fatty acids, partition into the aqueous or micellar phase. These compounds are nonabsorbable, nontoxic, nondegradable by digestive or bacterial enzymes, and do not influence the normal absorption of dietary aqueous nutrients or fat.

In mammals, passage rate of lipids through the pyloric valve and into the small intestine is nearly half that for aqueous components (Jian et al. 1982, Meyer et al. 1986). If a meal is homogenized prior to ingestion, however, both lipids and aqueous components empty the stomach together (Cortot et al. 1979).

Using lipid-phase and aqueous-phase markers, we examined gastrointestinal transit in chicks of the Antarctic Giant-Petrel (*Macronectes giganteus*) and Gentoo Penguin (*Pygoscelis papua*). These species represent the two major orders of seabirds in the Southern Ocean. The young of both species are fed at the nest by their parents until full grown. Giant-petrels usually locate their nests near penguin breeding colonies and feed their young a diet of (in decreasing order of importance) penguins, krill (*Euphausia superba*), seals, squid, and small seabirds (Hunter 1983, 1985). The chicks of both giant-petrels and Gentoo Penguins are usually fed 1–2 meals daily. The diet of Gentoo Penguins consists primarily of krill and, to a lesser extent, fish (Croxall and Prince 1980). Nestling giant-petrels store stomach oils at an early age and are adept at regurgitating oil on potential predators. Penguins do not store stomach oils.

We measured relative transit times of the two phases in penguins to ascertain the extent of differential passage rates in an avian species which lacks stomach oils. By comparing passage rates and gut morphology in penguins and petrels, we hoped to elucidate mechanisms responsible for stomach oil formation in the latter.

Materials and Methods

Radiolabels and fluors.—We used [^{14}C]-polyethylene glycol (molecular weight 4000, 15 mCi/g) from Amersham (Arlington Heights, Illinois) without further purification. Fluors were ACS II (Amersham, Arlington Heights, Illinois) and Biosafe II (Research Products International, Mount Prospect, Illinois). Duplicate samples were counted on a Beckman LS 3801 scintillation counter. A correction ("quench") curve was derived to determine counting efficiency for different extracts and tissue types using Compton edge ("H number") calibration (Beckmann Instruments). Counting efficiency for ^{14}C in the samples varied from 88.0–75.4% and that for ^{3}H from 22.0–6.0%. Counting times (2–10 min) were chosen to ensure at least 95% counting accuracy. The coefficient of variation for replicate samples averaged 3.0% (SD = 1.3) for tritium and 1.9% (SD = 0.9) for carbon-14. All radioactivities are expressed in disintegrations per min (DPM).

Tracer synthesis.—Bachem Bioscience Inc. (Philadelphia, Pennsylvania) synthesized the glycerol triether [1-(9 cis-octadecenyl) 2,3 didodecyl glycerol triether] as described by Morgan and Hofmann (1970). The tritiated glycerol triether (^{3}H-GTE) was prepared by reduction with platinum as a catalyst (New England Nuclear, Boston, Massachusetts). Purified ^{3}H-GTE (>98% radiopurity) was obtained by chromatography on a silicic acid column eluted with hexane/diethyl ether 85:15 (v/v). Solvent was removed with nitrogen evaporation and the purified ^{3}H-GTE dissolved in absolute ethanol to a specific activity of 1 mCi/ml.

Study area and subjects.—Feeding (including excreta collection) trials were conducted during January and February, 1986, at Ardley Island (62°13'S, 58°55'W), a ca. 18-ha island off King George Island, South Shetland Islands, where ca. 9,000 pairs of Gentoo Penguins and 15 pairs of Antarctic Giant-Petrels nest (Roby et al. 1986b). Six nestlings of each species were removed from nests and held in enclosures for 12–20 h before ingesting the labeled meal. Subjects were ca. 4 weeks old, judging from wing-length and body-mass measurements (Hunter 1984, Volkman et al. 1980). All were developed sufficiently to thermoregulate at ambient temperatures (ca. 0–5°C) independently of their parents. Body mass of subjects used in feeding trials ranged from 0.98–2.2 kg and averaged 1.5 kg. Average mass of subjects did not differ significantly between the two species (t = 0.344, P > 0.05, Table 1).

Prior to feeding the radiolabeled meal, we pumped the stomachs of giant-petrel chicks to remove any

TABLE 1. Morphological measurements of the gastrointestinal tracts of Gentoo Penguin ($n = 6$) and Antarctic Giant-Petrel chicks ($n = 6$).

	Gentoo Penguin	Antarctic Giant-Petrel
Mass		
Total chick mass (kg)	1.56 ± 0.39[a]	1.49 ± 0.31[a]
Proventriculus (g)	16.3 ± 3.90	22.3 ± 1.94
Gizzard (g)	11.5 ± 2.12	8.0 ± 0.69
Small intestine (g)	48.8 ± 15.5	18.7 ± 3.05
Duodenum	20.3 ± 6.97	6.36 ± 1.26
Ileum (g)	28.5 ± 8.64	12.3 ± 2.48
Colon and ceca (g)	3.99 ± 1.33	1.47 ± 0.20
Length (cm)		
Proventriculus	4.7 ± 0.32	20.2 ± 0.82
Gizzard	5.6 ± 0.97	4.9 ± 0.58
Small intestine	178 ± 16.0[a]	192 ± 7.70[a]
Duodenum	59.8 ± 5.77	38.0 ± 3.44
Ileum	118 ± 12.1	154 ± 9.32
Colon	7.8 ± 0.82[a]	7.4 ± 0.49[a]
Cecum	1.9 ± 0.13	0.9 ± 0.10
Area (cm^2)		
Proventriculus	40.4 ± 4.34	292 ± 44.9
Gizzard	44.3 ± 8.56	28.8 ± 5.34
Small intestine	297 ± 48.2[a]	254 ± 18.1[a]
Duodenum	120 ± 21.9	72.6 ± 7.54
Ileum	178 ± 29.3[a]	182 ± 19.1[a]
Colon	11.7 ± 1.74	9.6 ± 1.59

[a] Not significantly different at the 0.05 level.

residual stomach oils from the proventriculus. All subjects were weighed to the nearest 0.01 kg using a Pesola spring scale. We plucked the down feathers around the cloaca and glued a plastic cylinder around the cloaca of each subject with superglue. A plastic bag was taped to the cylinder to collect excreta and additional tape was used to help secure the cylinder and collection bag to the chick. Changing to a new collection bag was accomplished by removal of the tape from the plastic cylinder and retaping a new bag onto the cylinder.

Meal composition and feeding studies.—We used stabilized triglyceride emulsion (Sigma Lipase Substrate, No. 800-1) as a carrier to assure consistent ratios of the two markers in meals fed to subjects in Antarctica. Prior to departure for Antarctica, we added 10 μCi of [^{3}H-GTE), 2.5 μCi of [^{14}C-PEG], and 10 mg of PEG-4000 to each ml of the emulsion, mixed for 10 min on a vortex agitator, and counted aliquots in a liquid scintillation counter. Upon return to the U.S., additional aliquots were counted. The specific activity (11.4 ± 0.22 μCi/ml [^{3}H-GTE] and 2.91 ± 0.028 μCi/ml [^{14}C-PEG]) of the two markers and their isotopic ratio (3.92 ± 0.069, $n = 10$) were identical before and after the experiments were conducted.

Efforts to induce adult Gentoo Penguins to regurgitate chick meals were unsuccessful. Consequently, locally available canned fish ("caballa," presumably *Scomber japonicus peruvianus*) was used to prepare the labeled meal. Composition of the canned fish was analyzed later in the laboratory in air-dried samples at 60°C (constant mass) and by determining total lipids in separate samples (Bligh and Dyer 1959). The composition of the fish was 24.9% water (SD = 1.78, $n = 3$) and 3.7% lipid (SD = 0.04, $n = 3$). Canned fish, vegetable oil, and fresh water were thoroughly mixed in a 16:3:3 ratio (w:v:v) which yielded a 40% water and 13% lipid paste.

We warmed the fish paste to ca. 40°C and loaded ca. 30 ml into a 60-cm^3 disposable syringe. With a 1-cm^3 tuberculin syringe, we added 0.5 ml of the radiolabel-carrier mixture to the fish paste and filled the remainder of the 60-cm^3 syringe with fish paste. A single labeled meal was fed to subjects by forcing the paste through a 20-cm length of polyethylene tubing inserted into the esophagous. All chicks took the feeding without any regurgitation. After ingestion, each chick was placed in an outdoor, covered pen. At selected times post-ingestion chicks were quickly and humanely killed by stunning. Two giant-petrels and two penguins were killed at each of three trial periods: 4, 12, and 24 h post-ingestion. Subjects in the 24-h trials had their excreta collection bags replaced at either 10 h (penguins) or 12 h (petrels) post-ingestion.

Immediately after death, we plugged the esophagus of each subject with cotton to prevent regurgitation of the radiolabel. Excreta collection bags were removed and placed in double plastic bags. Abdominal and thoracic cavities were opened, photographed, and the entire gastrointestinal tract removed, measured, and photographed. Each tract was divided into eight parts: proventriculus, gizzard, anterior duodenum, posterior duodenum, anterior ileum, medial ileum, posterior ileum, and colon. The transition from duodenum to ileum was not externally apparent, and was separated arbitrarily at the splenic attachment. The duodenum was then divided at the midpoint and the ileum was separated into three equal segments. For the purposes of this study, the ileum was considered to be that portion of the intestine between the splenic attachment and the paired ceca. Each part of the gastrointestinal tract was opened lengthwise and the contents scraped into separate plastic bags. (In addition to digesta, scraping removes some intestinal epithelium). All samples were kept chilled in a snowbank for a maximum of 48 h before being transported to freezer facilities on King George Island. Samples were kept frozen during shipping to the United States, where they were stored at −70°C until analyzed.

Measurements of gastrointestinal tracts.—Each part of the gastrointestinal tract was thawed, weighed to the nearest 0.1 g, and measured to the nearest mm. We considered the proventriculus to be only that portion of the gastric region covered with secretory cells. The gizzard was considered the part of the gastric region

between the proventriculus and the pylorus. Prior to weighing, we removed the mucus lining of the gizzard. For both proventriculus and gizzard, maximal length and width (opened) were used to estimate area. Length of each intestinal part was measured with the segment fully extended but not stretched. Width of each intestinal part was measured on the opened segment at the midpoint. Area was estimated from the product of length and width.

Marker recovery and distribution.—We weighed each sample of digesta to the nearest 0.1 g and homogenized with a Brinkmann Polytron homogenizer with PTA 10TS generator until a uniform emulsion was obtained. Samples were diluted with deionized water to specified volumes and aliquots removed immediately for scintillation counting. We measured amounts of the two radiolabels in digesta from each gastrointestinal segment of each subject. Accumulated excreta in each collection bag was diluted with deionized water and homogenized as described for the digesta. Data were expressed as cummulative percentages per hour of the total amount of marker recovered. The mean retention time (t) was calculated from the equation:

$$t = \sum_{i=1}^{n} x_i t_i / \sum_{i=1}^{n} x_i, \quad (1)$$

where x_i is the amount of marker excreted at time t_i (Warner 1981). Food retention time was also estimated using the logistic function (Patton and Krause 1972):

$$\eta_t = \frac{\text{Total \% marker recovered}}{1 + \exp[-R(t - \tau)/25]}, \quad (2)$$

where η_t is the percentage of marker excreted from initial administration to time t, R is the maximum rate of passage of the marker, and τ is the time required for one-half of the marker to be excreted (mean retention time). In the original equation of Patton and Krause (1972), the percentage of recovered marker (Total % marker recovered) was set to 100. We did not impose such a restriction to fit curves. Estimates for R, τ, and Total % marker recovered were obtained by a nonlinear least squares iterative procedure (modified Gauss-Newton method; Johnson et al. 1981). Gastric emptying was modeled from the exponential function (Stubbs 1977, Smith et al. 1984):

$$V_t = V_i(e^{-t \cdot b}) \quad (3)$$

where V_t is the gastric volume at time t, V_i the initial volume fed, and b the exponential rate of emptying. We used the quantity (in μCi) of the two markers at each time interval sampled to estimate the meal volume present in the stomach at time t.

Statistics.—Results are expressed as the sample mean $\pm$ the standard deviation; n represents the number of measurements. Comparisons involving percentages were performed on arcsin transformed data. Differences were considered significant when $P < 0.05$, except for comparisons involving ratios of disintegrations per minute (DPM) of two markers; in this case, $P < 0.001$ was chosen. In all cases, ratios were calculated from a minimum of 1,000 DPM per sample for either isotope. Fitted curves were calculated by the modified Gauss-Newton method (Johnson et al. 1981). Other statistical procedures used are identifed in the text.

RESULTS

Marker recovery and phase specificity.—Recovery of the two markers, as a percentage of ingested marker, was statistically indistinguishable between species. Recovery of the aqueous marker, ^{14}C-PEG, was 91.2% (SD = 18.9%, n = 6) in the petrel chicks and 86.6% (SD = 18.9%, n = 6) in the penguin chicks. Recovery of the lipid marker, ^{3}H-GTE, was 78.5% (SD = 17.3%, n = 6) in the petrels and 70.6% (SD = 11.1%, n = 6) in the penguins. A paired comparison of marker recoveries within each individual indicated that recovery of aqueous marker (88.9%, SD = 18.2%, n = 12) was significantly greater than recovery of lipid marker (75.3%, SD = 14.0%, $n = 12$, $t_{11} = 3.023$, $P = 0.017$). Water used to extract digesta lowered recovery of the lipid marker because it was water insoluble and prone to adsorption by surfaces (glassware, digesta, intestinal lining, etc.).

To test the phase specificity of each marker, several samples of homogenized digesta and excreta were subjected to the Bligh and Dyer (1959) extraction method. Ninety-six percent (SD = 5.3%, n = 8) of ^{14}C-PEG radioactivity was recovered in the aqueous phase while 95% (SD = 2.5%, n = 8) of the ^{3}H-GTE radioactivity was recovered in the lower chloroform layer. Thin layer chromatography of both excreted markers indicated that neither had been substantially metabolized (i.e. radiopurity was at least 95% of that prior to ingestion).

Gastrointestinal transit of lipid and aqueous components.—Ratio of lipid marker to aqueous marker (^{3}H-DPM : ^{14}C-DPM) was used as an indicator of differential transit of lipid and aqueous components in the gastrointestinal tract. Recovered amounts of lipid-phase and aqueous-phase markers and the ratio of specific activity of the markers are shown in Table 2. At 4 h post-ingestion, >80% of either marker was still found in petrel chick stomachs. In contrast,

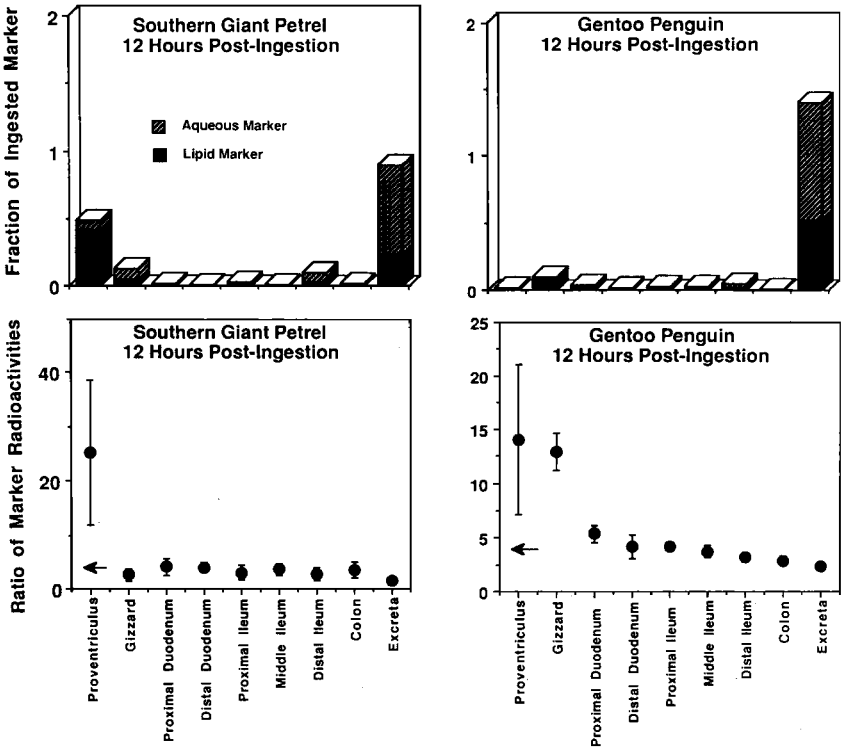

Fig. 1. Distribution of lipid-phase and aqueous-phase markers along the gastrointestinal tract 12 h after feeding Antarctic Giant-Petrel and Gentoo Penguin chicks. The top figures represent the fraction of ingested marker recovered from each segment of the gastrointestinal tract. The lower figures represent the ratio of the lipid-phase marker to the aqueous-phase marker in each segment. The aqueous-phase marker ([1-^{14}C] PEG) was recovered at 96.3% (SD = 8.3%; n = 4) and the lipid-phase marker ([^{3}H]-GTE) at 76.4% (SD = 10.6%, n = 4). The arrow indicates the ratio at the time of ingestion.

much of the meal fed to the penguin chicks had already emptied into the intestine. Distribution of the two markers along the gastrointestinal tract at 12 h post-ingestion is shown in Fig. 1. In petrel chicks >45% of the lipid marker was recovered from stomach oils removed from the proventriculus (Fig. 1). In penguin chicks <5% of the lipid marker was recovered from the proventriculus (Fig. 1). In both petrel and penguin chicks, ratios of lipid- to aqueous-phase markers in excreta were significantly lower than the ratio in the meal ($P < 0.001$), even if corrected for nonquantitative recovery of the two markers. This reflects the shorter transit time for aqueous components compared with lipids in both species. The decrease in the ratio of the two markers was gradual from the proventriculus to the colon in both species (Fig. 1). After 24 h, only 46.4% of the lipid marker was recovered in petrel excreta and the ratio of the markers was 2.4. Penguin chicks had excreted most of both lipid-phase and aqueous-phase markers and the ratio of the two markers was not significantly different from the ratio in the meal (3.6 ± 0.4 vs. 3.9 ± 0.07).

Gastric emptying.—We plotted the fraction of recovered marker found in the stomach (proventriculus and gizzard combined) over time (Fig. 2). In a pairwise comparison within each bird, significantly more lipid-phase than aqueous-phase marker was found in the stomach, which indicates slower gastric emptying of lipids. In petrel chicks, aqueous-phase marker emptied at 11.3%·h^{-1} while lipid-phase marker emptied at 5.5%·h^{-1}. The half-time ($T_{½}$) for aqueous component emptying from the stomach was 6.1 h while the $T_{½}$ for lipid emptying was 12.5 h. In penguin chicks, the aqueous marker emptied at 56.1%·h^{-1} while the lipid marker emptied at 25.4%·h^{-1}. This is equivalent

TABLE 2. Gastrointestinal transit of lipid-phase and aqueous-phase markers in 4-week-old Antarctic Giant-Petrel and Gentoo Penguin chicks. The percentage of marker recovered in stomach vs. excreta at 4, 12 and 24 h post-ingestion are presented (standard deviation in parentheses).

	Antarctic Giant-Petrel (n = 6)		Gentoo Penguin (n = 6)	
4 h				
Proventriculus & gizzard				
Lipid	80.2%	(12.0%)	32.2%	(20.2%)
Aqueous	80.7%	(22.2%)	10.5%	(6.8%)
Ratio[a]	3.99	(0.52)	9.52	(4.65)
Excreta				
Lipid	0.4%	(0.1%)	8.4%	(8.2%)
Aqueous	0.5%	(0.1%)	13.9%	(16.1%)
Ratio[a]	2.11	(1.05)	12.2	(0.78)
12 h				
Proventriculus & gizzard				
Lipid	56.5%	(3.3%)	2.7%	(0.9%)
Aqueous	16.7%	(2.2%)	11.4%	(10.3%)
Ratio[a]	25.7	(13.3)	14.1	(6.96)
Excreta				
Lipid	24.1%	(3.9%)	51.9%	(5.4%)
Aqueous	65.8%	(1.3%)	88.0%	(1.9%)
Ratio[a]	1.43	(0.22)	2.30	(0.20)
24 h				
Proventriculus & gizzard				
Lipid	25.3%	(0.7%)	6.0%	(5.9%)
Aqueous	6.5%	(5.8%)	5.3%	(4.1%)
Ratio[a]	64.2	(48.0)	5.0	(1.2)
Excreta				
Lipid	46.4%	(1.5%)	87.4%	(9.7%)
Aqueous	78.3%	(4.6%)	88.9%	(7.6%)
Ratio[a]	2.4	(0.27)	3.60	(0.38)

[a] Represents the ratio of lipid-phase marker specific activity to aqueous-phase marker specific activity.

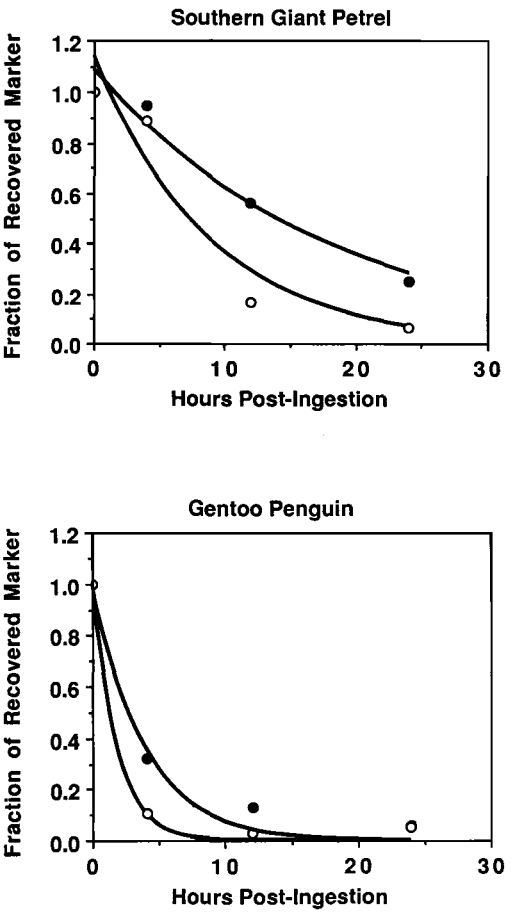

Fig. 2. Gastric emptying of the aqueous-phase marker [1-^{14}C] PEG and the lipid-phase marker [^{3}H]-GTE by Antarctic Giant Petrel and Gentoo Penguin chicks. The lines represent the fitted exponential function (Eq. 3) best describing the rate of gastric emptying.

to a $T_{½}$ of 1.2 h for aqueous components and a $T_{½}$ of 2.7 h for lipid components.

Cumulative excretion.—Cumulative excretion data for the two markers were compared using a pairwise comparison within each bird. Aqueous-phase marker was excreted at a significantly greater rate than lipid-phase marker (Fig. 3). In petrel chicks (Fig. 3), mean retention time for aqueous components was 10.7 h with a maximum excretion rate of 18.5%·h^{-1}. The fitted logistic curve for lipid excretion was suspect because only 60% of lipid marker was excreted by 24 h and there was no accurate estimate of the asymptote. Assuming nearly complete excretion of marker by 196 h (i.e. 100% recovery of marker), estimated mean retention time would be 19.5 h. Similar estimates (ca. 20 h) were obtained by linear interpolation between 12 h and 24 h data points and by using Equation 1 to estimate mean retention time. Mean retenton time (τ) for aqueous-phase and lipid-phase markers in the gastrointestinal tract of penguin chicks (Fig. 3) was 7.6 h and 8.9 h, respectively. Maximum rates of excretion (R) of the two markers were statistically indistinguishable (12.9%·h^{-1} and 12.2%·h^{-1}, respectively).

Results for cumulative excretion are consistent with findings for gastric emptying. First, aqueous components were excreted at a higher

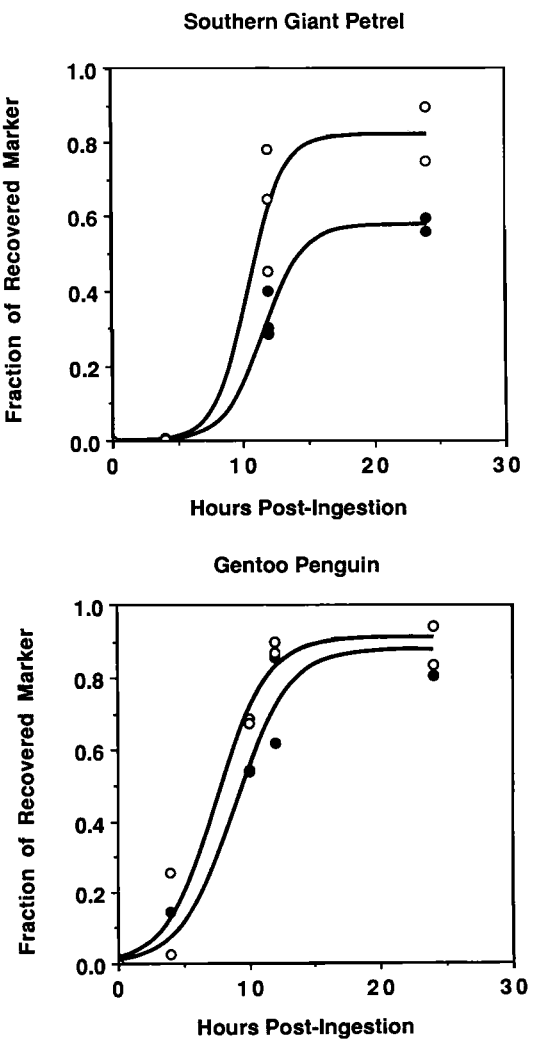

Fig. 3. Excretion of the aqueous-phase marker [1-^{14}C] PEG and the lipid-phase marker [^{3}H]-GTE by six Antarctic Giant Petrel and six Gentoo Penguin chicks. The lines drawn represent the fitted logistic function (Eq. 2) best describing the rate of gastrointestinal emptying.

rate than lipid components in both species and, second, excretion of both components, especially lipids, was slower in petrels than in penguins.

Gastrointestinal gross anatomy.—The proventriculus was the most striking difference between the gastrointestinal tracts of the two species. In petrel chicks, the proventriculus was very large, filled most of the abdominal cavity, extended posterior to the cloaca, and partly encircled the gizzard (Fig. 4A). In penguin chicks the proventriculus was much smaller; similar in size and mass to the gizzard (Fig. 4B). Petrel proventriculus walls were thin (ca. 0.5 mm); in penguins they were thick (ca. 5 mm) and muscular. Average mass of the petrel proventriculus was 35% greater than that of penguins and average surface area of the petrel proventriculus was more than 7 times that of penguins.

In contrast to the proventriculus, gizzards of the two species were similar in size (Table 1). Gross anatomy of the gizzard was similar to that of other avian carnivores, such as raptors (Duke 1985) and herons (Rhoades and Duke 1975), and lacked the opposing pairs of thin and thick muscles characteristic of fowl and most other avian species (Duke 1986a). Most of the penguin gizzards were filled with stones whereas most of the petrel gizzards were filled with penguin feathers. In petrels the pyloric valve was ventral to both the gizzard and proventriculus, while in penguins it was dorsal (Fig. 4). Neither species had a well-developed pyloric sphincter, but in penguins there was a glotus-like projection over the pylorus.

The length of the small intestine was similar in the two species but, due to a thicker wall, the average mass of penguin intestines was more than twice that of petrels (Table 1). Small intestine volume was ca. 50% greater in penguins due to a larger duodenum (Table 1). The penguin duodenum was coiled tightly around a large pancreas (ca. 13 g), while the petrel duodenum was looped around a smaller pancreas (ca. 7 g, Fig. 4). The colon was slightly larger in the penguin chicks, while the ceca of both species were small and apparently nonfunctional.

Discussion

The avian proventriculus and gizzard play a major role in chemical and mechanical digestion of solid food. Consequently, gastric emptying is a major component of gastrointestinal passage time. In three domestic species (goose, turkey, and chicken), 48.5% ± 24.5% (n = 6) of the total mean residence time of a meal (7.1 ± 2.25 h) involved gastric emptying (Warner 1981). The data from seabirds are limited, but in Jackass Penguins (*Spheniscus demersus*) fed fish, 23% of the mean residence time of 11 h involved gastric emptying (Wilson et al. 1985, Laugksch

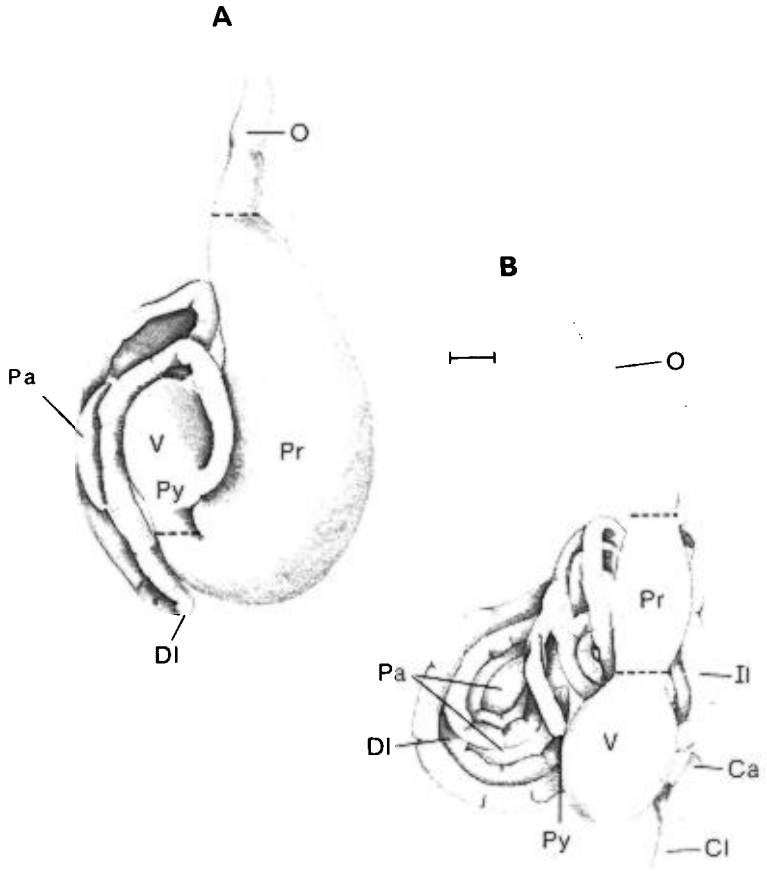

Fig. 4. Ventral views of the gastrointestinal tract of 4-week-old Antarctic Giant Petrels (A) and Gentoo Penguins (B). The sternum, abdominal wall, and liver have been removed. The marker is 1 cm. O = esophagus; Pr = proventriculus; V = gizzard; Py = pylorus; Dl = duodenal loop; Pa = pancreas; Il = ileum; Ca = ceca; Cl = colon.

and Duffy 1986). Fish meals are known to empty the stomach more rapidly than meals of squid or krill (Laugksch and Duffy 1986).

We have shown in birds that aqueous components of a meal are emptied from the stomach at a higher rate than lipids. This differential emptying occurs in both species despite striking differences in digestive anatomy. Petrels exhibit a low overall passage rate; gastric emptying comprised 57.8% of the 10.6 h mean transit time for aqueous components and 62.5% of the 20 h mean transit time for lipids. In penguins only 16% of the 7.5 h mean transit time for aqueous components and 30.3% of the 8.9 h mean transit time for lipids was a function of gastric emptying. In general, it appears that food transit time in seabirds (Jackass Penguins and Cape Gannets [*Morus capensis*]) is slower than in other birds (Laugksch and Duffy 1986). A specialized gastrointestinal anatomy is associated with the petrel's low gastric emptying rate. The petrel proventriculus is relatively large, and entire meals reside in the proventriculus for extended periods, as reflected in the low rates of gastric emptying. The functon of the proventriculus in procellariiforms (i.e. thorough chemical digestion of ingesta) is unique among birds. In other species, including penguins, food passes rap-

idly through the proventriculus and chemical, as well as mechanical, digestion occurs primarily in the gizzard (Duke 1986b).

The pattern of gastric motility in procellariiforms is also unique among birds. The proventriculus is relatively inactive during a digestive contraction cycle in Leach's Storm-Petrel (*Oceanodroma leucorhoa*) chicks (Duke et al. 1989). Proventricular contractions are observed only along the ventral surface (Duke et al. 1989). This is in contrast to the vigorous, coordinated muscle activity observed between the gizzard and proventriculus in fowl (Dziuk and Duke 1972, Duke 1982). The inactivity of the procellariiform proventriculus allows gastric lipids and aqueous components to form and remain in separate phases. The denser aqueous digesta accumulates in the ventral portions of the proventriculus and in the gizzard. The pylorus is ventral to the gizzard (and proventriculus) in the petrels and, consequently, aqueous digesta enters the duodenum first while lipid is retained in the stomach. Low gastric motility, slow gastric emptying, and the position of the pylorus relative to the proventriculus result in stomach function analogous to a separatory funnel.

In penguins, like most birds, the proventriculus is neither large nor distensible and is cranial to the gizzard. Food items in the distensible crop are subjected to acidic proteolysis sequentially as they enter the small proventriculus. The relatively high gastric emptying rate removes liquid digesta from the stomach before separation into a biphasic system can occur. The pylorus is situated dorsal to the gizzard, so if phase separation occurred, lipids would tend to pass through the pylorus first. The large pancreas and muscular duodenum of penguins are consistent with a rapid gastric evacuation of food.

The crucial roles of both anatomy and physiology in the formation of stomach oils are exemplified by diving petrels, the only procellariiforms which do not store stomach oils. In adult South Georgia diving petrels (*Pelecanoides georgicus*), in contrast to giant-petrel chicks, the gizzard and pylorus are situated more dorsal to the large, distensible proventriculus and would tend to empty less dense digesta first. Moreover, passage rates of digesta through the gastrointestinal tracts of diving petrels are so high as to preclude the formation of stomach oils (Roby et al. 1986a). For six common diving petrel (*P. urinatrix*) and four South Georgia diving petrel chicks, the average gastric emptying rate for lipids was $30\% \cdot h^{-1}$ (SE = $5.8\% \cdot h^{-1}$, $n = 10$) and the mean retention time for labeled lipid in the stomach was 2.3 h (SE = 0.1 h, $n = 10$). This compares with a gastric emptying rate of $5.5\% \cdot h^{-1}$ and a mean retention time of 12.5 h in the giant-petrel chicks we report. Diving petrels are an order of magnitude smaller in size than giant-petrels, so higher passage rates for dietary lipids are expected in the former. However, in Antarctic Prions (*Pachyptila desolata*), a small procellariiform similar in size to diving petrels and known to store stomach oils, the average gastric emptying rate for lipids was $4.6\% \cdot h^{-1}$ (SE = $1.1\% \cdot h^{-1}$, $n = 2$) and the mean retention time was 15.0 h (SE = 3.5 h, $n = 2$; Roby et al. 1986a). This provides strong evidence that both low passage rates and suitable gastric anatomy are necessary for stomach oil formation.

Warham (1977) speculated that the formation of stomach oil is an adaptation for exploitation of a pelagic food supply which is patchily distributed, requiring long journeys between feeding sites and necessitating extended fasting periods for both adults and chicks. Others (Ashmole 1971, Laugksch and Duffy 1986, Obst 1986) emphasized the energetic advantages to both adults and chicks of delivering chick meals which consist mostly of stomach oils, particularly with regard to the increase in potential foraging range of adults. Giant-petrel chicks, however, are fed at least as frequently as Gentoo Penguin chicks, presumably because of the proximity of penguin colonies where most chick food is obtained. In the case of giant-petrels, there seems to be little advantage either for adults to concentrate the lipid content of chick meals or for chicks to store stomach oils for long fasts between meals.

Diving-petrels are apparently unique among procellariiforms in having lost the ability to form stomach oils (Roby 1986). Diving petrels, like penguins, are pursuit-divers that exploit a more concentrated and presumably more predictable food supply. The energetically inefficient mode of flight in diving petrels suggests that they rarely, if ever, travel far to find food (Roby and Ricklefs 1986). A potential disadvantage of stomach oil formation in pursuit-divers is the requisite low passage rate of digesta and, consequently, low assimilation rate. Energy ex-

penditure rates of adult diving petrels are known to be high relative to other procellariiform seabirds and similar to penguins (Roby and Ricklefs 1986). Consequently, the ability to rapidly digest food and assimilate ingested energy would be advantageous.

For seabirds that frequently experience fasting periods, an energetic advantage can be realized by metabolizing stomach oils in preference to fat from adipose tissue. Metabolizing stomach oils precludes the energy cost of synthesizing fat depots from assimilated fatty acids and of later mobilizing those energy reserves, costs which amount to ca. 25–30% of the assimilated energy (Ricklefs 1974, Spady et al. 1976). This may be particularly significant for adults during the nonbreeding period if food supplies are patchily distributed in time and space, necessitating periodic fasts and energy conservation.

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