PHYSIOLOGICAL BASIS OF STOMACH OIL FORMATION IN LEACH'S STORM-PETREL (*OCEANODROMA LEUCORHOA*)

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ABSTRACT.—We examined the composition of stomach oils and the gastrointestinal adaptations responsible for their formation in chicks of Leach's Storm-Petrel, *Oceanodroma leucorhoa*. We used tritium-labeled glycerol triether (a nonabsorbable lipid-phase marker) and carbon-14 labeled polyethylene glycol (a nonabsorbable aqueous-phase marker) to follow the gastrointestinal transit of a homogenized meal fed to chicks. Dietary lipids remained in the gastrointestinal tract significantly longer (126 h mean retention time) than aqueous components (12 h) due largely to different rates of gastric emptying. Mean gastric retention times were 0.35 h for aqueous solutions and 70 h for neutral lipids. Stomach oils from Leach's Storm-Petrel chicks and adults consist largely (>90%) of neutral lipids (triglycerides, wax esters, and glycerol ethers). The composition is dynamic, and components are partitioned between an oil phase and aqueous phase based largely on water-lipid solubilities. The paucity of fatty acids and phospholipids in stomach oils derives from limited solubility in neutral lipids and rapid gastric emptying of polar lipid constituents. Proventricular lipolysis of neutral lipids is low (<3%) and we found no evidence of proventricular lipid secretion. During the week or two before fledging, Leach's Storm-Petrel chicks regulate the amount of stomach oil by restricting the rate of gastric lipid emptying. *Received 20 January 1989, accepted 10 June 1989.*

THE ORDER Procellariiformes is entirely pelagic. Most species feed on lipid-rich prey, and adults may commute long distances between breeding sites and feeding areas. With the exception of diving petrels (Pelecanoididae), all Procellariiformes possess stomach oils (Warham 1977, Jacob 1982). These oils are found in chicks and adults, breeders and nonbreeders, in birds captured at sea or at breeding colonies (Jacob 1982). The ability to concentrate dietary items into high-energy meals that occupy smaller volumes and have lower osmotic loads may provide an advantage for both adults and chicks. Stomach oils are important to the breeding ecology of these pelagic seabirds (Ashmole 1971). Nearly 80% of the energy delivered to chicks of Wilson's Storm-Petrel (*Oceanites oceanicus*) resides in stomach oils (Obst 1986) and 30% of the energy metabolized by incubating Leach's Storm-Petrels (*Oceanodroma leucorhoa*) resides in stomach oils (Ricklefs et al. 1986).

Cheah and Hansen (1970b) thought that oils accumulate in the stomach because lipids are digested more slowly than proteins. Warham (1977) suggested that the physical arrangement of the gastrointestinal tract in petrels slowed the drainage of oil into the intestine. The denser food and aqueous materials tend to separate as a bottom layer that blocks the access of oils to the pyloric opening. Those species that regurgitate stomach contents as a defense (e.g. fulmars) eject the lighter oil first, and they eject solid or partly digested material only after the oil supply is exhausted. Recent studies on gastric motility in Leach's Storm-Petrel chicks (Duke et al. 1989) and on gastrointestinal passage of dietary lipids and aqueous components in Gentoo Penguins (*Pygoscelis papua*) and southern Giant Petrels (*Macronectes giganteus*) (Roby et al. 1989) established that aqueous dietary components empty rapidly from the proventriculus of procellariiforms whereas dietary neutral lipids are retained. Moreover, mean retention times in petrels for lipid phase (20 h) and for aqueous phase (10.7 h) were significantly longer than in penguins (8.9 h for lipid phase, and 7.6 h for aqueous phase) (Roby et al. 1989).

We describe the mechanisms and kinetics of stomach oil formation in a representative pro-

cellariiform, Leach's Storm-Petrel. We relate the mechanics of stomach oil formation to the process by which procellariiforms are able to use the dietary lipids. We show how birds concentrate food into a store, the composition of which is determined largely by properties of the meals, the lipids, and a specialized gastrointestinal morphology.

MATERIALS AND METHODS

Radiolabels and fluors.—Tri[1-^{14}C]olein (122 mCi/mmol), L-[^{14}C(U)]proline (266.4 mCi/mmol), [1-^{14}C] L-α-dioleoyl, [dioleoyl-1-^{14}C]-phosphatidylcholine (114 mCi/mmol), and L-α-1-palmitoyl-2-oleoyl-[oleoyl-1-^{14}C]-phosphatidylcholine (52.6 mCi/mmol) from New England Nuclear (Boston, Massachusetts), [1-^{14}C] cetyl alcohol (24 mCi/mmol), D-[^{14}C(U)] glucose (4.28 mCi/mmol), n-[1-^{14}C]-hexadecane (61 mCi/mmol) from Amersham (Arlington Heights, Illinois), [1-^{14}C] stearic acid (52 mCi/mmol) from Research Products International (Mount Prospect, Illinois) were found to have radiopurities >98% by thin layer chromatography. We used [1,2-^{3}H] polyethylene glycol (M.W. 4000, 1.4 mCi/mmol) from New England Nuclear (Boston, Massachusetts) and [U-^{14}C] polyethylene glycol (M.W. 4000, 15 mCi/g) from Amersham (Arlington Heights, Illinois) without further purification. Scintillation fluors were ACS II (Amersham, Arlington Heights, Illinois) and Biosafe II (Research Products International, Mount Prospect, Illinois). Duplicate samples were counted twice on a Beckman LS 3801 scintillation counter, and a quench correction curve was derived for different extracts and tissue types using an H number calibration. The counting efficiency for ^{14}C in the samples varied from 88.0% to 75.4%, and that for ^{3}H from 24.0% to 11.0%. The coefficient of variation for replicate samples averaged 3.0 ± 1.3% (±SD) for tritium and 1.9 ± 0.9% for carbon-14. All radioactivities are expressed in μCi (1.00 microCurie = 37.0 kilobequerals).

Tracer and carrier wax ester synthesis.—Labeled wax ester ([1-^{14}C] cetyl oleate) and carrier cetyl oleate was synthesized (Place and Roby 1986). The radio purity (2.2 mCi/mmol) was 98.7%, the overall yield ranged from 45% to 75%. The chemical purity was >98%. Glycerol triether [1-(9 *cis*-octadecenyl) 2,3 didodecyl glycerol triether] was synthesized as described (Morgan and Hofmann 1970) by BACHEM. The tritiated triether (^{3}H-GTE) was prepared by reduction with platinum as a catalyst (New England Nuclear, Boston, Massachusetts) and purified (Roby et al. 1989). Carrier triacylglycerol was purified from olive oil (Place and Roby 1986).

Study areas.—Laboratory experiments were conducted in 1986 and 1987 at the Mt. Desert Island Marine Biological Laboratory on Mt. Desert Island, Maine. Storm-petrel chicks were obtained from a nesting colony on nearby Little Duck Island. Depending on the experiment, chicks were either taken to the laboratory for feeding experiments and then returned at the experiment's conclusion, or they were fed labeled meals in the field and then replaced in their nest burrows (where they remained). All the chicks from Little Duck Island were of known age. Other studies were conducted in 1986 at the Bowdoin Scientific Station on Kent Island, New Brunswick, just south of Grand Manan Island at the mouth of the Bay of Fundy. Chicks were removed from burrows and kept in a laboratory on the island until the conclusion of the study. The ages of Kent Island chicks were estimated from measurements of wing cord (Ricklefs et al. 1985).

Diet of captive subjects.—During laboratory experiments, chicks were fed freeze-dried krill (*Euphausia superba*) and freeze-dried copepods (predominantly *Limnocalanus macrurus*, 87% of biomass). A 20% (w/v) homogenate of either of these items was made with water and supplemented with either 35% (w/w) olive oil (triglyceride supplement) or 35% (w/w) cetyl oleate (wax supplement). Nightly meals of 5–8 cc (ca. 88–141 kJ/meal) were delivered with a disposable 5-ml syringe attached to a 10-cm length of polyethylene tubing inserted through the esophagus into the stomach. All chicks accepted meals without regurgitation and exhibited normal development, based on daily measurements of wing growth.

Monitoring aqueous-lipid phase partitioning.—Our basic strategy was to monitor the movement of carbon-14 labeled dietary constituents in relation to tritium labeled aqueous-phase and lipid-phase markers. In such studies it is important that each marker have similar properties to components of the phase under study (Wiggins and Dawson 1961; Carlson and Bayley 1972a, b). Polyethylene glycol (M.W. 4000) is a widely accepted aqueous-phase marker (Wingate et al. 1972) and glycerol triether is a suitable neutral lipid-phase marker (Morgan and Hofmann 1970; Carlson and Bayley 1972a, b; Meyer et al. 1986; Roby et al. 1989). These compounds are nonabsorbable, nontoxic, nondegradable by digestive or bacterial enzymes, and they do not influence the normal absorption of dietary aqueous nutrients or fat.

To test the phase specificity of each marker, several samples of stomach oil and excreta were subjected to Bligh and Dyer extraction (1959). Of the ^{3}H-GTE radioactivity, 93 ± 1.2% (n = 4) was recovered in the chloroform layer while 96 ± 2.5 % (n = 4) of ^{14}C-PEG radioactivity appeared in the aqueous phase. Thin layer chromatography of both excreted markers indicated that neither had been substantially metabolized (i.e. radiopurity of each was at least 95% of that prior to ingestion).

Sampling proventriculus contents.—We obtained stomach samples with a 5-ml syringe used to gently suck contents through a 10-cm length of polyethylene tubing from the esophagus into the proventriculus (Grubb 1971). Stomach oils were separated from the

aqueous and particulate phases by centrifugation at 10,000 × *g* for 10 min.

Gastrointestinal transit times for lipid and aqueous components.—Four storm-petrel chicks (age = 33–37 days, mass = 58 ± 4.98 g) were fed a radioactively tagged, homogenized meal (2 g) of calanoid copepods. We added the nonabsorbable water soluble marker [1-^{14}C] polyethylene glycol (M.W. 4000, PEG) and 50 mg/ml of unlabeled PEG to mark the aqueous phase (1.3 μCi/meal). To label the lipid phase, we added (3.7 μCi/meal) [^{3}H]-labeled glycerol triether (1-octadecyl-2,3-diodecyl glycerol, GTE). The ratio of [^{3}H] μCi/^{14}C μCi of the food was 2.83 ± 0.08 (n = 4). The meals were delivered by tube and syringe. After feeding, each chick was placed on a polyethylene mesh platform suspended in a 2-gal polyethylene container to collect excreta. Containers were kept in the dark and maintained at 14 ± 3°C which simulated the nest environment. Chicks were periodically transferred to clean containers, and the excreta was collected. Unlabeled meals (5–8 g) were fed nightly to chicks. Accumulated excreta in each container were extracted with 50 ml of chloroform : methanol (2:1) and filtered on glass fiber filters to obtain a single phase extract. Aliquots (5 ml) were placed in scintillation vials and the solvent was removed under nitrogen evaporation. Fluor was added and the vials counted. Food retention time was estimated using the exponential function:

$$n_t = b_0 - e^{[-b_1 \times (t - b_2)]} \quad (1)$$

where n_t is the proportion of marker excreted from initial administration to time t (Hove 1984). The parameter b_0 is the asymptotic recovery of marker, b_1 is the rate of excretion (per hour), and b_2 is the delay in hours before marker is recovered. The total mean retention time (TMRT) is the sum of b_2 and the reciprocal of b_1. Estimates and 95% confidence intervals for the three parameters were obtained by nonlinear least squares regression weighted by the variance of replicates (Johnson et al. 1981).

Aqueous and lipid emptying rates of the proventriculus.—To calculate the contribution of proventriculus emptying time to transit time, we used a double-isotope dilution technique (Diamond et al. 1986). To measure aqueous emptying rates, 8 chicks (age = 64.1 ± 5.0 days, mass = 71.3 ± 10.0 g) were first fed 5 ml of an isotonic (300 mOsm/l, according to Schmidt-Nielsen [1960]) glucose/water solution labeled with^{14}C-polyethylene glycol (50 mg/ml PEG; 0.017 μCi/ml). After 5 min (t = 0), an aliquot was removed and dilution of label determined. After 1, 2, 4, or 8 h, we fed 2 ml of an isotonic glucose/water solution labeled with a second marker, ^{3}H-polyethylene glycol (50 mg/ml PEG; 0.071 μCi/ml). Again, 5 min after each feeding, an aliquot was removed and dilution of the label determined. To measure the emptying rate of lipid components in the proventriculus, 6 chicks (age = 48.5 ± 4.6 days, mass = 76.3 ± 9.65 g) were fed 3 ml of a 1:1 (v/v) mixture of purified olive oil and cetyl oleate, with glycerol tri [9,10 (n)-^{3}H]oleate (12 μCi/ml) added. Aliquots were taken after 15 min, and the chicks were returned to their burrows with the entrances blocked to prevent adults from returning with food. After 24 h, the chicks were fed 3 ml of 1:1 (v/v) purified olive oil and cetyl oleate with labeled glycerol tri [1-^{14}C]oleate (0.3 μCi/ml) added. Samples were removed after 15 min. We used labeled triglycerides to estimate lipid volume because little (<1.0%) lipolysis occurs in the proventriculus for up to 36 h (Place and Roby 1986).

We calculated the apparent volume of aqueous or lipid components of the proventriculus, (V) at time t from marker dilution by the expression $(V_s C_i / C_s) - V_i$, where V_s is the analyzed sample volume, V_i is the volume of the marker fed, C_s is disintegrations/min (DPM) in the sample volume, and C_i is the DPM in the fed solution (Diamond et al. 1986). Gastric emptying was modeled using the exponential function (Stubbs 1977, Smith et al. 1984),

$$V_t = V_i \times e^{-(t \times b)} \quad (2)$$

where V_t is the gastric volume at time t, V_i is the initial volume fed, and b is the exponential rate of emptying.

Gastric lipolysis of stomach oils.—Six chicks from Kent Island (age = 68.6 ± 3.14 days, mass = 73.5 ± 12.5 g) and 4 chicks from Little Duck Island (age = 58 ± 3.5 days, mass = 64.3 ± 2.5 g) were fed 2 g of lipid containing 12.5 μCi of [^{3}H-GTE]-marker and 5 μCi of [^{14}C]-lipid. The carbon-14 labeled lipids were either glycerol tri [1-^{14}C]oleate in olive oil or [1-^{14}C]cetyl oleate in cetyl oleate. After 15 min, a sample of stomach oil was taken and the excreta collected. An additional stomach oil sample was taken 24 h after feeding. Aliquots of stomach oils were counted for dual label radioactivity as described above.

As a more sensitive test of differential lipolysis of waxes *and* triglycerides, 6 chicks on Little Duck Island (age = 42.3 ± 1.37 days, mass = 66 ± 9.25 g) were fed 3 ml of 1:1 (v/v) mixture of purified olive oil and cetyl oleate with glycerol tri [9,10 (n)-^{3}H]oleate (7.7 μCi/ml) and [1-^{14}C]cetyl oleate (0.14 μCi/ml) added. Samples were taken after 15 min, and birds were returned to their burrows. The chicks were removed at 4, 24, and 48 h after feeding for further sampling of stomach oils. The chicks were allowed to receive meals from their parents. Aliquots were analyzed for radioactivity and chemical composition.

Stomach oil composition.—Samples were taken from 12 Kent Island chicks (age = 66.2 ± 3.52 days, mass = 62.0 ± 5.75 g) that had been removed from their burrows and kept in the laboratory for 24 h. We withdrew stomach oil from these birds 5 min after they were fed labeled glucose or proline, and then again after 24 h. We also took samples from randomly selected chicks (age = 50.3 ± 1.42 days, mass = 80.3 ±

7.20 g) on Little Duck Island for stomach oil composition analysis.

Field observations indicated that chicks retain significant (5–8 ml) quantities of stomach oil during prefledging weight loss. An experiment was performed with 6 chicks (age = 62 ± 0.95 days, mass = 66 ± 2.44 g, wing cord = 158 ± 1.7 mm) to determine whether stomach oil composition and volume might be regulated in older chicks. The 6 chicks were fed a meal of 20% (w/w) homogenized krill containing 35% (w/w) of a 1:1 mixture of cetyl oleate and olive oil. [^{3}H]-GTE (0.48 μCi/g) and tri [1-^{14}C]oleate (0.16 μCi/g). Stomach oil was sampled 15 min later. Chicks were placed in polyethylene containers to collect excreta. On the subsequent 6 nights, the chicks were fed 3 g of unlabeled homogenized krill containing 35% (w/w) olive oil. Before feeding, we took stomach oil samples for lipid and radioactivity analysis, weighed the chicks, and measured wing cords. An additional experiment was conducted with 7 chicks that would have fledged if not in captivity.

Polar and nonpolar lipid repartitioning.—Labeled phospholipids, L-α-12-palmitoyl-2-oleoyl-[oleoyl-1-^{14}C] phosphatidylcholine and L-α-dioleoyl, [dioleoyl-1-^{14}C] phosphatidylcholine, were used in two separate experiments on phospholipid gastric emptying. We added 5 μCi of labeled phospholipid and 50 μCi ^{3}H-GTE to 49.5 g of homogenized copepods. We used 6 birds (age = 42.2 ± 1.47 days, mass = 76.7 ± 14.18 g) for each experiment; and we fed each bird a 5-cc meal. We withdrew samples 5 min after the meal was administered, and 1, 3, 5, 24, and 48 h thereafter. The 5-min sample provided an initial ratio of the labels for comparison with subsequent ratios. Because endogenous phospholipase activity was high in the homogenized copepod meal, we repeated the experiment using the same labels added to pure olive oil. In these experiments, the ratio of labeled phosphatidylcholine to glycerol triether was 5:1. Each chick (age = 45 ± 3.95 days, mass = 80.2 ± 12.3 g) was fed 3 ml of labeled lipid, and proventricular samples were taken at 1, 2, and 3 h.

Six chicks (age = 45.5 ± 2.5 days, mass = 77.2 ± 4.9 g) were fed 5 ml of a homogenized copepod meal containing [1-^{14}C] stearic acid (0.31 μCi/ml) and ^{3}H-GTE (1.85 μCi/ml) to determine fatty acid emptying rates. Stomach oil samples were taken at 5 min, 2 h, 5 h, and 24 h. To test whether nonpolar lipids (i.e. alkanes) accumulate in the proventriculus, we fed 6 chicks (age = 41.3 ± 1.03 days, mass = 70.5 ± 8.12 g) 0.5 ml of a copepod meal labeled with n-[1-^{14}C] hexadecane (1.16 μCi/ml) and ^{3}H-GTE (1.74 μCi/ml), and we withdrew aliquots at 5 min, 24 h, and 48 h.

Meal and stomach oil samples were centrifuged for 10 min to separate aqueous and lipid phases before we removed aliquots for scintillation counting and lipid analysis.

Tracer recovery and distribution.—We determined label distribution among various lipid classes in stomach oils and excreta by thin layer chromatography (TLC). Aliquots containing equivalent counts were spotted on the preabsorbent area of channeled silica G plates (Uniplates, Analtech), developed with hexane : diethyl ether : acetic acid (80:20:1), and scanned with a BioScan 100 radiometric scanner. This solvent system resolves wax esters, triacylglycerols, fatty acids, fatty alcohols, 1,3-diacylglycerols, 1,2-diacylglycerols, monoacylglycerols, and complex lipids, in order of decreasing relative mobility (R_f). To separate cholesterol esters from wax esters, double development with hexane : diethyl ether (98:2) was necessary (Christie 1982). The carrier gas used in the radiometric scanning was P-10 (90% argon, 10% methane) at a flow rate 0.5–1.0 l/min. The spatial resolution for each scan was set at 4 mm. The distribution of label among lipid classes was estimated by integration of the counts under each peak after subtraction for background. The overall counting efficiency for ^{14}C averaged 10.5% whereas the counting efficiency for ^{3}H averaged 0.5% across the plate. Labeled cetyl oleate, cholesterol oleate, triolein, oleic acid, and cetyl alcohol were used as standards to determine the R_f of these major lipid classes.

Quantification of lipids using TLC-flame ionization detection.—Operating conditions for the Iatroscan TH-10 analyzer (Iatron Laboratories, Tokyo) were as described by Rigler et al. (1983). Samples were spotted on type S-II chromarods (Iatron Laboratories) which were activated by scanning through a hydrogen flame twice. Integration was performed by a Hewlett-Packard 3390-A integrator (Avondale, Pennsylvania). Chromarods were developed in equilibrated filter paper-lined glass tanks containing 75 ml of solvent (Harvey and Patton 1981, Kaitarana and Ackman 1981, Parrish and Ackman 1983). Samples were prefocused with acetone. Samples of stomach oil were filtered, centrifuged, and 10 μl was added to 500 μl of chloroform. Total lipid content was determined by developing the rods in chloroform : methanol (1:1, v/v) along with a lipid standard. The solvent front was run to 2 cm, and quantification was performed by TLC-FID. Lipid quantities were determined by comparison with a standard curve for concentrations of olive oil between 1 and 20 μg. For determination of stomach oil lipid compositions, rods were developed in one of several solvent systems. The first was 85 ml hexane : 15 ml diethyl ether : 0.1 ml formic acid for 50 min. Phospholipid composition was determined with double sequential development. Rods were first developed for 50 min in the above solvent, dried in the oven, and burned halfway. These rods were then developed a second time with 75 ml $CHCl_3$: 35 ml MeOH : 3.5 ml H_2O. Finally, samples were analyzed for glycerol ether components using a double development system. Samples were prefocused twice in acetone, and air-dried between each prefocusing. Rods were then developed in hexane : diethyl ether : formic acid (99:1:0.05) for 25 min, air-dried for 5 min,

and then redeveloped for 20 min. After drying at 100°C for 5 min, the rods were burned to a point behind the wax esters. After this partial burning, rods were dried and then developed for 40 min in hexane : diethyl ether : formic acid (80:20:0.1); they were then burned the entire length of the rod. A standard curve was generated for each major class of lipids observed (wax esters, dialkyl glycerol ethers, triglycerides, fatty acids, fatty alcohols, mono- and diglycerides, and phospholipids).

Enzymatic determination of lipid components.—Cholesterol concentrations were determined with cholesterol oxidase (Sigma Diagnostics, Procedure No. 351). Triglyceride concentrations were determined using glycerol phosphate oxidase after lipase production of free glycerol (Sigma Diagnostics, Triglyceride [GPO-Trinder] Procedure No. 338).

Statistics.—Results are expressed as means ± standard error of the mean ($\bar{x}$ ± SEM); n represents the number of measurements. Comparisons that involve percentages were performed on arcsin transformed data. Differences were considered significant when $P \leq 0.05$, except for comparisons involving ratios of disintegrations/minute (DPM) of two markers; in this case, $P \leq 0.001$ was chosen. In no case were fewer than 1,000 DPM/sample observed for either isotope. Lines were calculated by a nonlinear least squares iterative procedure (modified Gauss-Newton method) (Johnson et al. 1981). Parameter estimates are given with their 95% confidence limits in brackets. Other statistical procedures used are identified in the text.

Results

Gastrointestinal transit times for lipid and aqueous components.—The excretion curves differed for lipid and aqueous markers (Fig. 1). The aqueous marker was excreted significantly faster than the lipid marker. By 24 h, 83.7 ± 1.8% (n = 4) of the aqueous marker was recovered compared with only 11 ± 1.1% (n = 4) of lipid marker. After 4.5 days, nearly all of the aqueous marker was recovered (94.3 ± 0.9%, n = 4) while only 44 ± 2.1% (n = 4) of the lipid marker was recovered. A major portion (38 ± 2.4%, n = 4) of the unrecovered lipid marker resided in stomach oils aspirated from the proventriculus.

The fitted lines (Fig. 1) best describe the rate of gastrointestinal emptying according to Equation 1. The time for first appearance (b_2) of either marker was similar: 1.4 h [0.4, 2.5] for lipids and 0.9 h [0.7, 1.1] for aqueous components. The rate of passage (b_1) for aqueous components was 0.09 · $\cdot h^{-1}$ [0.08, 0.10] while that for lipids was 0.008 · h^{-1} [0.007, 0.009]. The total mean retention time (TMRT) was 12.0 h for aqueous components and 126 h for lipid components.

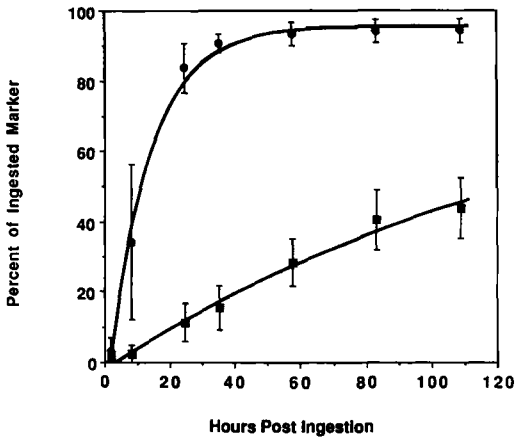


Fig. 1. Time courses of gastrointestinal emptying of aqueous (■) and lipid (●) phases in 4 young (34-day-old) Leach's Storm-Petrel chicks. The lines represent the fitted exponential function (Eq. 1) that best described the rate of gastrointestinal emptying.

When 65-day-old chicks were fed an identical meal, 86.0 ± 0.34% (n = 12) of the aqueous marker was recovered after 24 h while only 1.8 ± 0.2% (n = 12) of the lipid marker was recovered. The unexcreted lipid marker was found in stomach oils. Older chicks with substantial stomach oil (i.e. 9.1 ± 1.5 ml, n = 6) excrete significantly more lipid marker (31.4 ± 7.2%, n = 6). This higher excretion rate was accompanied by a low lipid assimilation efficiency (46.1 ± 1.88%, n = 6) as compared with 94 ± 0.35%, n = 12) (Place and Roby 1986, Place et al. 1986, Roby and Place 1986). The unassimilated lipids are excreted largely unhydrolyzed in older chicks.

Aqueous- and lipid-phase emptying rates of the proventriculus.—Initial aqueous proventricular volumes for eight 65-day-old chicks varied from 0.9–5.1 ml. After 1 h, the gastric aqueous volume decreased to 0.65 ± 0.19 ml. We assume this indicated a proventriculus empty of aqueous components. The proventriculus still contained 3.42 ± 2.09 ml of stomach oil. The average aqueous volume emptied was 6.6 ± 1.2 ml, and the mean emptying time was 0.35 h. When the volume of aqueous material in the proventriculus exceeded 8 ml, emptying was not complete by 1 hour.

Gastric emptying of lipid was significantly slower. The initial stomach oil volume was 1.9 ± 0.5 ml (n = 6). After 24 h, the volume was reduced to 1.5 ± 0.2 ml (n = 6) with a mean emptying time of nearly 70 hours.

TABLE 1. The [^{3}H]-GTE to ^{14}C-labeled lipid ratio in stomach oils from Leach's Storm-Petrel chicks 24 h after feeding.

Kent Island (n = 4)		Little Duck Island (n = 6)	
Initial ratio	Final ratio	Initial ratio	Final ratio
2.8 ± 0.03[a]	2.9 ± 0.006	2.5 ± 0.02	2.5 ± 0.07
3.1 ± 0.02[b]	3.2 ± 0.114		

[a] Wax ester supplemented diet.
[b] Triglyceride supplemented diet.

TABLE 2. Stomach oil composition (as weight percentages) of Leach's Storm-Petrel chicks from Kent Island and Little Duck Island.

Lipid	Kent Island (n = 18)	Little Duck Island (n = 12)
Wax ester	5.8 ± 0.6	28.6 ± 3.5
Triglyceride	65.6 ± 2.0	35.9 ± 3.3
Glycerol ether	0.8 ± 0.2	28.7 ± 10.6
Fatty acid	6.3 ± 0.3	1.8 ± 0.18
Cholesterol	9.5 ± 0.9	2.0 ± 0.2
Polar lipid[a]	12 ± 0.6	2.9 ± 0.43

[a] Predominantly monoglycerides.

Gastric lipolysis of stomach oils.—Because the tritium label resided in a nonmetabolizable, nonabsorbable lipid marker while the carbon-14 label resided in neutral lipid (triglyceride or wax ester), lipolysis would lead to an increased [^{3}H]/[^{14}C] ratio as neutral lipid was hydrolyzed and carbon-14 label emptied from the proventriculus. There was no significant change in the ratio after 24 h on either Kent Island or Little Duck Island (Table 1).

Similar results were recorded when chicks received meals from their parents. The volume of stomach oil after we fed chicks 3 ml of a 1:1 (w/w) mixture of olive oil and cetyl oleate was 5.9 ± 0.4 ml (n = 6). On the subsequent two nights, chicks received meals from their parents. The ratio of triglyceride to wax ester radioactivity remained constant for the first 24 h despite a nearly threefold dilution of both markers (addition of unlabeled lipid from meals fed by their parents diluted the markers). At 48 h post-ingestion, wax esters apparently were retained in stomach oils at higher levels than triglycerides. However, < 0.4% of either labeled lipid was present, and the significance of this finding is questionable. Significantly faster gastric emptying was observed in chicks fed by their parents than in those fed artificially. We attribute this to larger and fresher natural meals.

Stomach oil composition.—In stomach oil samples taken from randomly chosen chicks on Kent Island in 1986 (Table 2), the oil contained mostly triglycerides, with only a small proportion of wax esters (Fig. 2). Cholesterol concentrations, determined by TLC-FID (0.12 ± 0.008 mM, n = 14) and enzymatically (0.14 ± 0.01 mM, n = 14), were statistically indistinguishable (paired t-test, t = 0.575, df = 13, P = 0.575). Triglyceride concentrations determined by TLC-FID (0.94 ± 0.03 M, n = 10) were significantly higher (paired t-test, t = 3.96, df = 10, P = 0.0033) than those determined enzymatically (0.89 ± 0.01 M, n = 10). We believe the difference is due to glyceryl ether diesters which co-migrate with triglycerides in our solvent system and which are not assayed enzymatically. The organic phosphate content of oil indicated a phospholipid level of 0.29 ± 0.26% (n = 10). The relative proportions of each lipid component in stomach oils did not change significantly after 24 h (i.e. in a repeated measures ANOVA, the amount (μg) of each component in the 24-h sample and the initial sample was not significantly different).

The composition of stomach oil samples from chicks on Little Duck in 1986 and 1987 was different from that of samples taken from Kent Island chicks (Fig. 2). Wax ester and triglyceride content were nearly equivalent. A component with chromatographic properties similar to diacyl glyceryl ether was detected. Estimated phospholipid content was 0.21 ± 0.26% (n = 12). As with the Kent Island chicks, the relative proportions of each lipid component did not change significantly after 24 h in unfed chicks. Thus, the composition of stomach oil is stable for at least 24 h provided no dietary lipids are eaten. We believe that lipid secretion by the proventriculus, if present, is extremely low.

The composition of stomach oil changed when chicks were fed nightly meals (Fig. 3: A and B). After an initial meal of homogenized krill supplemented with a 1:1 mixture of triglyceride and wax ester (and containing labeled triglyceride and triether), we fed subsequent meals of homogenized krill supplemented with triglyceride. Each meal (53 kJ) contained ca. 1.0 g of lipid. With each meal, triglyceride content of stomach oil increased (3.68 ± 0.93%/day, R^2 = 0.246) while wax ester content decreased (8.36 ± 2.52%/day, R^2 = 0.319). The free fatty acid content remained constant while the cholester-

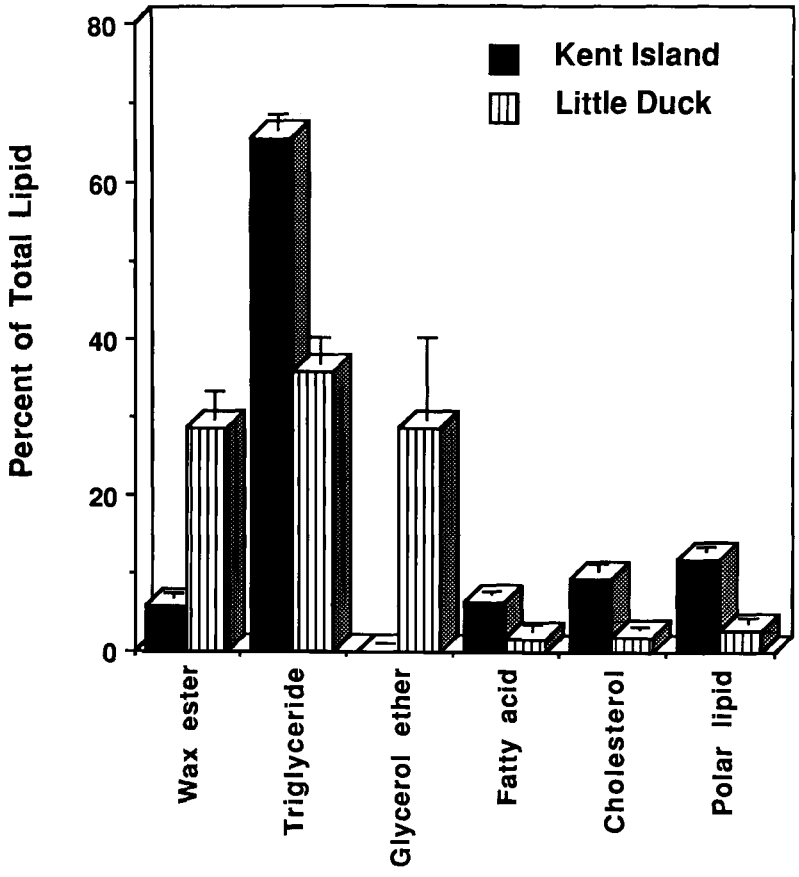
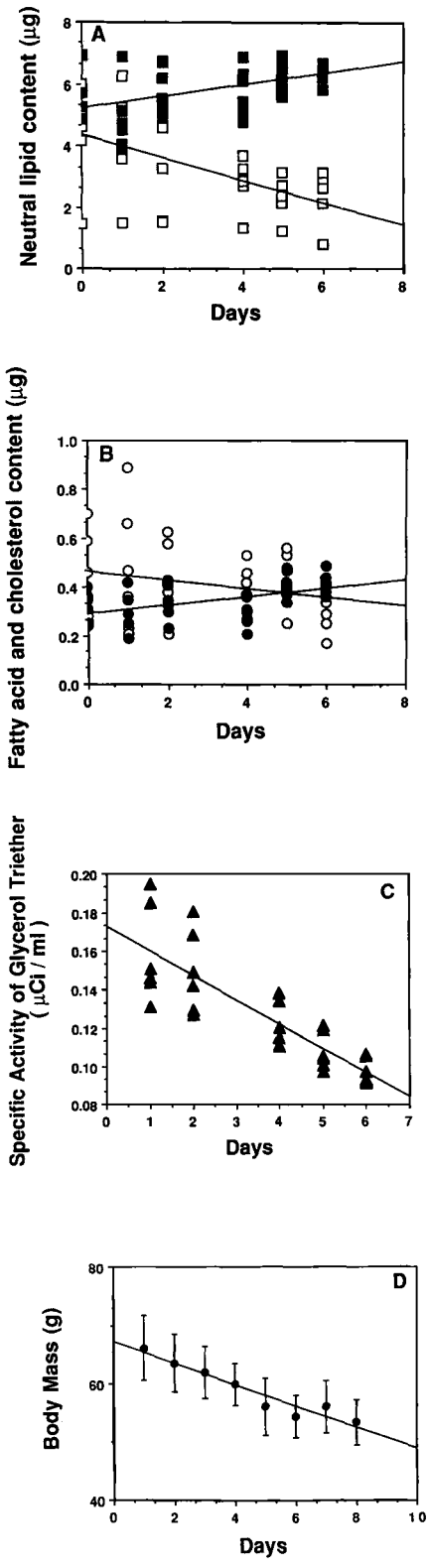


Fig. 2. Stomach oil composition from Leach's Storm-Petrel chicks on Little Duck Island (1986 and 1987) and Kent Island (1986).

ol content increased (6.1 ± 1.69%/day, R^2 = 0.267). These changes in lipid composition are consistent with replacement of the volume sampled (0.364 ± 0.014 ml, n = 30) by dietary triglyceride (assuming constant volume). The initial volume of stomach oil was 7.45 ± 0.459 ml (n = 6). Sampling removed 4.9% of the stomach oil per day. From fecal collections on the first two days, 2.3 ± 0.53% (n = 12) of [^{3}H]-GTE was excreted with chicks assimilating 94.2 ± 0.58% (n = 12) of labeled triglyceride. The daily decrease (7.35 ± 0.85%/day, R^2 = 0.705) in specific activity of [^{3}H]-GTE is consistent with addition of dietary lipid equivalent to the volume removed in sampling plus the volume emptied from the proventriculus for assimilation by the chick (Fig. 3C). This storage of gastric lipid occurs as body weight decreases (Fig. 3D). Except for one individual, all chicks had completed growth. The daily weight loss (1.81 ± 0.21 g/day, R^2 = 0.93) observed is similar to that observed in the field for Leach's Storm-Petrels prior to fledging (Ricklefs et al. 1980a, b, 1985).

Additional evidence for volume regulation of stomach oil was observed in daily weight gains exhibited by 7 chicks (70 days old) after removal of all stomach oil. Each chick was weighed prior to being fed nightly meals of krill supplemented with triglylceride (2.2 g/meal). Average body mass increased linearly for 7 days (1.12 ± 0.06 g/day, R^2 = 0.97) until each chick gained on average 8 g. Subsequent weighings indicated no additional change in mass. Aspiration of the proventriculus on day 9 provided 7.75 ± 1.46 g of lipid, and no net change in body mass from the initial weighing at the start of the experiment.

Fate of polar lipids and hydrocarbons in stomach oils.—Phospholipids rapidly repartitioned out of stomach oils (Fig. 4: A and B). In contrast, fatty acids and alkanes slowly repartitioned out of stomach oils (Fig. 4: C and D).



DISCUSSION

In Leach's Storm-Petrel, we found that stomach oils originate in the diet, but their composition need not match that of the diet precisely. We determined that extensive differential lipolysis of wax esters vs. triglycerides in the proventriculus was not responsible for the prevalence of wax esters in the stomach oils of some species, and that the proventriculus secretes little, if any, neutral lipid. The lipid composition of stomach oils reflects not only the lipid composition of prior meals but also the relative solubility each class of lipids has in the stomach oils previously accumulated. Finally, during the week or two prior to fledging, chicks regulate the amount of stomach oil by restricting the rate of gastric emptying.

Physiological basis of stomach oil formation.— Gastric emptying in animals is a highly regulated and coordinated process. Gastric emptying is slow when the stomach contains nutrient-rich food (such as lipids) and is more rapid when the contents are less energy rich. In humans, the half-time for gastric emptying of a non-nutrient saline solution is only 7.9 ± 1 min while that for a homogenous meal is on the order of 60 min (Smith et al. 1984). In other mammals, gastric emptying of lipids is nearly half that for aqueous components (Jian et al. 1982, Meyer et al. 1986). Comparable differences exist between passage rates of lipids and aqueous components in seabirds (Roby et al. 1989). In Leach's Storm-Petrel chicks, the half-time for gastric emptying of neutral lipids is 70 h while that for aqueous solutions is only 0.35 h. A unique gastrointestinal anatomy and corresponding gastrointestinal motility are important determinants of this slower gastric emptying of dietary lipid (Duke et al. 1989, Roby et al. 1989). We have described previously the gastrointestinal anatomy and motility pattern following a meal in Leach's Storm-Petrels (Duke et al. 1989). Low gastric motility, slow gastric emptying, and the position of the pylorus relative to the proventriculus all contribute to stomach oil accumulation.

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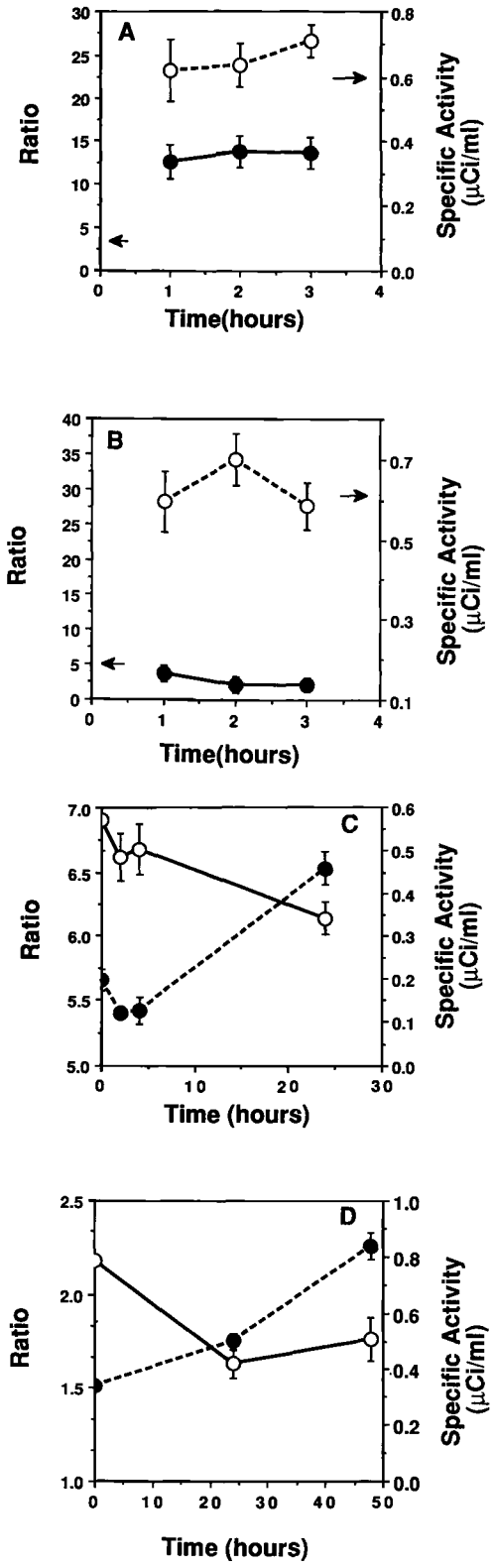
Fig. 3. Changes in the content of stomach oils from 62-day-old chicks fed nightly: **(A)** triglyceride (■) and wax ester (□); **(B)** fatty acid (○) and cholesterol (●). **(C)** The glycerol triether marker (▲) was diluted by dietary lipids. **(D)** Prefledging weight loss occurs even with nightly feedings.



The mammalian stomach is the site of differential emptying of lipids and aqueous components, and it also plays an important role in neutral lipid emulsification. Ingested fats must be emulsified or solubilized in the stomach prior to the formation of micelles in the aqueous contents of the duodenum. Potential emulsifiers that can function in the acid milieu of the stomach include peptic digests of dietary protein, complex polysaccharides, and dietary phospholipids (Carey et al. 1983). Some enzymatic hydrolysis of triglycerides is known to occur which results in digestion of up to 30% of dietary fats (Carey et al. 1983). The monoglycerides formed during this gastric lipolysis aid in the emulsification process.

In seabirds, we found very little gastric lipolysis (Roby et al. 1986, Place and Roby 1986). A possible explanation of the efficient assimilation of nonpolar lipids is the unique character of the "enterogastric reflex." In fowl, as in mammals, gastric motility is inhibited by intraduodenal injections of 0.1 N HCL, 1600 mOsm solutions of NaCl, corn oil, amino acids, or by intraduodenal balloon inflation (Duke and Evanson 1972, Duke et al. 1973, Sklan et al. 1978). An aspect of this feedback regulatory mechanism peculiar to birds, including seabirds, is the occurrence of one or more intestinal refluxes during the period of gastric motility inhibition (Duke et al. 1973, 1989). Intestinal refluxes occur approximately three times more often in Leach's Storm-Petrels than in fowl (Duke et al. 1989) and involve the movement of intestinal contents back to the proventriculus. Thus, gastric emptying is tied closely to the receptiveness of the duodenum for additional digesta, and the reflux returns the digesta (both gastric and duodenal) for further processing in the gizzard. In the process, duodenal products like monoglycerides and fatty acids are refluxed to the gizzard along with biliary (bile salts, phospholipids, and

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Fig. 4. Repartitioning of dietary phospholipids **(A)** L-α-12-palmitoyl-2-oleoyl-[oleoyl-1-^{14}C] phosphatidylcholine and **(B)** L-α-dioleoyl, [dioleoyl-1-^{14}C] phosphatidylcholine, fatty acids **(C)** ([1-^{14}C] stearic acid) and hydrocarbons **(D)** (n-[1-^{14}C] hexadecane) from stomach oils. All comparisons are made relative to [^{3}H]-GTE. The ratio of [^{3}H] μCi/^{14}C μCi in stomach oils is given on the left ordinate while the specific activity (μCi/ml) of ^{14}C is given on the right ordinate. The arrow indicates the initial values at the time of ingestion.

triglycerides) and pancreatic products (lipases). Gastric production of lipid emulsifiers is replaced in birds by products of normal intestinal lipolysis which are refluxed to a highly efficient emulsification mill (the gizzard). We believe that intestinal reflux is unique to birds and permits high assimilation efficiencies for such nonpolar lipids as wax esters (Roby et al. 1986, Place and Roby 1986). We also believe that intestinal reflux is responsible for gastric emptying of polar lipids dissolved in stomach oils.

Effects of solubility on stomach oil composition.—Lewis (1966) found glyceryl ether diesters to dominate stomach oils from Leach's Storm-Petrels. Lewis (1966) felt the presence of glyceryl ether diesters supported a secretory origin, because these compounds were not common in zooplankton and other marine organisms. Our results establish that the composition of stomach oils in Leach's Storm-Petrels is determined by dietary lipid and the composition can be highly variable (e.g. Fig. 2). Stomach oil composition is further influenced by the solubility of each dietary lipid class in previously accumulated stomach oils. When major dietary lipids are changed, the composition of the resident stomach oil changes but not in a manner necessarily reflecting the lipid composition of a meal (e.g. Fig. 3). The absence or low abundance of polar lipids (such as fatty acids and phospholipids in stomach oils) is due to limited solubility in stomach oils and rapid gastric emptying of these polar lipids with the aqueous phase (Fig. 4).

Lipid analysis of meals delivered to chicks on Kent Island recorded nearly 21.7 ± 10.5% SD phospholipid (Place and Roby 1986) yet the phospholipid content of stomach oil was negligible (<1%). We can explain this from the physical properties of phospholipids. Phospholipids are insoluble in neutral lipids (Sandermann 1979) and partition into the aqueous phase either as monomers or as micelles. Partitioning enriches neutral lipids as the aqueous phase of a meal empties rapidly from the stomach. The solubilities of other polar and nonpolar lipids in stomach oils (such as long chain fatty acids, alcohols, alkanes, triglycerides, and chlorinated aromatic and aliphatic hydrocarbons) are determined largely by their melting points. Above their melting temperature (i.e. as liquids), all of these compounds are theoretically miscible with liquid trioleoylglycerol (Patton et al. 1984). Below their melting temperature, the solubility of these compounds can be estimated by the equation:

$$\text{Log(mole fraction solubility)} = \frac{-\Delta S_f(Tm - T)}{2.303RT}, \quad (3)$$

where ΔS_f is the entropy of fusion, and Tm is the melting point (Patton et al. 1984). For example, palmitic acid (melting point 61.8°C) has a 0.76% (w/w) solubility at 14°C while at 37°C the solubility is 4.72% (w/w) in trioleoylglycerol.

Recently, the presence of fossil fuel hydrocarbons in stomach oils has been used to monitor water quality in the marine environment (Boersma 1986). Our findings with n-hexadecane indicate that ingested hydrocarbons persist in stomach oils of Leach's Storm-Petrels at least as long as the nonmetabolizable lipid marker (TMRT > 70 h). Because residue from a single contaminated meal may persist in stomach oils for extended periods, care must be taken in assessing frequency of contamination.

Physiological advantages of stomach oil formation.—An energetic advantage can be realized by metabolizing stomach oils in preference to fat from adipose tissue during fasts (Roby et al. 1989). Such gastric storage of lipids precludes the energy costs of synthesizing triglycerides from assimilated fatty acids, transporting the triglycerides to the fat depots, and of later mobilizing those energy reserves. These energy costs amount to ca. 25–30% of the assimilated energy (Ricklefs 1974, Spady et al. 1976).

A correlate of increasing the energy density of a meal through concentration of dietary lipids is a lower dietary osmotic (i.e. salt) load for the chick. Marine prey items (particularly invertebrates) impose large salt loads. Excess dietary salts are excreted from the nasal salt glands through energy dependent mechanisms which might consume reserves otherwise available for maintenance and growth. Leach's Storm-Petrels produce highly concentrated nasal secretions (Schmidt-Nielsen 1960). Through inclusion of stomach oils in chick meals, procellariiform chicks may experience lower costs of digesting marine prey items.

Salt load reduction also lowers a meal's calcium load, which for marine prey items can be high. In fat absorption, high dietary calcium levels frequently result in calcium soap formation of fatty acids and subsequent poor ab-

sorption of fats (Carey et al. 1983). Calcium soaps are poorly solubilized by bile salts (Graham and Sakman 1983) and up to 50% of fecal fat in steatorrhea has been shown to be calcium soaps (Bliss et al. 1972).

Gastric lipid storage could also affect the digestive efficiency of nonlipid dietary components. Isocaloric substitution of calories from fat for nonlipid calories in the diet improves feed efficiency, increases weight gain, or both in chickens (Fuller and Rendon 1977). In hens, a clear positive correlation exists between the percent lipid in a meal and first appearance of a nonmetabolizable marker (Mateos and Sell 1981, Mateos et al. 1982). This slowing of gastrointestinal transit in response to added dietary fat may explain improved feed utilization in chickens that receive lipid supplements. By retaining neutral lipids in the proventriculus, procellariiforms may slow gastrointestinal transit uniformly and thereby increase assimilation efficiency of nonlipid dietary components uniformly.

Regulation of stomach oil volume.—An unexpected observation was the apparent regulation of stomach oil volume by Leach's Storm-Petrel chicks nearing fledging. While experiencing prefledging weight loss, chicks fed nightly meals retained significant dietary lipid in the proventriculus (ca. 8 ml). In 7 chicks which had completed growth and attained adult body mass (38.2 ± 1.60 g), a previously emptied proventriculus could be refilled with stomach oil within 8 days if fed nightly. The ecological significance of departing the nest with nearly 7-days energy needs (if indeed the fledglings retain the oil rather than dumping the extra ballast before flight) remains to be ascertained.

Prior work on stomach oil formation.—Early studies (Smith 1911; Carter 1921, 1928; Carter and Malcolm 1927; Rosenheim and Webster 1927) described the major lipid component in stomach oils as wax esters. Such early work, supplemented by other studies which failed to find wax esters in littoral and pelagic food sources (Imber 1976, Lewis 1966), led to the speculation that stomach oils resulted from proventricular secretions or were the result of ingested secretions from the preen gland (Carter and Malcolm 1927). Matthews (1949) provided morphological and histological evidence that supported a secretory origin of stomach oils. Hagerup (1926) argued for a dietary origin.

A secretory origin for stomach oils was generally accepted until analyses of mesopelagic and bathypelagic marine organisms were found to contain high concentrations of wax esters (Nevenzel et al. 1965, Lewis 1967) and Imber (1973) found primarily mesopelagic and bathypelagic organisms of fresh petrel meals. In support of a dietary origin was the wide variation in lipid composition found in additional studies (Lewis 1969; Cheah and Hanson 1970a, b; Watts and Warham 1976; Clarke and Prince 1976). For example, Cheah and Hansen (1970a) showed that stomach oil of *Fulmaris glacialis* contained mostly triglycerides, whereas stomach oils of *Puffinus tenirostris* showed mostly wax esters. Watts and Warham (1976) found various mixtures of triglycerides, wax esters, and diacylglyceryl ether in 16 species of petrel. This extreme variability strongly supported a dietary origin as proventricular secretions would show much more uniformity in lipid composition. Current opinion now holds that these oils are the product of food breakdown in the acidic and glandular proventriculus (Warham 1977, Jacob, 1982). We clearly establish a dietary origin for stomach oils in chicks of Leach's Storm-Petrel.

Acknowledgments

We are grateful to S. W. Kress and the National Audubon Society for access to Little Duck Island Sanctuary. We are grateful to C. E. Huntington and the Bowdoin Scientific Station for the use of facilities on Kent Island. The authors also wish to acknowledge the laboratory assistance of Tong Ma and the generous gift of the tritiated glycerol triether from James Meyer, Veteran's Administration, Sepulveda, California. Helpful comments on improving earlier versions of the manuscript were provided by D. D. Roby and A. H. Brush. This study was supported by the National Science Foundation (USA) DCB87-09340, a Lucille Markey Fellowship, and a Department of Navy Contract (N00014-86-K-0696) to Place and by the Endangered and Nongame Species Program of the Maine Department of Inland Fisheries and Wildlife (Butler). This is contribution No. 116 from the Center of Marine Biotechnology, University of Maryland.

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