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

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Birds of the Order Procellariiformes (albatrosses, fulmars, shearwaters and other petrels) are unique in being able to store oil in their large, glandular and distensible fore-guts or proventriculi. The oil is found in chicks and adults, as well as breeders and nonbreeders, and in birds captured at sea or on land. Originally thought to be the result of proventricular secretion (Matthews, *The Ibis*, 91:373-392, 1949), the oil is now considered to be of dietary origin (Warham, *Proc. New Zealand Ecol. Soc.* 24:84-93, 1977).

An equally unique character of procellariiformes involves the gross anatomy of the gastrointestinal tract. Forbes (*Challenger Repts*.4:1,1882) described the enormous distensible proventriculus; 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. This arrangement of viscera is unique to the group and is thought to be responsible for the storage of stomach oils. In the proventriculus, the heavy, more aqueous material would settle and empty first, followed by the lighter, oily lipid. This assumes substantial layering of a lipid phase above an aqueous phase prior to proventricular emptying. In humans, although lipids were found to empty the stomach more slowly (14 ± 3%/hr) than aqueous (34.3 ± 1.8%/hr) or solid components (22 ±1.8%/hr) (Jian *et al., Dig. Dis, Sci*. 27:705-711, 1982), no evidence of substantial layering of lipids above nonlipids was found.

To test whether lipids and aqueous components had differential transit times through the gut of Leach's storm-petrel chicks, we fed four chicks a radioactively tagged homogenized meal (2g) of calanoid copepods. To mark the aqueous phase of this meal we added the nonabsorbable water soluble marker [1-^{14}C] polyethylene glycol, M.W. 4000 and to mark the lipid phase we added [^{3}H]-labeled glycerol triether (1-hexadecyl-2,3-didodecyl glycerol (1-hexadecoxy-2,3-didodecoxypropane)). The meals were delivered with a disposable 5 ml syringe attached to a 10-cm length of polyethylene tubing inserted into the esophagus. All chicks took the feeding without any regurgitation. After ingestion, each chick was placed on a polyethylene mesh (1/4-in) platform suspended in a 2-gallon polyethylene container to collect excreta. Containers were kept in the dark and maintained at 14 ± 3° to simulate the nest enviroment as much as possible. At selected times the chicks were transferred to a clean container so the excreta from the previous time interval could be analyzed. Unlabeled meals (5 to 8 g) were fed to the chicks nightly.

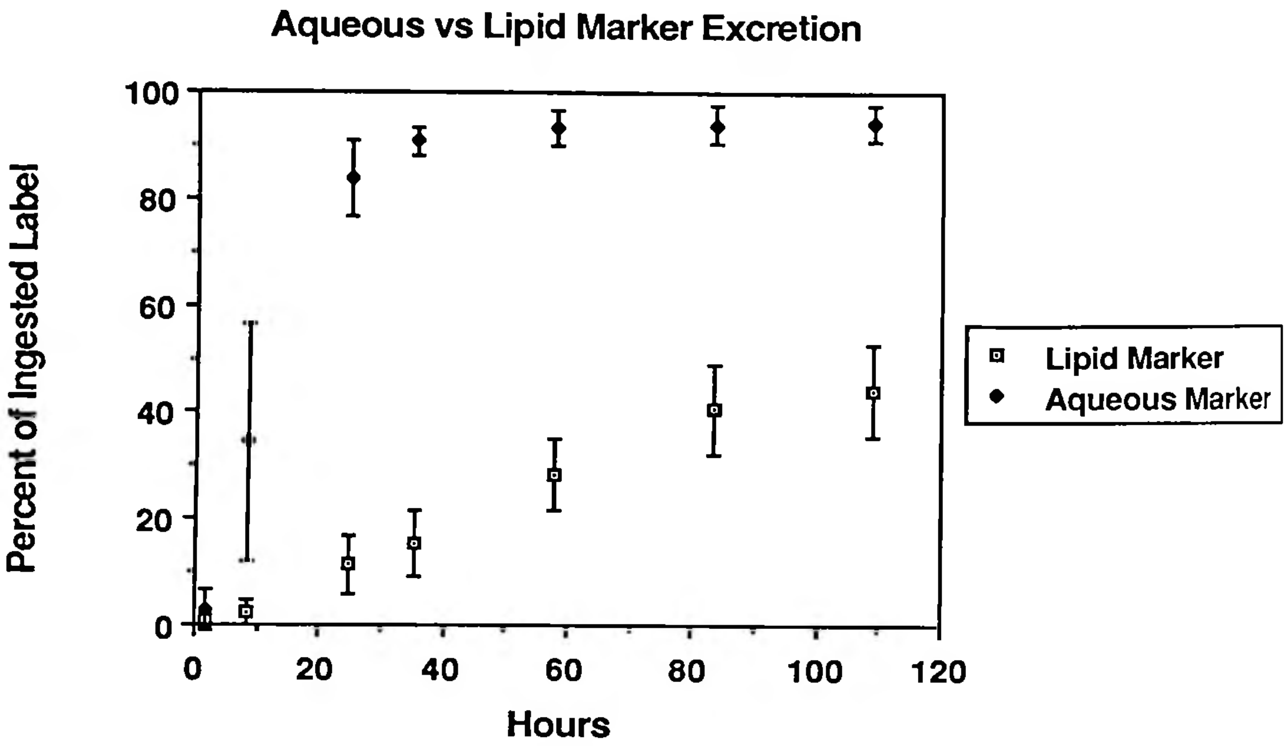

Figure 1. Time-courses of gastrointestinal emptying of aqueous and lipid phases in four Leach's storm-petrel chicks.

Accumulated excreta in each container were extracted by the Bligh and Dyer technique (*Can. J. Biochem. Physiol.* 37:911-917, 1959). Figure 1 presents the findings from this experiment. By 24 hours 83.7% (S.E. 7.0%) of the aqueous marker was recovered while only 11% (S.E. 5.5%) of lipid marker was recovered in the same time period. By 4.5 days only 44 % (S.E. 8.57%) of the lipid marker was recovered whereas nearly all the aqueous marker was recovered (94.3 %; S.E.3.4%). A major portion (approximately 30%) of the unrecovered lipid marker was found to reside in the stomach oils aspirated from the proventriculus. This represents nearly a 10 fold slower transit time for lipid components compared to transit time for aqueous components. Gastrointestinal transit times for slower components are 1-3 hours for most bird species, and 2- 150 hours for most mammals and lizards (Karasov *et al. J. Comp. Physiol. B,* 156:599-609, 1986).

To calculate the contribution of the proventriculus-emptying time to the transit time, we used a double-isotope dilution technique (Diamond *et al., Nature* 320:62-63, 1986) for measuring proventriculus volumes at various times after an aqueous meal. Birds were first fed 5 mls of ^{14}C-polyethylene glycol labelled isotonic (300 mOsm) sugar water. After five minutes an aliquot was removed and the dilution of label determined so the proventriculus volume at t = 0 could be measured. At times of 1, 2, 4 or 8 hours each bird was fed a second 2 ml liquid meal labeled with ^{3}H-polyethylene glycol. Again after a further five minutes to allow uniform mixing of the two isotopes an aliquot was removed and dilution of the label determined. The apparent volume of the proventriculus (V_t) at time t was calculated from marker dilution as $(V_sC_i/C_s)-V_i$, where V_s is the analyzed sample volume, V_i is the volume of marker fed, C_s is the d.p.m in the sample volume and C_i is the d.p.m in the fed solution. Figure 2 presents the findings from this experiment.

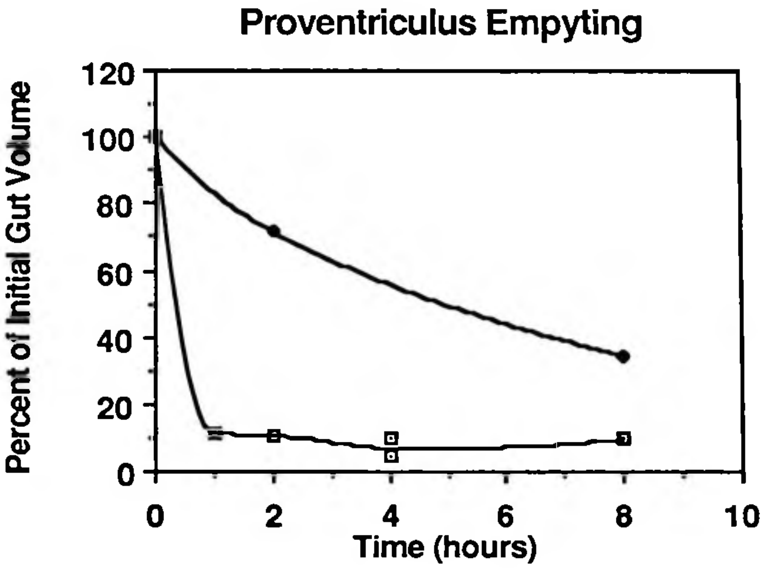

Figure 2. Proventricular emptying rates of an aqueous phase marker in Leach's storm-petrel chicks. Open symbols represent the data from five chicks which show nearly complete emptying while the solid symbols are data from two chicks which exhibited partial emptying.

Initial aqueous proventricular volumes varied from 5.13 to 0.9 mls (not including the 5 mls fed). In five of the chicks, the volume had decreased to 0.65 ± 0.19 mls by 1 hour. We assume this is an empty proventriculus. (It should be noted that the proventriculus would still contain 3.42 ± 2.09 mls of stomach oil). The average volume emptied was 6.63 ± 1.15 mls. There appears to be a maximum volume of about 7 to 8 mls which can be empytied at one time. Only after this volume has been processed in the intestine can a further volume be emptied.

From these two studies we conclude that the lipid and aqueous phases of Leach's storm-petrel meals exhibit large differential transit times through the gastrointestinal tract. We believe the large distensible and secretory proventriculus in these birds plays an important role in this phenomenon. Chicks 40-60 days old are fed on the average 8.5 g of food per night or approximately 12-14 % body mass (Ricklefs *et al.*, *J. Anim. Ecol.* 54:883-898, 1985). The average meal fed to a chick can have a lipid content (ave. 27% wet weight) of nearly 60% wet weight (Place and Roby, *J. Exp. Zool.* 240:149-161,1986). In the acid environment of the glandular proventriculus (contents pH = 2.57 ± 0.5) fatty acids would be protonated and relatively insoluble. There is little lipolytic activity in the proventriculus (Place and Roby, *J. Exp. Zool.* 240:149-161, 1986), so neutral lipids like triglyceride and wax esters would remain intact. However, peptilytic activity is high, so preliminary breakdown of proteins would proceed. In this environment, low density water insoluble components would tend to layer on top of the aqueous phase. Free amino acids released by pepsinolysis would stimulate empyting of the proventriculus until the lipid phase reached the bottom of the proventriculus because lipids of 12 to 18 carbons in length are the most powerful inhibitors of gastric emptying (Mateos *et al.*, *Poult. Sci.*, 61:94-103, 1982). Once lipids entered the small intestine and lipolysis proceeded, gastric emptying would be slowed. This inhibitory process would lead to an accumulation of stomach oils. The small ascending loop of the duodenum distal to the pylorus would help stop the oil draining down to the intestine. Thus, we believe that the large distensible proventriculus seen in Procellariiformes is an evolved adaptation for dealing with large meals high in lipid. This work was supported by a Markey Fellowship from the Lucille Markey Charitable Trust to A. R. Place during the summer of 1986.