THE IMPORTANCE OF BILE SALTS TO WAX ESTER ASSIMILATION IN LEACH'S STORM-PETREL, *OCEANODROMA LEUCORHOA*

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For many pelagic sea birds, particularly species that forage at high latitudes, wax esters are a major component of the diet (Warham *et al.*, *J. Exp. Mar. Biol. Ecol.* 23:1-13, 1976; Clarke and Prince, *J. Exp. Mar. Biol. Ecol.* 23:15-30, 1976; Warham, *Proc. New Zealand Ecol. Soc.*, 24:84-93, 1977). In species that feed on plankton, up to 63% of the energy content of meals fed to growing chicks resides in wax esters (Roby, Place, and Ricklefs, *J. Exp. Zool.*, 238:29-41, 1986). Although wax esters are generally considered to be a poor food source (Patton *et al.*, *Lipids*, 10:575-583, 1975; Patton and Benson, *Comp. Biochem. Physiol.*, 52B:111-116, 1975; Verbiscar *et al.*, *J. Agric. Food Chem.*, 28:571-578, 1980; Tocher and Sargent, *Comp. Biochem. Physiol.*, 77B:561-571, 1984), we have shown that Leach's storm-petrel chicks efficiently hydrolyze and assimilate (better than 95%) both the fatty alcohol and fatty acid moiety of wax esters (Place and Roby, *J. Exp. Zool.*, 240:149-161,1986). Since wax esters are classified as non-polar lipids (Carey and Small, *Am. J. Med.*, 49:590-608, 1970), we expect their intestinal absorption to be similar to another non-polar lipid class, cholesteryl esters. When bile acids are diverted from the intestinal lumen cholesterol uptake is nearly abolished while absorption of the longer-chain-length fatty acids is reduced by only 15 to 30% (Westergaard, H., and Dietschy, *J. Clin. Invest.* 54:718-732, 1974). Our plan was to lower the intestinal bile salt concentration and measure the resultant assimilation efficiencies of wax esters and triglycerides. We selected the bile salt sequestrant, cholestyramine, as our vehicle for reducing the intestinal bile salt concentrations.

Leach's storm-petrel chicks of known ages (35- 50 days) were removed from their burrows and fed for several days prior to intitiating the experiment a diet of homogenized calanoid copepods supplemented with either (65% w/w) triolein or (65% w/w) cetyl oleate. 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 and based on wing growth exhibited normal development. 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 24 hour intervals 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 until the start of the experiment. Cholestyramine was added to meals on a percent weight basis after hydration in water.

To estimate lipid absorption efficiencies we adopted an isotope ratio procedure based on a nonabsorbable lipid marker fed together with the radioactive lipid of interest. This procedure circumvents the problems in chemical balance studies of nondietary (endogenous) lipids being measured as well removing the requirement that complete fecal collections be obtained. As a nonabsorbable lipid marker we used [^{3}H]-labeled glycerol triether (1-hexadecyl-2,3-didodecyl glycerol (1-hexadecoxy-2,3-didodecoxypropane)) (Morgan and Hofmann, *J. Lipid Res.*, 11:223-230, 1970). The ^{3}H label is in the 9 and 10 positions of the hexadecyl moiety. Both dietary lipids, triolein and cetyl oleate, were lableled with ^{14}C. Each of the fatty acids moieties of triolein was labeled in C-1 position while the C-1 fatty alcohol position of cetyl oleate was labeled. Each bird was fed a meal containing 10 mCi of [^{3}H]-marker and 5 mCi [^{14}C]-lipid. Accumulated excreta in each container were extracted by the Bligh and Dyer technique (*Can. J. Biochem. Physiol.* 37:911-917, 1959). Absorption of [^{14}C]-lipid was calculated from the ratio of [^{3}H]-marker to [^{14}C]-marker in the daily fecal collection by the formula:

$$\% \text{ lipid absorbed} = 1 - [{}^{3}H/{}^{14}C \text{ in test meal per } {}^{3}H/{}^{14}C \text{ in daily fecal collection}]) \times 100$$

Figure 1 presents the findings from this experiment.

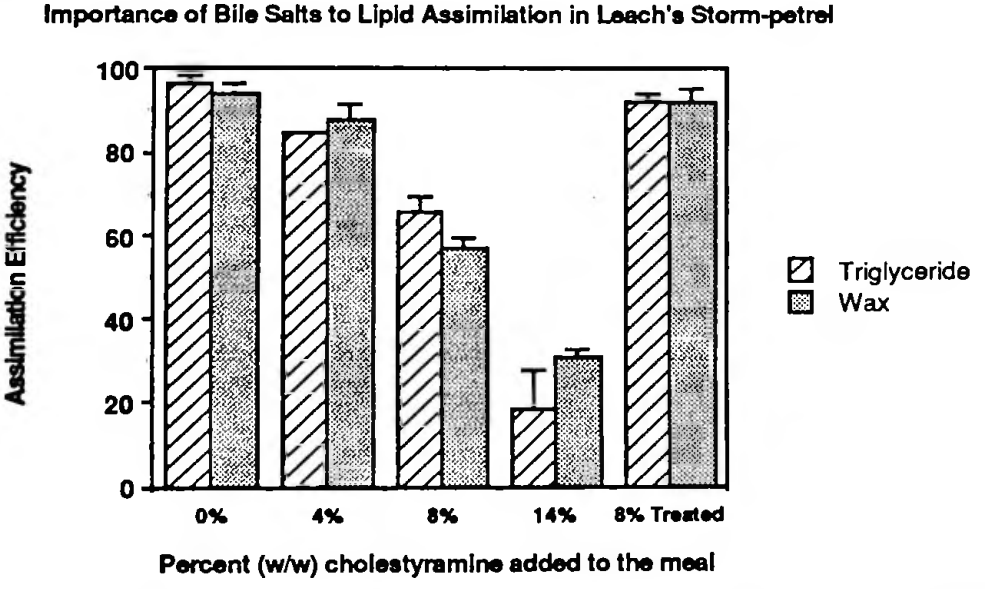

Figure 1. The effect of cholestyramine on wax and triglyceride assimilation in Leach's storm-petrel chicks.

The average assimilation efficiencies of triolein and cetyl oleate (96 ± 2.11% and 94 ± 2.5%, respectively) in control chicks were indistinguishable, corroborating our earlier findings from isotope recovery studies (Place and Roby, *J. Exp. Zool.*, 240:149-161,1986). Based on radiometric TLC tracings of fecal extracts, the [^{3}H]-GTE marker was recovered intact. Both wax ester and triglyceride absorption decreased with increasing amounts of resin, and with 14% cholestyramine in the diet less than 30% of the lipids were retained. A large portion of the lipids passed through the gastrointestinal tract without being hydrolyzed. There were no statistically significant differences between wax fed and triglyceride fed chicks in any treatment. When chicks were fed 8% cholestyramine pretreated with sodium taurocholate to saturate all the bile salt binding capacity of the resin, lipid absorption efficiencies were indistinguishable to control chicks. Moreover, glucose assimilaton efficiencies, determined by the isotope ratio technique using ^{3}H PEG (M.W. 4000) as a nonabsorbable marker and ^{14}C D-glucose (25 mM ; isotonic solution), for chicks fed meals containing 8% cholestyramine were 91.4 ± 3.8 %. Thus the effects of cholestryamine observed, appear to be specific to lipid absorption and involve bile salt binding. These results are similar to the findings in the rat (Harkins *et al.*, *Nutrition*, 87:85-92, 1965; Morgan and Hofmann, *J. Lipid. Res.*, 11:231-236, 1970) and in the chicken (Garrett and Young, *J. Nutrition* 105:827-838, 1975) for fatty acid absorption. With the chicken, identical fatty acid absorption patterns were observed in both bile duct cannulated treated birds and cholestyramine treated birds (Garrett and Young, *J. Nutrition* 105:827-838, 1975).

Since cholestyramine feeding has been shown to bring about hypolipidemia and hypocholesterolemia in other organisms (Huff *et al.*, *Proc. Soc. Exp. Biol. Med.* 114:352-355, 1963) , we examine the blood levels of glucose, triglycerides, and cholesterol in our cholestyramine treated chicks. Figure 2 presents our findings. Cholestyramine had no effect on either blood glucose levels or blood cholesterol levels. However, the resin did significantly lower blood triglyceride levels. The serum levels of glucose (10.7 ± 1.19 mM; n =15); cholesterol (12.7 ± 2.74 mM; n = 18), and triglyceride (5.47 ± 3.91 mM; n =12) in randomly chosen chicks in the wild were comparable to the measured concentrations in the control chicks. From this study we conclude that bile salts are essential for normal fat absorption in Leach's storm-petrel chicks. This is in contrast to studies done on man (Porter*et al.*, *Gastroenterology* 60:1008-1019, 1971) and the rat (Gallagher *et al.*, *Clin. Sci.*, 29:73-82, 1965) with individuals possessing biliary fistulas. The reduction in fat absorption is 15-25% despite almost complete absence of bile salts in the intestine

Since little is known about avian bile and its constituents, we analyzed the gall bladder contents of 8 Leach's storm-petrel chicks sacrificed at 4, 8, 12 and 24 hours after being fed 5 mls of pure lipid. The results are found in Table 1. The

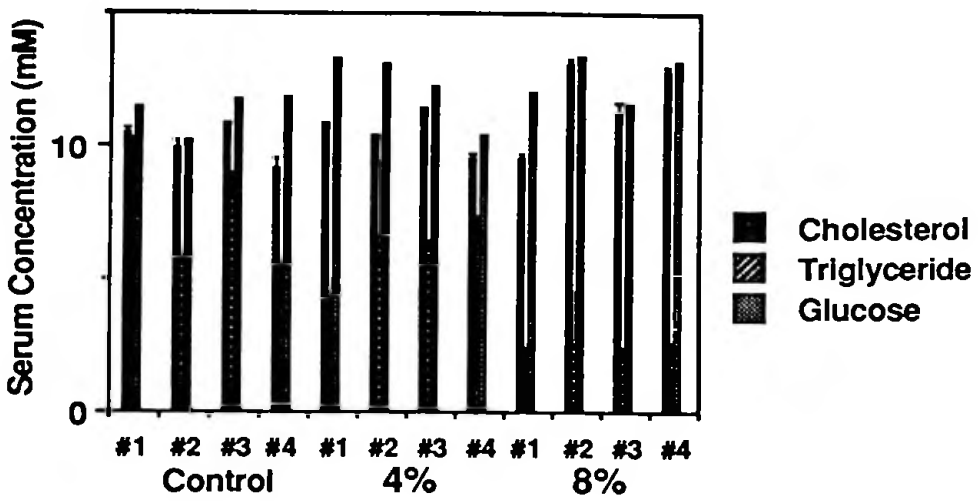

Figure 2. Serum cholesterol, triglyceride and glucose levels in control (n = 4) chicks and chicks fed meals containing 4% (w/w, n = 4) and 8% (w/w, n = 4) cholestryamine. Chicks #1 and #3 were fed triolein supplemented meals and chicks #2 and #4 were fed cetyl oleate supplemented meals. Then means of three replicate determinations for each metabolite are plotted. Serum glucose, cholesterol and triglyceride were assayed colorimetricly using Sigma Diagnostic Kits.

concentrations of bile salts found in the gall bladder are the highest recorded in nature. They are almost exclusively taurine conjugates of chenodeoxycholate and cholate, with less than 1% secondary bile salts. The phospholipid and cholesterol levels are within the range observed in humans. The serum levels of total bile salts was 1.94 ± 0.65 mM. We would predict that the luminal concentration of bile salts in these chicks would be 5-8 times higher than that observed in man or the rat. We would argue these elevated levels of bile salts are essential to high efficiency assimilation of nonpolar lipids like waxes and similar findings would be expected in any sea bird which ingests these materials.
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Table 1

Leach's Storm-petrel's
(*Oceanodroma leucorhoa*)
Bile Composition

Constituent	Mean
Total Bile Acid Salts (mM)	500 ± 83.8
(> 98% Tauro conjugates of primary salts)	**[75.3 ± 32.3]**
Phospholipid (mM)	24.9 ± 7.2 **[33.3 ± 16.5]**
Cholesterol (mM)	3.3 ± 0.1 **[10.4 ± 5.48]**
Total Protein (mg/ml)	6.1 ± 0.5 **[26.7 ± 18.2]**
D/T Ratio (molar ratio: total dihydroxy/total trihydroxy bile acids)	5.5 ± 2.2 **[0.77 ± 1.17]**
Osmolarity (mOsm/kg)	470 ± 13.8 **[349 ± 40]**

Values in brackets are normal values for humans. Total bile salts were assayed with 3 a-hydroxysteriod dehydrogenase (EC 1.1.150) (Coleman *et al., Biochem. J.* 178:201-208). Bile salt chromatography was performed by TLC (Goppelt and Resch, *Anal. Biochem.* 140:152-156, 1984) and HPLC (Goto *et al., J. Chromat.* **3**:645-655, 1980). Lipid extracts (Bligh and Dyer,*Can. J. Biochem. Physiol.* 37:911-917, 1959) of bile were assayed for phosholipid phosphorous (Petitou *et al., Anal. Biochem.* 91:350-353, 1978) and cholesterol (Christie, *Lipid Analysis,* 1982). Total protein was assayed according to Bradford (*Anal Biochem.* 72:248-256, 1976).