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Crustacean Intestinal Detergent Promotes Sterol Solubilization

Abstract. *Although crustacean tissue cholesterol content is high, Crustacea, like other arthropods, are incapable of cholesterol synthesis, and presumably are dependent for maintaining tissue cholesterol stores on the intestinal absorption of ingested sterol. A detergent,* N-*(*N-*dodecanoylsarcosyl)taurine, representative of a set of detergents synthesized by the crustacean hepatopancreas and secreted into the intestine, is capable of efficient cholesterol solubilization, and thus of promoting sterol absorption.*

Micellar solubilization is an essential step in the biliary secretion and efficient intestinal absorption of endogenous and dietary lipids (*1*). In vertebrates aqueous solubility of lipids is enhanced by mixed micelle formation with bile salts, a family of steroidal hepatobiliary detergents derived from cholesterol (*2*). The formation in bile of mixed micelles of bile salts with phospholipids and cholesterol promotes the excretion of these otherwise insoluble lipids. Similarly, the formation in the intestine of mixed micelles of bile salts with fatty acids, monoglycerides, and cholesterol augments the solution concentration of these luminal lipids and thus promotes their absorption from the gut (*3*).

Less is known about the intestinal absorption of essential lipid in subvertebrate species (*4*). While the cholesterol concentrations of crustacean tissues are among the highest recorded for animals (*5*), Crustacea, like many other arthropods, are incapable of cholesterol synthesis from nonsteroidal precursors, such as acetate and mevalonate (*6*). Also, some marine invertebrates contain a wide variety of sterols in addition to cholesterol (*7*). We therefore propose that the sterols of crustacean tissue are derived from the diet. Since dietary sterols are largely insoluble in water, it seemed reasonable, by analogy to vertebrate physiology, to seek a mechanism for sterol solubilization in the crustacean intestine. The crustacean hepatopancreas, unlike the vertebrate liver, is incapable of bile salt synthesis (*8*) although enzyme systems capable of catalyzing the conversion of phytosterols to cholesterol and of cholesterol to a variety of steroid hormones are present (*9*). However, a mixture of straight-chain fatty acid detergents containing predominantly *cis*-dodec-5-enoic acid conjugated with the dipeptide sarcosyltaurine has been isolated from crustacean (*Cancer* spp.) gut contents (*10*). These detergents have been shown to be synthesized endogenously from acetate (*11*) and have been assumed to aid lipolysis by playing a role in the intestinal emulsification of dietary fat (*12*).

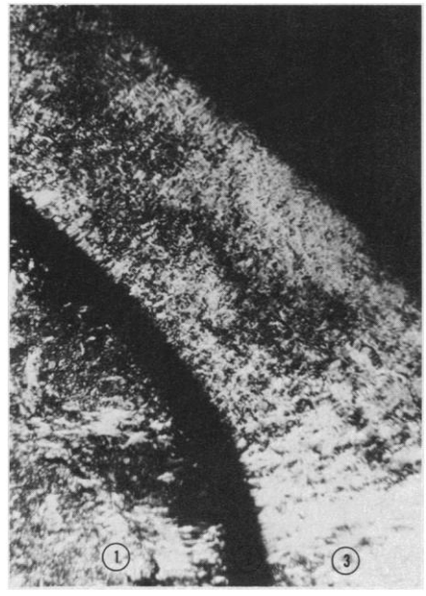

Fig. 1. Photomicrograph of phases observed during flooding of DST with water (crossed nicols; (× 55) 23°C. Phases: (1) Neat phase; (2) viscous isotropic phase (cubic); (3) middle phase texture (cloudy); (4) isotropic solution. This photograph demonstrates the microscopic textural appearances of DST as progressive hydration occurs. A small quantity of crystalline DST was placed between slide and cover slip, and a drop of water at *p*H 6.8 was added. The changes in the preparation were observed within a few seconds of hydration. As the water comes in contact with the sample, a water gradient is created with the outer border being the most hydrated and the center of the preparation remaining dry. Zones in between contain more and more water as one approaches the outside border. This simple method is helpful in determining the number and texture of the phases found in a detergent water mixture, provided the ambient temperature is above the critical micellar or penetration temperature of the detergent (*17*).

We hypothesize that crustacean detergent is essential for the intestinal micellization of endogenous and ingested sterols and thus permits their efficient solubilization and absorption. In order to examine this possibility we have studied the interactions between the sodium salt of *N*-(*N*-dodecanoylsarcosyl)taurine (DST), a representative model for the class of crustacean detergents, and cholesterol in water. For this purpose, a mixed anhydride of the benzyloxycarbonyl derivative of sarcosine was reacted with taurine, and, after removal of the blocking group and crystallization from ethanol, the resultant sarcosyltaurine was reacted with dodecanoic acid (*13*). The synthesized product was purified by reversed-phase column chromatography, and no traces of dodecanoic acid, sarcosine, taurine, or sarcosyltaurine were discovered by thin-layer chromatography (TLC), proton titration, or amino acid analysis. The melting point of the sodium salt of DST was 190° to 192°C. The critical micellar temperature (*14*) was below 0°C, as indicated by the fact that a 1 percent solution showed no trace of turbidity on prolonged cooling (0°C). The apparent *pK* (by titration with HCl, 1 percent solution) was 2.2 (*15*). DST exhibited lyotropic mesomorphism (liquid crystalline phases) comparable to that demonstrated by soaps and synthetic straight-chain detergents (*16*). Polarizing microscopy (*17*) revealed that, upon hydration of dry samples of DST, alternating sharply delineated, texturally characteristic, birefringent and isotropic bands corresponding to crystalline hydrates, neat (possibly lamellar), viscous isotropic (cubic), and middle (possibly hexagonal) liquid crystalline phases, and a micellar phase were demonstrable at increasing distance from the unhydrated material (Fig. 1).

Cholesterol solubilization by DST micellar solutions was demonstrated in binary mixtures in an excess of aqueous solvent (Fig. 2). At concentrations of 20 m*M* DST or less, there was no solubilization of cholesterol. Increasing amounts of cholesterol were solubilized at concentrations of

DST of 30 mM or greater. The solutions were Tyndall-negative, thermodynamically stable, and isotropic under crossed nicols at a magnification of $\times$ 500. By extrapolation, the data suggest that micelles capable of cholesterol solubilization begin to form at 21 mM DST, a figure similar to the critical micellar concentration (CMC) of decanoylsarcosyltaurine as estimated by surface tension under somewhat different conditions (*12*). It can be calculated from the slope of the solubility line and the CMC under these conditions that 14 molecules of micellar DST are necessary to solubilize 1 molecule of cholesterol. In similar experiments, it was possible to establish that 60 molecules of the bile salt sodium taurocholate (NaTC) and 100 molecules of the common paraffin chain detergent sodium dodecyl sulfate (SDS) were required to solubilize 1 molecule of cholesterol in mixed micellar solutions.

In a series of emulsification experiments, 0.5 ml of pure triolein was mixed with varying amounts of aqueous DST, NaTC, and SDS in water at *p*H 6.8, to give 2 ml of total mixture. After vigorous treatment in a Vortex for 1 minute, the breaking times of the emulsions were measured arbitrarily. DST and the bile salt were very poor emulsifiers, the emulsions breaking within 1 minute. However, SDS produced a relatively stable emulsion which persisted for several hours.

Cholesterol is mixed with lecithin in both the crustacean and vertebrate intestinal luminal contents (*12, 18*). The phase behavior of ternary systems of mixtures of cholesterol, lecithin, and DST in excess water was, therefore, determined (Fig. 3). The single phase micellar zone was defined and compared with that obtained when NaTC and SDS were substituted for DST. The addition of lecithin (egg yolk, grade 1, Lipid Products, Surrey, U.K.) to each detergent increased cholesterol solubilization. Maximum cholesterol solubilization was 10 percent [percent = (moles cholesterol solubilized divided by the total moles of all lipids) $\times$ 100] for the system containing DST and lecithin, 6 percent for the system containing NaTC and lecithin, and 4.5 percent for the system containing SDS and lecithin.

The concentration of the constituents in fasting intestinal juice of the crustacean species *Cancer borealis* was determined. The mean concentration of total solids equaled 6.9 g/100 ml with 40 percent acid precipitable material. The total cation concentration was 335 mM, and the electrolyte concentrations resembled those in seawater (*19*), with 91 mg/dl of Mg and 49 mg/dl Ca (*20*). The mean sterol concentration was 0.06 g/100 ml, and was shown to be exclusively cholesterol by hexane extraction and GLC–mass spectroscopy. The mean phospholipid concentration was 0.12 g/100 ml, and, as shown by TLC, consisted entirely of lecithin. Hydrolysis and GLC of the lecithin fatty acids (as methyl esters) established that approximately 50 percent were saturated and monounsaturated C_{14} to C_{20} even-chain fatty acids, with the remainder being C_{22}, C_{24}, and C_{26} polyunsaturated fatty acids (*21*). A mixture of C_{10} to C_{14} *N*-acylsarcosyltaurines, lecithin, and cholesterol were the only constituents of deproteinated fasting intestinal juice identifiable on TLC. No measurable hydrocarbons, glycerides, or free fatty acids were detected. When the relative concentrations of detergents, lecithin, and cholesterol from seven samples of juice from different animals were quantitated by conventional methods (*22*) and plotted on triangular coordinates, all values fell within the predicted single phase micellar zone (Fig. 3).

The results establish that crustacean intestinal detergent is a very poor emulsifier of triolein but solubilizes both lecithin and cholesterol as mixed micelles. The capability for cholesterol solubilization by DST alone and in the presence of small amounts of lecithin is not only in excess of that of an analogous paraffin chain detergent (SDS) but also exceeds that of the vertebrate hepatic steroidal detergent taurocholate. However, the maximum capacity of DST for lecithin solubilization is much less than that of the bile salt (Fig. 3). For these reasons the shape of the micellar zone is significantly different when compared with that of taurocholate and SDS. The reasons for the greater cholesterol solubilizing capability of DST as compared with SDS and taurocholate are not entirely certain. Superficially, both DST and SDS are straight-chain detergents with identical paraffin chains and acidic sulfate or sulfonate head groups. The interposition of the peptide bonds and the carbon atoms of sarcosine and taurine between the fatty acid and charged terminus makes DST a longer and more polar molecule than SDS. These

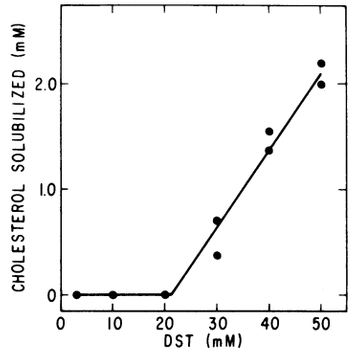

Fig. 2. Cholesterol solubilization by DST. Dried mixtures of DST and [^{14}C]cholesterol, total lipid concentration, 55 mM, were hydrated in 1 ml of 0.15M phosphate buffer, *p*H 7.4, mixed, equilibrated for 24 hours at room temperature (23°C) under N_2, and centrifuged at 25,000 rev/min; the cholesterol concentration was determined by scintillation counting of the clear supernatant. Cholesterol concentration in micellar solution is plotted as a function of the DST concentration. Below an estimated DST concentration of about 21 mM, the amount of cholesterol solubilized is unmeasurable. Once this concentration is exceeded, the solution concentration of cholesterol increases linearly. An estimate of the CMC of the system is given by the DST concentration at the intersection of the straight lines. The ratio of the number of DST to cholesterol molecules in micellar solution is obtained from the slope of the steep part of the curve with correction for the concentration of DST molecules present as monomers.

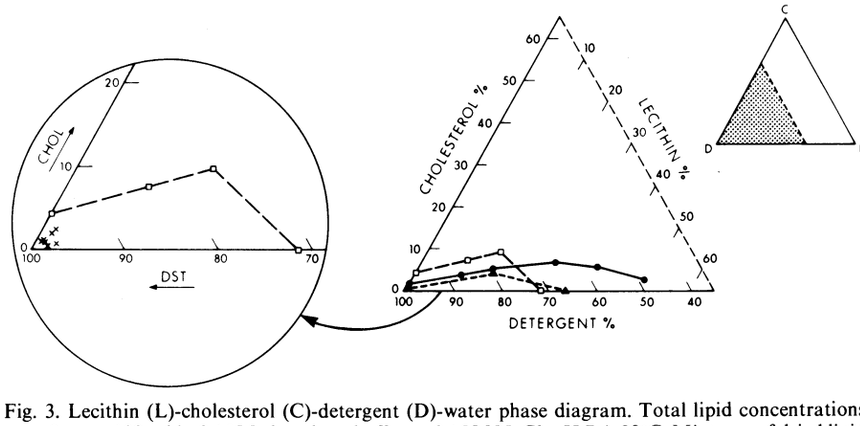

Fig. 3. Lecithin (L)-cholesterol (C)-detergent (D)-water phase diagram. Total lipid concentrations were 3 g per 100 ml in 0.15M phosphate buffer or 0.15M NaCl, *p*H 7.4, 23°C. Mixtures of dried lipid were hydrated, mixed, equilibrated for 24 to 96 hours under N_2, and examined under a strong point light source by polarizing microscopy. The shaded area of the small triangle on the right shows the relevant segment of the phase diagram. The triangle in the center shows this segment expanded, with the single phase micellar zone demarcated for the detergents DST (□ - - □), taurocholate (● —— ●), or SDS (▲---▲). The encircled insert on the left shows the relative concentrations of cholesterol, lecithin, and DST in deproteinated, fasting *C. borealis* gut juice (×). Note that all values fall within the single phase micellar zone.

properties differentiate the physical chemical characteristics of DST from SDS. The CMC of SDS was 4 m*M* under the conditions of these experiments, whereas the CMC of DST was 21 m*M*, and cholesterol was more efficiently solubilized by the longer DST molecule. The bulky hydrated head group should stabilize the DST micelle, and reduce the hydrophobic chain interactions, thus opening the palisade layer of the micelle for interactions with the bulky, nonpolar parts of sterols. For the same reason, the strong, bulky, polar head group may render the molecules so soluble in water that they are poor oil-water emulsifiers. The rigidity of the steroidal hydrophobic parts of simple bile salt micelles reduces their efficiency for cholesterol solubilization. However, once lecithin is incorporated into the micelles, the acquired liquid hydrocarbon core increases cholesterol solubility significantly (Fig. 3).

Whatever the precise explanation for those differences, the results establish that the crustacean detergent is not an efficient emulsifier but exhibits a marked capacity to solubilize cholesterol and lecithin as mixed micelles. While further studies will have to be performed to see the effect of free fatty acid and other constituents of the postcibal intestinal milieu on solubilization, our results support our hypothesis that these detergents promote the intestinal absorption of ingested sterol. The high capacity of crustacean detergent for cholesterol solubilization ensures the maintenance of cholesterol in solution in the exocrine secretion of hepatopancreas even at low concentrations of lecithin, and promotes the efficient solubilization of dietary sterols prior to absorption. The results also suggest that DST may serve as a model for detergent replacement in bile salt deficiency syndromes in humans.

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Arteriovenous Anastomoses in the Skin of the Weddell Seal, *Leptonychotes weddelli*

Abstract. *Arteriovenous anastomoses of epithelioid type were demonstrated in Weddell seal skin. The majority occurred just beneath the epidermis and among the hair follicles. There was no significant variation in density of these anastomoses between body and flipper skin. These observations suggest that arteriovenous anastomoses are important in thermoregulation in the Weddell seal, particularly as heat dissipating structures when the animal is out of the water, and that the entire body surface is involved rather than specific regions such as the flippers.*

In this report we describe the structure, distribution, and density of arteriovenous anastomoses (AVA's) of epithelioid type (*1*) in the skin of the Weddell seal, *Leptonychotes weddelli*. To our knowledge, AVA's have not been described previously in the skin of marine mammals, although their presence was suspected in two species of seals (*Callorhinus ursinus* and *Phoca vitulina*) by Tarasoff and Fisher (*2*).

Skin samples of a 2-day-old female pup and an adult female Weddell seal were taken from the dorsal midline between the scapulae, and from the dorsal aspect of the