6. S. D. Hires *et al.*, *SAE Pap. 780232* (1978), p. 1053.
7. R. M. May, *Nature (London)* **261**, 459 (1976); P. Collet and J.-P. Eckmann, *Iterated Maps on the Interval as Dynamical Systems* (Birkhauser, Boston, 1980); C. Preston, *Iterates of Maps on an Interval* (Springer-Verlag, New York, 1983).
8. M. J. Reilly and R. A. Schmitz, *AIChE J.* **12**, 153 (1966); R. B. Root and R. A. Schmitz, *ibid.* **15**, 670 (1969).
9. N. B. Le, thesis, University of Notre Dame (1982).
10. Supported by NSF grant CPE83-07293-01 and by the Henry and Camille Dreyfus Foundation.

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Copepod Fecal Pellets as a Source of Dihydrophytol in Marine Sediments

Abstract. *Dihydrophytol (3,7,11,15-tetramethylhexadecanol) was identified in the nonsaponified lipid fraction of fecal pellets from the copepod* Calanus helgolandicus *fed in the laboratory on a unialgal diet. Direct deposition in the fecal pellets of certain zooplankton species may explain the presence of dihydrophytol in marine sediments. Microbial reduction of phytol in sediment cores does not account for the origin of this compound in all sedimentary environments.*

Phytol [3,7(*R*),11(*R*),15-tetramethylhexadec-2(*E*)-enol], the ester-linked isoprenoid side chain of chlorophylls a and b, is an important and abundant algal lipid. It is recognized as the precursor of a variety of acyclic isoprenoid compounds of 20 or fewer carbon atoms detected in aquatic organisms (*1–3*) and bottom sediments (*4*).

Sever and Parker (*5*) first detected dihydrophytol (3,7,11,15-tetramethylhexadecanol) in aquatic sediments and suggested that it was formed by hydrogenation of phytol under reducing conditions. Speculation was not made as to what mediates this reduction. Other researchers (*6, 7*) subsequently noted dihydrophytol in freshwater, estuarine, and marine sediments and proposed rapid microbial reduction of phytol in the surfacemost sediment as its most likely derivation.

We now report that dihydrophytol can be directly introduced into particular sediments through the deposition of fecal pellets released by certain species of zooplankton. In situ microbial production from phytol (*8*) is not a requirement for the presence of this compound in all aquatic sediments.

Controlled feeding experiments were carried out in the laboratory with two different species of zooplankton, stage 5 of the copepod *Calanus helgolandicus* and nauplii of the barnacle *Elminius modestus*. The copepods were collected fresh by tow net and the nauplii were from adults obtained in the sea area of Plymouth Sound, England. Fecal pellets were obtained from an experiment in which the copepod and the barnacle nauplii were individually fed the green alga *Dunaliella primolecta* and from an experiment in which the copepod was fed the same alga but with both organisms treated overnight with antibiotics (benzopenicillin and streptomycin, each 50 mg/liter) before the start of feeding. Each experiment was conducted over a period of 1 day, after which the fecal pellets, animals, and uneaten algal cells were separated by sequential filtering through nylon meshes (*9, 10*).

All samples, including a portion of the original algal food stock, were then collected on combusted glass fiber filters and stored under methanol in a freezer until chemical extraction (*11*). Before storage, the animals were transferred to filtered seawater and starved overnight to eliminate any gut contents; half of the fecal pellet sample obtained from each of the two copepod experiments was transferred to stoppered glass tubes containing oxic, filtered seawater and aged for 2 weeks at 10°C.

Results of the analyses of the total fatty alcohol fractions (*12*) of the alga and of each pellet sample are summarized in Table 1. Phytol was the only alcohol detected in the alga (*9*). It also dominated the total fatty alcohol composition in the copepod (*9*) and barnacle nauplii pellets. Phytol was not detectable in the total alcohol fraction of either animal, even with multiple ion detection gas chromatography–mass spectrometry, although calanid copepods are known to accumulate metabolites of phytol in their total lipid pool (*13*). Dihydrophytol was observed only in the copepod pellets and not in those of the barnacle nauplii, in either animal, or in the algal diet. The presence of dihydrophytol in copepod fecal pellets and its absence from the algal diet strongly suggests that it is a metabolite of dietary phytol in this animal.

The stereochemistry of the dihydrophytol in the fecal pellets was determined by gas chromatography (Fig. 1) and is designated as 3(*R*),7(*R*),11(*R*),15-tetramethylhexadecanol. The presence of only one isomer of dihydrophytol is consistent with its formation through a biologically controlled reduction of the double bond of phytol (*6*). An abiotic reduction would occur nonstereospecifically and yield an approximately equal mixture of *SRR* and *RRR* isomers (*14*).

Our observation of dihydrophytol in the fecal pellets of *C. helgolandicus* fed a unialgal diet demonstrates that in situ microbial production from phytol is not the only process responsible for the appearance of this compound in sediment deposits. Van Vleet and Quinn (*7*) suggested that the exclusively *RRR* isomer of dihydrophytol observed in recent sediments from the Gulf of Maine is formed

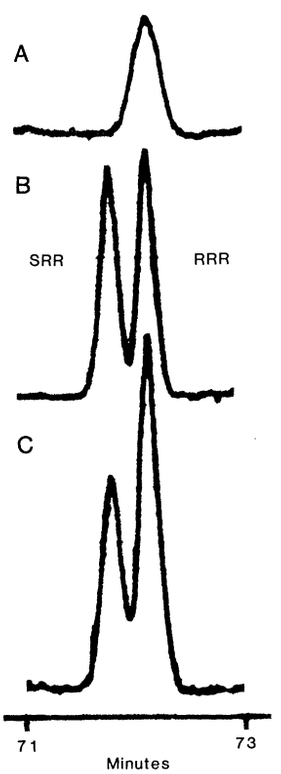

Fig. 1. Stereochemistry of dihydrophytol in copepod fecal pellets. (A) The total alcohol fraction of the untreated, unaged copepod fecal pellet sample, chromatographed as acetate derivatives on a polar glass capillary column [DEGS/PEGS, 50 m by 0.3 mm (inner diameter)] with hydrogen carrier gas (1.8 kg/cm^2) and temperature programming (20° to 125°C at 4°C per minute), yields a single peak for dihydrophytol. (B) A 1:1 mixture of *SRR* and *RRR* isomers, made by reducing commercial phytol with hydrogen and PtO_2 as catalyst, resolves into its two diastereomeric components under these chromatographic conditions. (C) The dihydrophytol of the sample coinjects with the second eluting component (*RRR*) of the standard mixture. Although the *SSS* enantiomer represents a possible structural assignment, the *RRR* configuration is assigned in view of the 2(*E*),7(*R*),11(*R*) stereochemistry of algal phytol.

Table 1. Total phytol and dihydrophytol concentrations (absolute and relative) in the algal diet and fecal pellets of two zooplankton species.

Sample	Concentration*		Phytol/ dihydrophytol
	Phytol	Dihydrophytol	
Danaliella primolecta	2.4		
Calanus helgolandicus feces			
No antibiotics or aging	2.0	0.033	61
Aging but no antibiotics	2.3	0.041	56
Antibiotics but no aging	1.7	0.015	110
Antibiotics plus aging	1.5	0.016	94
Elminius modestus feces	0.13		

*Nanograms per 10^4 cells (alga) and nanograms per fecal pellet (zooplankton).

microbially from phytol in the sediment, although concentration profiles of dihydrophytol and its precursor both remain relatively constant with depth down the core. They stated that in situ production has occurred in the surfacemost sediment on a shorter time scale than represented by the core sections examined. We draw attention to the fact that the dominant organism (in terms of biomass) in a mixed zooplankton sample collected from the Gulf of Maine in another study (*15*) was *Calanus finmarchicus*, a species closely related to our study organism. Thus, in view of our results, it seems more reasonable that the dihydrophytol in the Gulf of Maine sediments reflects a "preformed" sedimentary input in copepod fecal pellets rather than production in the bottom deposit. A relatively steady depositional input would account for the observed constant concentration profiles of dihydrophytol and phytol with sediment depth. Input of dihydrophytol to sediments through fecal pellets may be a geographically widespread phenomenon, since calanid copepods are abundant in most temperate to cold waters.

Is biological reduction of phytol to dihydrophytol mediated microbially by gut flora or enzymatically by the animal? Evidence that the animal is responsible was obtained from the experiment with *Calanus* treated with antibiotics in which dihydrophytol was still detected in the fecal pellets (Table 1). However, the absolute concentration of dihydrophytol and its concentration relative to phytol both changed in this experiment, consistent with the alternative interpretation that the microbial production of dihydrophytol in the copepod gut is only retarded, not blocked, by the antibiotic treatment. Accordingly, experiments were carried out in which the copepod pellets were aged in seawater to allow any endogenous gut flora of the copepod released in the feces to further effect lipid changes. The absolute and relative concentrations of dihydrophytol and phytol in the aged pellets showed no marked change from those in corresponding unaged samples (Table 1). This supports the interpretation that the dihydrophytol in these samples is produced by the copepod itself.

The presence of dihydrophytol in the fecal pellets of the copepod has an important biochemical implication for a metabolic pathway by which dietary phytol is converted to pristane in this organism (Fig. 2). Pristane (2,6,10,14-tetramethylpentadecane), although measurable in most zooplankton (*16*), typically shows high enrichment in calanid copepods (*2*). In addition, phytanic acid (3,7,11,15-tetramethylhexadecanoic acid) and 4,8,12-trimethyltridecanoic acid are also important lipids in species of *Calanus* (*3, 9, 13*). The product-precursor relation between the pristane and phytanic acid in two *Calanus* species and dietary phytol was shown in an earlier study (*13*) in which [U-^{14}C]phytol was used as a tracer.

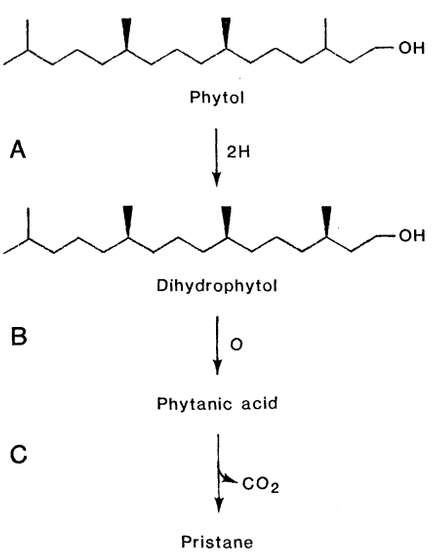

Fig. 2. Proposed metabolic pathway of phytol to pristane in calanid copepods. (A) Reduction of the double bond of phytol, producing dihydrophytol. (B) Oxidation of this alcohol to phytanic acid. (C) Decarboxylation of the acid to the C_{19} hydrocarbon.

The proposed pathway by which phytol is converted to pristane in these zooplankton was previously suggested by Blumer *et al.* (*2*) but was not substantiated, since dihydrophytol was undetected in the animal. Our observation of dihydrophytol in *C. helgolandicus* fecal pellets is evidence for this metabolic route (*17*), assuming that dihydrophytol is a short-lived intermediate in the animal. The simplest explanation for the absence of dihydrophytol in the total lipid fraction of the copepod is that reduction of dietary phytol occurs rapidly in the gut and that the reduced product is assimilated and quickly transformed to products such as phytanic acid and pristane, which accumulate to measurable levels. This route requires < 100 percent efficiency of dihydrophytol assimilation, since dihydrophytol is released in the fecal pellets. We do not know whether reduction of the phytol moiety occurs before or after hydrolysis of the ester bond in chlorophyll.

The ability of zooplankton in general to metabolize dietary phytol is not well established. Earlier studies showed that a high pristane content in the total body lipid fraction is characteristic of calanid copepods and atypical of most other types of zooplankton (*2*). Thus the ability to produce metabolites like dihydrophytol, phytanic acid, and pristane from phytol may be species-dependent. For example, in contrast to *C. helgolandicus*, fecal pellets from nauplii of *E. modestus* contained no dihydrophytol, only phytol (Table 1). Furthermore, no phytol-derived lipids were detectable in the total saponified lipid of the nauplii (*18*). Experiments with other herbivorous zooplankton may reveal that dihydrophytol is characteristic of the fecal pellets of only a small range of species. The dihydrophytol in many sediments could therefore pinpoint a fecal pellet input from a very limited group of pelagic zooplankton.

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References and Notes

1. M. Blumer and D. W. Thomas, *Science* **147**, 1148 (1965); *ibid.* **148**, 370 (1965).
2. M. Blumer, M. M. Mullin, D. W. Thomas, *Helgol. Wiss. Meeresunters.* **10**, 187 (1964).
3. M. Blumer and W. J. Cooper, *Science* **158**, 1463 (1967).
4. D. M. Didyk, B. R. T. Simoneit, S. C. Brassell, G. Eglinton, *Nature (London)* **272**, 216 (1978).

5. J. Sever and P. L. Parker, *Science* **164**, 1052 (1969).
6. P. W. Brooks and J. R. Maxwell, in *Advances in Organic Geochemistry*, B. Tissot and F. Bienner, Eds. (Technip, Paris, 1974), pp. 977–991.
7. E. S. Van Vleet and J. G. Quinn, *Geochim. Cosmochim. Acta* **43**, 289 (1979).
8. Dihydrophytylglyceryl ethers in halophilic bacteria provide another precursor in specialized sediment environments [I. McKean, G. Eglinton, K. Douraghi-Zadeh, R. G. Ackman, S. N. Hooper, *Nature (London)* **218**, 1019 (1968)].
9. F. G. Prahl *et al.*, *J. Mar. Biol. Assoc. U.K.*, in press.
10. J. K. Volkman, E. D. S. Corner, G. Eglinton, *Colloq. Int. CNRS No. 293* (1980), pp. 185–197.
11. Samples were extracted ultrasonically in 50 percent methylene chloride in methanol. The extracts were rotary evaporated under reduced pressure and then saponified by reflux in a 20 percent aqueous solution of 1 percent KOH in methanol. The neutral lipids were fractionated into 10 percent diethyl ether in hexane and chromatographed on silica gel by thin-layer chromatography, with 16 percent ethyl acetate in hexane being used as the solvent system. The fatty alcohol band, defined by phytol as the thin-layer chromatography standard, was isolated and analyzed by gas chromatography.
12. Individual fatty alcohols in each mixture were quantified as trimethylsilyl derivatives by capillary/gas chromatography on an apolar fused silica column [OV-1; 25 m by 0.3 mm (inner diameter)] with helium carrier gas (1.2 kg/cm^2), temperature programming (80° to 280°C at 4°C per minute), and 5-α-(H)-cholestane as an internal injection standard. The identity of phytol or dihydrophytol in a sample was confirmed by gas chromatography–mass spectrometry through comparison of sample spectra with those of authentic standards.
13. J. Avigan and M. Blumer, *J. Lipid Res.* **9**, 350 (1968).
14. M. Blumer and W. D. Snyder, *Science* **150**, 1588 (1965).
15. M. Blumer, M. M. Mullin, D. W. Thomas, *ibid.* **140**, 974 (1963).
16. J. R. Sargent, R. F. Lee, J. C. Nevenzel, in *Chemistry and Biochemistry of Natural Waxes*, P. E. Kollattukudy, Ed. (Elsevier, Amsterdam, 1976), pp. 50–91.
17. Our scheme is not necessarily exclusive. For example, a pathway for the degradation of phytol through phytenic acid that does not involve dihydrophytol as an intermediate is recognized in mammals [J. H. Baxter, D. Steinburg, C. E. Mize, J. Avigan, *Biochim. Biophys. Acta* **137**, 277 (1967)] and conceivably may occur competitively in the copepod.
18. F. G. Prahl, unpublished results.
19. Supported by Natural Environment Research Council grant GR3/5036. We thank C. A. Lewis for assistance in making the stereochemical assignment of dihydrophytol and the skippers and crews of R.V. *Gammarus* and R.V. *Sepia* for collecting the animals used in this study.
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Sodium-Coupled Sugar Transport: Effects on Intracellular Sodium Activities and Sodium-Pump Activity

Abstract. *Intracellular sodium activities, $(Na)_c$, were determined in* Necturus *small intestine before and after addition of galactose to the mucosal bathing solution. In the absence of galactose, $(Na)_c$ averaged 12 millimoles per liter. Within 2 minutes after the addition of galactose to the mucosal solution, $(Na)_c$ increased to a mean value of 20 millimoles per liter and then declined, in parallel with an increase in transcellular sodium transport, to a value that did not differ significantly from that observed in the absence of the sugar. The final steady state in the presence of galactose was characterized by a three- to fourfold increase in the rate of transcellular Na^+ transport in the absence of a significant increase in $(Na)_c$. Thus, the increase in steady-state basolateral pump activity cannot be attributed to an increase in the intracellular sodium transport pool.*

It is generally accepted that the entry of some sugars and all natural amino acids across the apical or luminal membranes of small intestine and renal proximal tubules in various animals throughout the phylogenetic scale is coupled to the entry of Na^+ and is energized by the electrochemical potential difference for Na^+ across those membranes (*1*). These coupled entry processes result in the "secondary active" absorption (*2*) of the sugars and amino acids and also bring about an increase in the rate of active Na^+ absorption by these epithelia. However, the effect of these processes on the intracellular Na^+ activity, $(Na)_c$, and the relation between $(Na)_c$ and the rate of active Na^+ absorption have not been clearly established.

In 1972, Lee and Armstrong (*3*) reported that the addition of 3-*O*-methylglucose to the solution bathing segments of bullfrog small intestine in vitro resulted in a decrease in $(Na)_c$ from an average value of 14.9 m*M* to one of 12.1 m*M*. Although the effect of this sugar on the rate of active Na^+ absorption was not reported, it is reasonable to infer from the results of earlier studies (*4*) that active Na^+ absorption was increased. Thus, one was confronted with two somewhat unexpected and paradoxical findings; namely (i) that $(Na)_c$ decreased despite an increase in the rate of Na^+ entry across the apical membrane and (ii) that the rate of active transcellular Na^+ absorption, which reflects the rate of Na^+ pump activity at the basolateral membrane, increased in the face of a decrease in $(Na)_c$. The single possible shortcoming of these studies was the method used to determine $(Na)_c$. Inasmuch as highly selective liquid ion-exchange resins for Na^+ were not available at that time, the investigators used two closed-tip K^+-selective microelectrodes with different selectivity coefficients for K^+ over Na^+ ($K_{K:Na}$) together with a conventional KCl-filled open-tipped microelectrode. The intracellular Na and K activities were calculated from the results of three separate sets of microelectrode impalements by simultaneously solving two Nicolsky equations (*5*). This approach, unavoidably, introduced a significant propagation of errors and some degree of uncertainty in the final mean values. Nonetheless, to the best of our knowledge, the study by Lee and Armstrong is the only one that directly addressed this issue, and the results have neither been confirmed nor challenged.

We used highly Na^+-selective liquid ion-exchanger microelectrodes to examine the effects on $(Na)_c$ of adding galactose to the solution bathing the mucosal surface of *Necturus* small intestine. Because the villus cells of this epithelium are electrically coupled (*6*), we simultaneously measured $(Na)_c$ and the electrical potential difference across the apical membrane (ψ^{mc}), the latter with conventional microelectrodes, by impaling two nearby cells. In several instances we succeeded in keeping the two single-barreled microelectrodes in place before and after the addition of the sugar to the mucosal solution, so that the results are internally paired.

Necturus maculosa (*7*) were stored in tap water at 4°C until use. The animals were anesthetized in tap water containing 0.1 percent tricainemethanesulfonate (Sigma) until all reflexes were absent. The proximal one-third of the small intestine was excised and stripped of its underlying musculature by blunt dissection, and a segment of this stripped preparation was mounted mucosal surface up in a perfusion chamber with an exposed surface area of 0.19 cm^2. The control perfusion solution contained (in millimoles per liter): Na^+, 110; Cl^-, 117; K^+, 2.5; Ca^{2+}, 1.2; Mg^{2+}, 1.2; MOPS (*7*), 2.5; and mannitol, 15. For the solutions containing galactose, 15 m*M* galactose was substituted for the mannitol. The *p*H of both solutions when equilibrated with air at 23°C was 7.2.

Conventional microelectrodes for the determination of ψ^{mc} were fabricated from glass capillary tubing (outer diameter, 1.5 mm) (*7*) and, when filled with 0.5*M* KCl, had tip resistances (measured in 0.5*M* KCl) of 40 to 60 megohms. The Na^+-selective microelectrodes were fabricated from the same capillary tubing, with the same microelectrode puller used at the same settings. These electrodes were dried by heating in a nitrogen-filled oven at 200°C for 1 hour; then 20 μl of dichlorodimethylsilane was injected into the oven through a gas-tight port and the electrodes were heated for another hour.