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Recycling of D-Glucose in Collagenous Cuticle: A Means of Nutrient Conservation?

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Summary. Transport by an epithelium, possessing an accumulating, saturable transport system in the apical membrane as well as a finite Fick permeability to the transported solute, was considered in the steady state in the case of zero *cis* concentration, and in the presence of a peripheral diffusion resistance in a layer apposing the *cis* face of the tissue (unstirred solution or structural coating). Under suitable conditions, the combination of peripheral diffusion resistance and accumulating epithelial transport may lead to recycling of solute at the *cis* face of the epithelium. This causes a decrease of the effective permeability to diffusional *trans-cis* flow across the tissue. The phenomenon is discussed in terms of epidermal D-glucose transport by the integument of aquatic animals with a collagenous cuticle, such as the seawater-acclimated polychaete worm *Nereis diversicolor*. The recycling phenomenon may be of significance to other epithelia with the function of maintaining large concentration gradients of permeating substances.

Key words: Recycling, organic solutes, D-glucose, epidermal permeability, cuticle (collagenous), unstirred layer, *Nereis diversicolor* (Annelida, Polychaeta)

List of Symbols and Fixed Parameter Values

C_m	Bulk medium solute concentration, *cis* face of epidermis	$C_m = 0$ mol cm^{-3}
C_i	Concentration of solute at interface between cuticle and unstirred medium	(mol cm^{-3})
C_s	Concentration of solute at *cis* face of apical epidermal membrane	(mol cm^{-3})
C_e	Concentration of solute in extracellular fluid, *trans*-side of epidermis	$C_e = 1.0 \times 10^{-6}$ mol cm^{-3}
D_m	Diffusion coefficient of solute in outside medium	$D_m = 6.7 \times 10^{-6}$ cm^2 sec^{-1}
D_c	Diffusion coefficient of solute in cuticle	$D_c = 7.4 \times 10^{-9}$ cm^2 sec^{-1}
δ_m	Operative thickness of unstirred medium layer	$\delta_m = 2.0 \times 10^{-2}$ cm
δ_c	Thickness of cuticle	$\delta_c = 2.0 \times 10^{-4}$ cm
J	Steady-state net flux of solute through cuticle or unstirred layer (flux is positive in direction *cis-trans*)	(mol cm^{-2} sec^{-1})
J_i^{max}	Maximal influx through saturable transport system in apical membrane	$J_i^{max} = 2.0 \times 10^{-12}$ mol cm^{-2} sec^{-1}
K_t	Transport constant, saturable system	$K_t = 1.0 \times 10^{-7}$ mol cm^{-3}
P	Epithelial permeability	(cm sec^{-1})

The fixed parameter values are based on Gomme (1981*a*). For comments on the choice of D_c, J_i^{max} and K_t, *see* Discussion.

The seawater-acclimated polychaete worm *Nereis diversicolor* absorbs D-glucose from micromolar concentrations across the integumentary surface. A previous paper (Gomme, 1981*a*) demonstrated that a phlorizin-sensitive, Na^+-dependent transport system in the apical epidermal membrane is rate-limiting to integumentary D-glucose uptake. However, peripheral integumentary structures (probably the collagenous cuticle) offers a significant resistance to D-glucose diffusion between the incubation medium and the transporting membrane. The epidermal permeability to simple (Fick) diffusion of D-glucose was estimated to be 8×10^{-8} cm sec^{-1}, part of it probably being due to the intercellular septate junctions. The large surface-to-volume ratio of *Nereis* may result in a considerable D-glucose leakage across the integument, since the concentration of the compound in the extracellular fluid is about three orders of magnitude higher than that in the ambient sea water. Comparison of net flux and influx measurements demonstrated, however, that diffusional outflux is actually quite small.

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The purpose of the present paper is to suggest a mechanism that may lower the effective permeability of the integument to D-glucose loss through the combined action of (1) the accumulating transport system in the apical epidermal membrane, and (2) the peripheral diffusion resistance: Recycling of D-glucose.

D-[6−^{3}H]glucose and ^{3}HHO diffusion in cuticle and tube walls were determined by following the steady-state rate of permeation of tracer through sheets of the isolated material. *Nereis* cuticle is not well suited to this kind of experiment, since it is perforated by gland openings and microvillar canals. Therefore, cuticle from the nematode *Ascaris suum* and tubes of the polychaetes *Chaetopterus sp.* and *Spirochaetopterus sp.* were used as models; neither of these are perforated. *Ascaris* cuticle, although it is certainly different in many respects from that of *Nereis*, has an analogous arrangement of collagen fibers in a protein-polysaccharide matrix (Bird, 1971; Lee & Atkinson, 1976). The tube of *Chaetopterus sp.* has been shown to have a similar ultrastructure as annelid cuticle, and, since the epidermis of this particular species is naked, the tube may be considered simply a shed cuticle (Brown & McGee-Russell, 1971). Investigations with the transmission electron microscope (Gomme, *unpublished observations*) have revealed a similar ultrastructure of the *Spirochaetopterus* tube. Tubes of *Chaetopterus* and *Spirochaetopterus* undoubtedly are better models of the *Nereis* cuticle than is the cuticle of *Ascaris*, but unfortunately the former materials were available only in small amounts.

The hypothesis was presented in a preliminary form at the Second International Congress of Ecology in Jerusalem, 1978.

Materials and Methods

Roundworms, *Ascaris suum* (Nematoda), were obtained at the slaughterhouse from the intestines of infected pigs. They were kept in Tyrode's solution (Altman & Dittmer, 1972) for a maximum of 5 days. For the preparation of cuticle, dorsal and ventral pieces of body wall from the mid-part of the worm were dissected free and soaked in distilled water for 1 hr. The soft tissues then were removed by a blunt forceps, taking care not to damage the cuticle. After re-equilibration in Tyrode's for at least 2 hr at 15 °C, the cuticle was mounted between two Perspex half-chambers, each holding 10 ml of Tyrode's. The exposed cuticle area was 0.68 cm^2. Labeled test substrate (^{3}HHO or D-[6-^{3}H]glucose, Radiochemical Centre, Amersham) was added in tracer quantities to one side, and a maximum of 12 pairs of samples (each sample of 10 µl) were taken from both chambers throughout a period of 24–200 hr. The samples were subjected to liquid scintillation counting (scintillant composition: 1000 ml toluene, 150 g naphthalene, 300 ml 2-ethoxyethanol, 7 g PPO). The rate of appearance of tracer on the 'weak' side was used to calculate the permeability of the cuticle. When the decrease of volume activity on the 'hot' side was significant in the course of the experiment, the permeability was determined by fitting the following equation to the data (Gomme, *unpublished*):

$$\frac{Q^*_{\text{weak}}}{Q^*_{\text{hot}}} = f(t) = \frac{1-\exp(-2PAt/V)}{1+\exp(-2PAt/V)}. \quad (1)$$

Here, the Q^*'s are the activities per 'hot' and 'weak' sample, respectively, at time t after addition of labeled compound to the 'hot' chamber. P is the permeability of the cuticle, A the exposed area, and V the volume of each half-chamber.

After the experiment, the exposed cuticle was freed and arranged flat between two glass cover slips. The cuticle thickness was taken as the difference between the thickness of the total assembly and that of the cover slides taken alone, using a micrometer caliper graduated to 0.001 cm. Values of about 0.01 cm (100 µm) were obtained. From the permeability and thickness, the 'apparent diffusion coefficient' was calculated, assuming the cuticle to be homogeneous and the partition coefficient between cuticular material and water to be unity.

The tube-dwelling polychaete worms *Chaetopterus sp.* and *Spirochaetopterus sp.* were kindly supplied by Dr. K.W. Ockelmann, Marine Biological Laboratory, University of Copenhagen. Permeability of the tube wall was determined as for *Ascaris* cuticle, except that 65% ASW (artifical sea water, *see* Gomme, 1981*a*) was substituted for Tyrode's solution. For determination of thickness, the tube wall (~0.005 cm) was placed under the microscope between a glass slide and cover slip, both having fine scratches on the side facing the tissue. The distance between the lacerated glass surfaces was determined by focusing and reading the scale on the focusing knob.

Simulation of solute recycling at the surface of *Nereis* epidermis was performed on the UNIVAC 1110 computer, using a program written in FORTRAN (ASCII). The program is available from the author on request.

Results

Diffusion Experiments

The data in Table 1 indicate that all three model materials restrict the diffusion of hydrophilic compounds, as compared to the diffusion in aqueous solution. For the following reason, the apparent D-glucose diffusion coefficient should be regarded as the approximate maximal value. Although the radiolabeled D-glucose was purified by thin-layer chromatography prior to use, it cannot be excluded that minute quantities of labeled impurities account for part of the extremely small *trans*-cuticular flux; such problems often are encountered in permeability studies on biological membranes (Christensen, 1975).

The *Ascaris* cuticle contains lipid in a thin superficial layer (Bird, 1957). In two additional experiments, ^{3}HHO permeability was investigated after 60-min extraction of lipid with 1% Triton X-100. The permeability was indistinguishable from that of untreated controls. Although this result does not exclude lipid involvement in the low permeability of *Ascaris* cuticle to D-glucose, it appears that the main restriction of the diffusion of hydrophilic solutes is due to the nonlipid part of the cuticle. This faci-

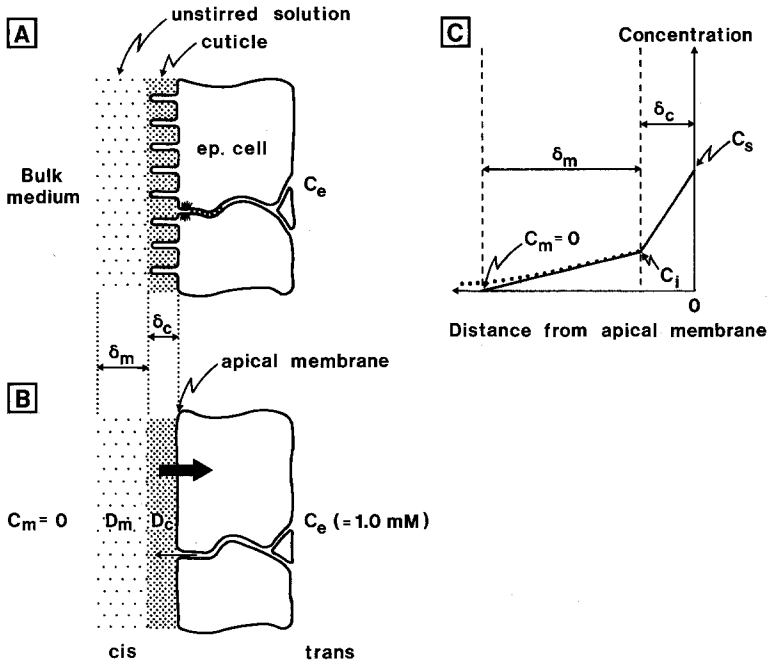

Fig. 1. Model of the *Nereis* integument. (*A*) Moderately simplified, showing epidermal cells with apical microvilli, cuticle, and an unstirred layer of bathing medium. (*B*) Level of simplification used for model calculations; microvilli ignored, cuticle assumed homogeneous. (*C*) Concentration profile in the layers peripheral to the apical epidermal membrane (cuticle and unstirred medium). The dotted curve indicates actual concentration profile in medium with gradually increasing convection in direction towards well-stirred bulk phase; the full line in the same region represents linear concentration profile resulting from concept of 'operational thickness of unstirred layer' (*cf.* Dainty & House, 1966)

Table 1. Diffusion resistance of collagen-polysaccharide cuticles and tube walls

Test molecule	Test material	Apparent Diff. coeff.	Diff. coeff.
		Material ($cm^2\ sec^{-1}$)	Free water ($cm^2\ sec^{-1}$)
H_2O	*Ascaris* cuticle	1.2×10^{-7} $\pm1.9\times10^{-8}$ (N=4)	2.4×10^{-5}
	Spirochaetopterus tube	2.1×10^{-8}	2.4×10^{-5}
	Chaetopterus tube	7.7×10^{-6}	2.4×10^{-5}
D-glucose	*Ascaris* cuticle	8.4×10^{-10}	6.7×10^{-6}

Standard values given in the right column are taken from Weast (1969) and from Wang, Robinson and Edelman (1953). Apparent diffusion coefficients are calculated from experimental permeabilities, assuming a unity partition coefficient. N=1, if not shown otherwise.

litates comparison with polychaete cuticle and tube wall, in which no lipid layer has been described.

In the previous paper (Gomme, 1981*a*), indirect evidence was presented, implicating the *Nereis* cuticle as a major determinant of the 'peripheral diffusion resistance'. An effective diffusion coefficient for D-glucose of the order of 10^{-9} $cm^2\ sec^{-1}$ was stipulated. The data of Table 1 show that effective D-glucose diffusion coefficients of this order or lower may well apply to at least some collagen-polysaccharide materials.

Computer Simulation

A simple model was established to evaluate the potential significance of the recycling phenomenon. Concentration of D-glucose in the medium was assumed to be zero in all calculations. With reference to Fig. 1, the following equation was used to describe the steady-state net flux J at any level peripheral to the apical membrane:

$$J=\frac{J_i^{\max}C_s}{K_t+C_s}+P(C_s-C_e)=\frac{D_c}{\delta_c}(C_i-C_s)$$
$$=\frac{D_m}{\delta_m}(C_m-C_i). \qquad (2)$$

For simplicity, influx across the apical membrane is assumed to obey simple Michaelis-Menten kinetics, overlaid by a linear diffusion component, $PC_s \cdot J_i^{\max}$ is the maximal value of influx via the saturable system, K_t is the transport constant. Elimination of C_i in Eq. (2) gives a quadratic expression for C_s:

$$KC_s^2+LC_s+M=0, \qquad (3)$$

in which

$$K=\frac{(D_c/\delta_c)(D_m/\delta_m)}{D_c/\delta_c+D_m/\delta_m}-P,$$

$$L=\frac{(D_c/\delta_c)(D_m/\delta_m)}{D_c/\delta_c+D_m/\delta_m}(C_m-K_t)+P(C_e-K_t)-J_i^{\max}$$

$$M=\frac{(D_c/\delta_c)(D_m/\delta_m)}{D_c/\delta_c+D_m/\delta_m}K_t C_m+PK_t C_e.$$

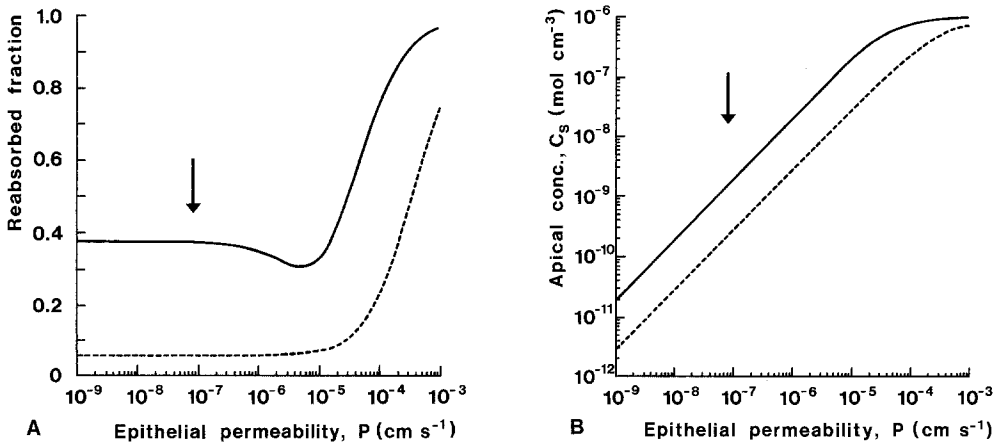

Fig. 2. Result of model calculations on integumentary D-glucose exchange in *Nereis* integument. Bulk medium concentration assumed to be zero. (*A*) Plotted as a function of epithelial permeability: The fraction of D-glucose initially lost from the apical surface of the epithelium, but reabsorbed through the apical cell membrane due to the presence of a peripheral diffusion resistance. Solid line: cuticle present; model parameters used are those given in parameter list. Broken line: cuticle not present ($D_c \to \infty$); other parameters unchanged. (*B*) Solute concentration just outside the apical epithelial membrane as a function or epithelial permeability. Symbols as in (*A*)

Solving for C_s and inserting the proper value into the left part of Eq. (2) gives the result for J, expressed as a function of the parameters J_i^{max}, K_t, P, D_c, δ_c, D_m, δ_m, and C_e. The computer program performs the necessary calculations and presents the results in tabular and graphic form.

According to the recycling concept, part of the D-glucose released from the apical epidermal surface by diffusion may be reabsorbed by the apical D-glucose transport system(s) to an extent dependent on the diffusion-restricting properties of the cuticle, and on the characteristics of the membrane transport system(s). We assume that all D-glucose outflux occurs through the septate intercellular junctions from an extracellular D-glucose concentration of 1.0 mM (*cf.* Gomme, 1981*a*; $C_e = 1.0 \times 10^{-6}$ mol cm^{-2}).

The solid line in Fig. 2(*A*) shows the fraction of reabsorbed D-glucose, $(PC_e - |J|)/PC_e$, plotted as a function of epidermal permeability P, but with constant peripheral diffusion resistance. The model parameters selected (and shown in the parameter list) are compatible with experimental data on D-glucose transport by *Nereis* integument (Gomme, 1981*a*), although the value of D_c is high; *see* Discussion. A special mention only need be made of the selected values of K_t and J_i^{max}: As discussed in the above paper, a conventional kinetic analysis of D-glucose influx is not possible for *Nereis* epidermis. For simplicity, however, I ascribe to the transport system standard Michaelis-Menten kinetics [*cf.* Eq. (2)], using values of K_t and J_i^{max} that give a reasonable fit to the observed data on concentration dependence of influx within the relevant range of C_s (and C_m) (Gomme, 1981*a*, Table 5).

When the permeability (P) of the epidermis is low ($< 10^{-6}$ cm sec^{-1} under the conditions of Fig. 2), the reabsorbed fraction is almost independent of P. For increasing permeabilities, the reabsorbed fraction is first diminished as the apical transport system is unable to cope with all the additional substrate exiting from the epidermis. Increasing P still further, however, leads to a sharp increase of the reabsorbed fraction, which eventually approximates 1. This increase is due to the peripheral diffusion resistance assuming a larger share of the total resistance of the 'cuticulo-epidermal complex', thereby allowing higher concentrations (C_s) to build up close to the apical membrane. Hence a larger concentration gradient is maintained across the cuticle. Values of C_s as a function of P are given by the solid line in Fig. 2(*B*).

The broken line in Fig. 2(*A*) represents the reabsorbed fraction when the cuticle is absent, and only the 200-μm unstirred solution layer is left to constitute the peripheral diffusion resistance. For the parameter values selected, the reabsorbed fraction is modest, except for very high permeabilities ($P > 10^{-4}$ cm sec^{-1}); the corresponding plot of C_s *vs.* P is shown by the broken line in Fig. 2(*B*).

The epidermal permeability is used as the independent variable in Fig. 2 only to illustrate the general properties of the model. The epidermal D-glucose permeability has been determined experimentally (8×10^{-8} cm sec^{-1}), and it is indicated in the Figure by arrows. For realistic values of all parameters, the model therefore predicts that close to 40% of the D-glucose initially released by diffusion across the epithelium is again reabsorbed. Due to the limitations of the model, however, this estimate should

not be taken as definitive; it merely indicates that solute recycling at the apical epidermal surface may be a significant factor in reducing the effective epidermal permeability to D-glucose.

Discussion

The data presented in Table 1 give an order-of-magnitude indication of the diffusion coefficients for H_2O and D-glucose in *Ascaris* cuticle[1] and polychaete tube walls. The protein-polysaccharide matrix seems to reduce the diffusivity of hydrophilic molecules by at least 2–3 orders of magnitude, when compared to free aqueous solution. As demonstrated earlier (Gomme, 1981*a*), a diffusion-restraining effect of this magnitude is sufficient to account for the 'peripheral diffusion resistance' of the *Nereis* integument. Further experiments are in progress to more accurately define the diffusion resistance of polychaete cuticle to small organic molecules.

The mathematical model used to evaluate the significance of D-glucose recycling has some shortcomings: (1) It ascribes simple Michaelis-Menten kinetics to D-glucose influx across the apical membrane, although the kinetic parameters used do not seem well-founded experimentally; however, the calculated influxes are in satisfactory agreement with experimental values in the relevant range of C_m (Gomme, 1981*a*). (2) The model assumes the physical equivalent of the peripheral diffusion resistance to be a slab of constant thickness throughout the epidermal surface. Since the cuticle undoubtedly accounts for most or all of the peripheral resistance, this assumption is only a rough approximation to reality due to the presence of epidermal microvilli (*see* Fig. 1). Any specific distribution of transport activity and permeability characteristics along the folded apical face of the epidermis cannot be taken into account.

The crudeness of the model, however, parallels the scant experimental knowledge of the system. We have no information on the actual distribution of transport and permeability properties at the apical epidermal face, and there would be no data to feed into an elaborate model, taking into account the exact geometry of the system. To not fortuitously exaggerate the diffusion-restraining effect of the cuticle, the value of D_c used here is rather high.

By intuition, the following predictions for the two limiting cases may be given: If the main part of epidermal D-glucose outflux occurs through the intercellular junctions or the nonmicrovillous part of the apical membrane, and if D-glucose transport activity is equally distributed over the entire apical membrane (microvilli included), the model will underestimate the reabsorbed fraction. If on the other hand, D-glucose outflux occurs preferentially across the tips of the microvilli (an unlikely situation), the capacity for reabsorption will be largely overestimated. In the latter case, D-glucose outflux will be from a smaller concentration (in the epidermal cells) than that prevailing in the extracellular fluid (Gomme, 1981*a*).

If the septate junctions are assumed to provide the major part of the integumentary D-glucose outflux, a D-glucose concentration gradient would develop parallel to the apical surface. The area of the apical membrane exposed to significant D-glucose concentrations would thereby be reduced. The effect of such a situation should be further explored: Another complication arises from the presence of gland openings, which perforate the cuticle. However, the density of these pores is believed to be small enough to have only an insignificant effect on solute recycling.

The experimental data presented, taken together with the model calculations, show that recycling of D-glucose within the 'epidermal-cuticular complex' is likely to be a factor in reducing the loss of this compound across the *Nereis* surface. Epidermal transport systems for monosaccharides and amino acids are generally present in marine polychaetes, and they occur in many other groups of aquatic invertebrates as well (Stephens, 1967, 1972; Jørgensen, 1976; Sepers, 1977; Stewart, 1979; Gomme, 1981*b*). Often, a structural coating (cuticle) is present on the transporting surface. The recycling concept presented in this report therefore provides a new perspective from which to evaluate the biological significance of nutrient transport by the surface membranes of aquatic invertebrates.

A large number of animal epithelia are involved in creating and/or maintaining large concentration gradients between aqueous phases. Recycling of solute at the low-concentration side of such epithelia may be operative in establishing such gradients. It should be emphasized that the presence of a structural coating on the epithelium (*viz.* cuticle) is not necessarily a prerequisite for the operation of such a mechanism: Unstirred layers are ubiquitously present along epithelial surfaces, and, although without much effect under physiological conditions in the example given above, they may be significant in other situations. Work is in progress in this laboratory to elucidate the applicability of the recycling concept to epithelia other than *Nereis* epidermis.

[1] The *Ascaris* integument has been reported to be almost impermeable to D-glucose (Castro & Fairbairn, 1969), which is compatible with the present results: For a cuticular thickness of 0.01 cm, the permeability is less than 8.4×10^{-8} cm sec^{-1}.

References

Altman, P.L., Dittmer, D.S. 1972. Biology Data Book. Vol. I, 2nd ed. Federation of American Societies for Experimental Biology, Bethesda, Maryland

Bird, A.F. 1957. Chemical composition of the nematode cuticle. Observations on individual layers and extracts from these layers in *Ascaris lumbricoides* cuticle. *Exp. Parasitol.* **6**:383–403

Bird, A.F. 1971. The structure of nematodes. Ch. 3. Academic Press, New York

Brown, S.C., McGee-Russell, S. 1971. *Chaetopterus* tubes. Ultrastructural architecture. *Tissue Cell* **3**:65–70

Castro, G.A., Fairbairn, D. 1969. Comparison of cuticular and intestinal absorption of D-glucose by adult *Ascaris lumbricoides*. *J. Parasitol.* **55**:13–16

Christensen, H.N. 1975. Biological Transport. Benjamin, Reading, Mass.

Dainty, J., House, C.R. 1966. 'Unstirred layers' in frog skin. *J. Physiol. (London)* **182**:66–78

Gomme, J. 1981*a*. D-glucose transport across the apical membrane of the surface epithelium in *Nereis diversicolor*. *J. Membrane Biol.* **62**:29–46

Gomme, J. 1981*b*. Epidermal nutrient absorption in marine invertebrates: A comparative analysis. *Am. Zool. (in press)*

Jørgensen, C.B. 1976. August Pütter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. *Biol. Rev.* **51**:291–329

Lee, D.L., Atkinson, H.J. 1976. The Physiology of Nematodes. McMillan Press, London

Sepers, A.B.J. 1977. The utilization of dissolved organic compounds in aquatic environments. *Hydrobiologica* **52**:39–54

Stephens, G.C. 1967. Dissolved organic material as a nutritional source for marine and estuarine invertebrates. *In:* Estuaries. G.H. Lauff, editor. pp. 367–373. American Association for the Advancement of Science, Washington

Stephens, G.C. 1972. Amino acid accumulation and assimilation in marine organisms. *In:* Nitrogen Metabolism and the Environment. J.W. Campbell and L. Goldstein, editors. pp. 155–184. Academic press, New York

Stewart, M.G. 1979. Absorption of dissolved organic nutrients by marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* **17**:163–192

Wang, J.H., Robinson, C.V., Edelman, I.S. 1953. Self-diffusion and structure of liquid water. III. Measurement of self-diffusion of liquid water with H^2, H^3, and O^{18} as tracers. *J. Am. Chem. Soc.* **75**:466

Weast, R.C. 1969. Handbook of Chemistry and Physics, 50th ed. The Chemical Rubber Co., Cleveland, Ohio

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