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TRANSPORT OF EXOGENOUS D-GLUCOSE BY THE INTEGUMENT OF A POLYCHAETE WORM (*NEREIS DIVERSICOLOR* MÜLLER)

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SUMMARY

1. Integumentary exchange of radio-labelled D-glucose in the brackish-water polychaete worm *Nereis diversicolor* (Annelida; Polychaeta) was investigated.

2. In animals acclimated to 50% sea water, the influx of D-glucose was measured, and shown to occur largely across the outward-facing membranes of the epidermal cells.

3. Transfer of exogenous D-glucose across the outer membranes occurs by facilitated transfer, involving two different transport systems. One of these has a K_t (transport constant) of $\simeq 5$ μM, i.e. of the order of magnitude of environmental D-glucose concentrations. The K_t for the other system is at least ten times higher.

4. The D-glucose is rapidly metabolized by the epidermal cells. Small amounts of unmetabolized D-glucose are released to the extracellular fluid, and probably to the medium.

5. The maximal D-glucose outflux through the apical border of the epidermal cells is at least 4 times smaller than the outflux through the baso-lateral border.

6. A maximum value is given for the diffusion permeability of D-glucose through the intercellular spaces of the integument. Applying this figure, the loss by intercellular diffusion was found not to exceed the D-glucose influx into the epidermal cells.

INTRODUCTION

The possible nutritional role for aquatic animals of organic material dissolved in natural waters has been a subject of considerable controversy since the beginning of this century (Pütter, 1909; Krogh, 1931; Jørgensen, 1966; Stephens, 1968; Johannes, Coward & Webb, 1969). Krogh (1930) found that some fresh-water animals could absorb D-glucose at concentrations ranging from 30 to 225 μM, but their simultaneous total loss of oxidizable organic compounds exceeded the input via the sugar alone. In a review of the early literature, Krogh (1931) concluded that in aquatic animals there was no evidence of a net uptake of dissolved organic matter (DOM) at naturally occurring environmental concentrations, and that the integument of these

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animals was generally impermeable to organic molecules. During the last decade, a considerable number of soft-bodied marine and estuarine invertebrates were shown to possess the capability of sequestering radioactively labelled monosaccharides, amino acids, and fatty acids in natural concentrations from sea water (Stephens, 1962, 1963, 1964, 1968, 1972; Ferguson, 1967, 1968, 1970; Ernst & Goerke, 1969; Little & Gupta, 1969; Southward & Southward, 1970; Péquignat, 1970, 1972; Testerman, 1972). This uptake was found to take place mainly or exclusively across the body surface (Stephens, 1962, 1963, 1968). The results have been interpreted by these authors as providing evidence for DOM playing a significant nutritional role to the animals concerned.

It is well known that the passage of a labelled compound across a biological membrane often does not disclose the rate – or even the direction – of *net* movement of the substance. Due to the large outwardly directed concentration gradient across the body surface of aquatic invertebrates, the efflux of osmotically active organic substances may well exceed the influx. It has therefore been questioned whether the mentioned uptake of radioactive organic compounds is due to absorption processes of nutritive significance (Johannes *et al.* 1969; Johannes & Webb, 1970). Further research, based on a tenable experimental approach, concerning the role of DOM as a source of energy to aquatic animals is therefore warranted.

The present study was conducted to examine the integumentary exchange of D-glucose in the brackish-water polychaete worm, *Nereis diversicolor* Müller. Results reported in this paper have been presented previously in a preliminary form (Gomme & Ahearn, 1972; Ahearn & Gomme, 1972).

MATERIALS AND METHODS

Animals. Animals were obtained from shallow water at Vellerup Vig, a small bay opening into the south-eastern region of the Isefjord, Zealand, Denmark. The ecology of this area has been described by Rasmussen (1973). A preliminary estimate of total carbohydrate in the water at the collection site was made by the phenol–sulphuric acid method (Strickland & Parsons, 1968): in the water immediately above the mud, values (expressed as D-glucose equivalents) ranged from 0·12 to 3·8 μM. Interstitial seepage water values were between 1·6 and 3·8 μM.

Worms were collected by means of hand shovels throughout the year, and were brought to the laboratory in chilled (approx. 5 °C) containers. The animals were kept at 15 °C in artificial sea water (Hale, 1955), diluted to 50%, corresponding to a salinity of 17·2‰. The worms took residence in small glass tubes (5 × 100 mm) which served as artificial burrows, permitting normal respiratory movements. The water was changed at intervals to prevent the build-up of organic material.

Since the uptake of exogenous D-glucose is known to depend upon the state of salinity acclimation (Gomme & Ahearn, in preparation), worms were maintained in 50% sea water for at least 5 days (but usually not longer than 15 days) before experimentation. The animals were not fed while in the laboratory. Only animals in the size range 150–500 mg were used. The mean weight was 326 mg.

General experimental procedures. Acclimated animals were weighed to the nearest mg on an analytical balance and transferred individually to 10 ml of the acclimation

medium in a glass vial, immersed in a water bath at 15 °C. After approximately 15 min, each animal was transferred to another vial in the bath, containing 10 ml of incubation medium. This consisted of autoclaved 50% sea water to which D-[6-^{3}H]-glucose or D-[U-^{14}C]glucose (New England Nuclear Corp.) had been added together with inactive D-glucose to concentrations between 1 and 100 μM, and activities of 100–300 μCi l^{-1}. Following incubation, usually of 1–30 min duration, the animals were removed, rinsed rapidly (10 sec) in unlabelled 50% sea water, and extracted for 48 h in 10 ml 70% ethanol at room temperature. The recovery of labelled material was 85–90%, and radioactivity incorporated into ethanol-insoluble compounds constituted less than 2% of the total. Extracts were counted in a liquid scintillation counter.

Samples for radioactivity determination were taken from the medium before and after incubation, and invariably the activity per volume was found to remain constant during the experiment.

In some cases, animals were incubated in a D-[6-^{3}H]glucose medium and rinsed as described, but were then transferred to 25 ml unlabelled 50% sea water. Samples were taken from this medium to investigate the time-course of ^{3}H-activity washout.

Alternatively, incubation was performed in a D-[U-^{14}C]glucose medium, and during washout the animals were kept in corked vessels, the medium being constantly aerated. CO_2 in the exhaled air stream was absorbed in an ethanolamine: ethyleneglycol monomethylether 'trapping solution' (1:2 v/v), and the activity determined by liquid scintillation counting.

When coelomic fluid samples were taken, the animals were dried with tissue-paper, and a tapering capillary with a tip diameter of approximately 0·1 mm was inserted through the dorsolateral body wall into the coelomic space. Cellular material in the sample was removed by centrifugation, and a measured volume of the supernatant taken by a Hamilton syringe for further analysis.

For collection of blood samples, the anterior end of the alimentary canal was exposed by cutting a longitudinal slit in the dorsal body wall through the first 10–15 segments. After careful removal of coelomic fluid, the blood vessels adjacent to the pharynx were severed, and the extravasating blood was drawn into a capillary. A bright red colour was taken to indicate that no significant contamination with the colourless coelomic fluid had occurred.

Analytical methods. Separation of non-volatile labelled compounds in ethanolic extracts and in samples of extracellular fluid was performed by thin-layer chromatography of silica gel G (Merck), using the solvent system isopropanol: n-butanol: redistilled H_2O (5:3:2). Samples were evaporated to dryness in a stream of N_2 and redissolved in 3 × 20 μl methanol for spotting. Scanning of the plates for radioactivity was conducted by transferring 0·5 cm units of the separation path directly to scintillation vials for counting. The ethanol-containing liquid scintillation fluid (see later) was capable of eluting the labelled material almost quantitatively, there being no significant adhesion of labelled material to the silica gel in the counting vial.

Radioactivity determinations. The radioactivity in the various types of samples was determined by liquid scintillation counting, using a Packard model 3320 liquid scintillation spectrometer. The common scintillation fluid contained 3·5 g PPO and 140 mg dimethyl-POPOP per litre of toluene: ethanol (100:43). When counting

samples containing inulin-^{3}H (see later), Bio-Solv BBS-3 (Beckman) was added to avoid precipitation of the labelled compound (Bray, 1970). For determination of $^{14}CO_2$-activity released during washout, 3 ml of 'trapping solution' was added to 15 ml of a scintillation fluid containing 5 g PPO and 333 ml ethyleneglycol monomethylether/l toluene (Rapkin, 1969). The same mixture was used when total ^{14}C-activity (including metabolically formed $^{14}CO_2$), and ^{14}C-activity associated exclusively with organic compounds, had to be measured in samples of coelomic fluid. The organic component was determined separately by evaporating a known volume of sample in a counting vial before addition of the counting mixture. In all cases, quench correction was performed by means of the external standard method.

Calculations. Statistical treatment of the data was carried out in the manner of Hald (1952). Regression analysis on the basis of linear and non-linear functions according to the principle of least squares was performed by means of a digital computer. Weights were assigned to the experimental values as indicated in the figure legends.

RESULTS

A. Evaluation of integumentary D-*glucose uptake*

After incubation of *N. diversicolor* in 50% sea water containing labelled D-glucose in micromolar concentrations, the activity of ethanolic extracts (expressed as dpm/mg animal) can be considered a linear function of time for incubations not exceeding 30 min. Fig. 1 illustrates the uptake of extractable activity from a 5·0 μM D-[6-^{3}H]-glucose medium with a specific activity of 236 dpm pmol^{-1} (107 Ci mol^{-1}). The slope of the straight line with zero intercept corresponds to a rate of increase in extract activity of 15·3 dpm/mg animal per min.

The extractable activity may have originated from more than one source. An inadequate removal of adhering medium or a specific adsorption of labelled substance to the surface structures of the animal should provide the same contribution to uptake values (dpm mg^{-1}) irrespective of the length of the incubation period, and therefore the uptake curve should show a positive intercept, which is not the case. Statistical analysis of the data shows that the smallest volume of adhering medium that would produce a detectable positive intercept is 0·8 μl per average-sized animal (300 mg). In contrast, the actual rate of increase in extractable radioactivity corresponds to each animal 'clearing' approximately 3·9 μl per minute or 117 μl during the experiment.

To investigate whether micro-organisms on the animal surface participate in the uptake of labelled material, 7 groups of 10 animals were pre-incubated for different periods in 50% sea water to which antibiotics were added (500 000 units l^{-1} penicillin, 200 mg l^{-1} streptomycin and 50 mg l^{-1} chloramphenicol). After 10–20 h pre-incubation, this mixture has been found effective in reducing the uptake of [^{14}C]glycine by micro-organisms on the exoskeleton of crustaceans (Anderson & Stephens, 1969). Comparison with a control group by means of a *t*-test demonstrated that up to 40 h pre-incubation of *N. diversicolor* did not affect the uptake during incubation. This, and the results of other experiments to be presented in this paper, seems to preclude any significant contribution by surface micro-organisms to the radioactivity of extracts.

To investigate drinking of the incubation medium as a contribution to the uptake of labelled material, a group of 9 animals was exposed for 30 min to 50% artificial

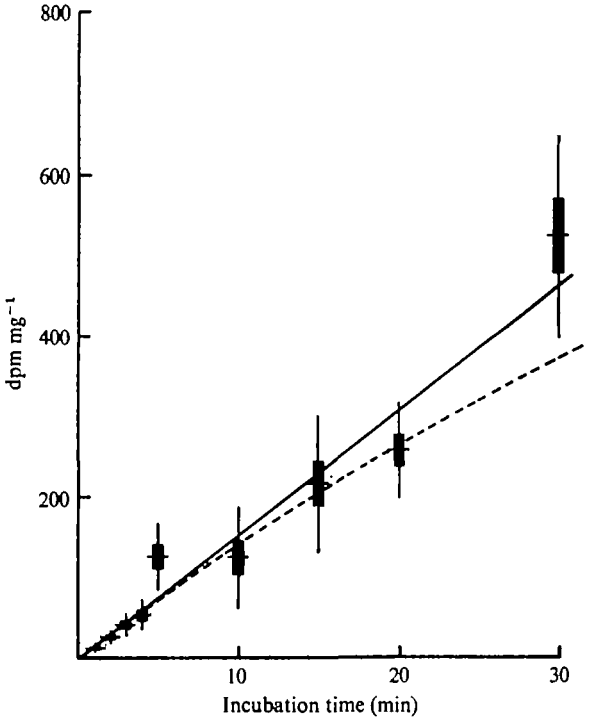

Fig. 1. Time-course of whole-animal radioactivity uptake on incubation in 5·0 μM D-[6-^{3}H]-glucose (1180 dpm/μl medium). The straight line was obtained by weighted least-squares linear regression analysis, assuming that the standard error in the dependent variable is a linear function of time. The intercept on the vertical axis is $-1{\cdot}9 \pm 6{\cdot}3$ dpm mg^{-1}, and the slope of the line is $15{\cdot}3 \pm 1{\cdot}6$ dpm mg^{-1} min^{-1} (95% limits). The curve was plotted according to equation (10) of the Appendix, using results presented in Section D. In this and subsequent figures, vertical lines and rectangles represent standard deviation and standard error, respectively. $N = 82$.

sea water containing [^{3}H]inulin. The animals were then washed in unlabelled medium as usual, killed by rapid freezing, and homogenized. From the activity of the homogenate supernatant, the average volume of medium associated with an animal after washing was found to be 2·65 μl. This amount includes material adhering to the external surface* as well as that swallowed. Comparison of this result with that mentioned in relation to Fig. 1 ('clearing' of 117 μl in 30 min) is convincing evidence that drinking does not significantly contribute to the uptake of labelled material.

In another experiment, 2 groups of 10 animals were exposed for 30 min to 5·0 μM D-[6-^{3}H]glucose. In one group, thin cotton thread secured around head and tail ends prevented connexion between the incubation medium and the intestinal tract. No significant difference between rates of uptake in the experimental and the control group could be detected. Drinking, therefore, plays a negligible role in activity uptake under the experimental conditions used throughout this study.

* Subsequent experiments have shown that the volume of incubation medium adhering to the surface after washing is only $\simeq 1$ μl (average 300 mg animal). Part of the [^{3}H]inulin associated with the animal in the present experiment was possibly trapped in the cuticle (see Fig. 12, Plate 1), since a washing period of 10 sec, due to the low diffusivity of the inulin molecule, may be insufficient to remove all [^{3}H]-inulin present after 30 min incubation.

Uptake of radioactive material from a medium containing labelled D-glucose thus represents the penetration of tracer molecules into or through the integument or parts of it. From the data shown in Fig. 1 it can be calculated that the activity taken up by a 300 mg worm during a 30 min exposure amounts to $\simeq$ 1% of the total activity present in the incubation vessel. The composition of the medium must therefore have remained essentially constant in both activity per volume and chemical identity of the labelled compound.

Furthermore, the linearity and zero intercept of the uptake curve (Fig. 1) suggest that for incubations shorter than 30 min, backflux to the medium of label is small relative to the influx. This is supported by the results of washout experiments which will be described in Section D.

On these grounds, the rate of activity uptake found from Fig. 1 (15·3 dpm mg^{-1} min^{-1}) can be considered a measure of D-glucose influx across some rate-limiting barrier in the surface of the animal. Using the specific activity of D-[6-^{3}H]glucose in the medium (236 dpm $pmol^{-1}$), the integumentary D-glucose influx was calculated to be 0·064 pmol mg^{-1} min^{-1}.

B. The internal distribution of labelled compounds during incubation in media containing radioactive D-*glucose*

Radioactivity in blood and coelomic fluid

In polychaetes, the coelomic space and the vascular system are morphologically distinct (Dales, 1968), and samples can be taken separately from these compartments. Fig. 2 illustrates the linear time-course of activity uptake into the coelomic fluid during incubation of animals in a medium with 5·0 μM D-[6-^{3}H]glucose: there was no statistically significant time-lag in the uptake. The rate of uptake was 26% of that into the whole animal (cf. Fig. 1).

The internal distribution of label was further investigated by comparing the activity of extracellular fluid samples with that of the total ethanol-extractable material (Fig. 3). First coelomic fluid and subsequently blood were drawn from the animals immediately upon removal from the incubation medium, and before ethanolic extraction. Sampling of coelomic fluid required 1–2 min, but the collection of an adequate volume of blood usually took 6–9 min, a relatively long period in comparison with the shortest incubation time used (15 min). The extent of any redistribution of labelled material within the animal during this interval is not known. The activity removed by sampling represented less than 5% of the total amount accumulated.

It is seen from Fig. 3 that the blood acquires a higher activity than the coelomic fluid, but both extracellular fluids are far below the whole animal average, as determined from the ethanolic extracts. The body surface is furnished with a fine net of capillaries, many of which are located just beneath the epidermis. The activity distribution observed is therefore in good agreement with an integumentary D-glucose uptake.

Using [U-^{14}C]sucrose as a marker, the extracellular volume was found to be approximately 40% of the total body volume (Gomme & Ahearn, 1975). If this value is arbitrarily divided into 25% coelomic space and 10% blood space (leaving 5% for tissue interstitial fluid), it can be estimated from Fig. 3 that only $\simeq$ 5% of the total

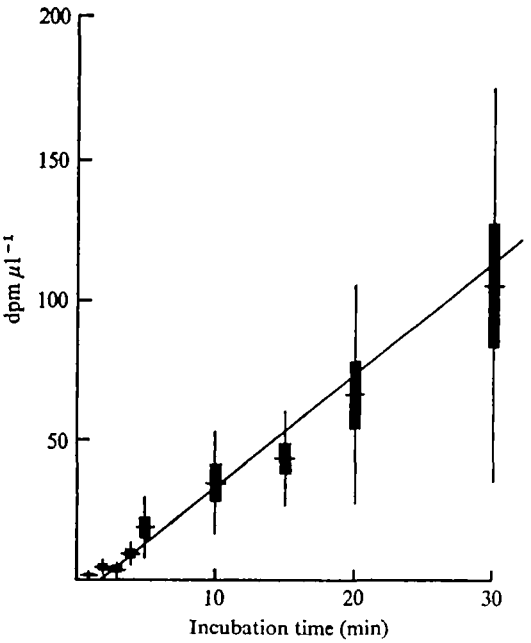

Fig. 2. Time-course of ^{3}H-activity entry into the coelomic space by incubation in 5·0 μM D-[6-^{3}H]glucose (specific activity: 236 dpm pmol^{-1}). The straight line was obtained as in Fig. 1. The y-intercept is $-7{\cdot}0 \pm 7{\cdot}6$ dpm μl^{-1} and the slope $4{\cdot}0 \pm 0{\cdot}5$ dpm μl^{-1} min^{-1} (95 % limits). $N = 76$.

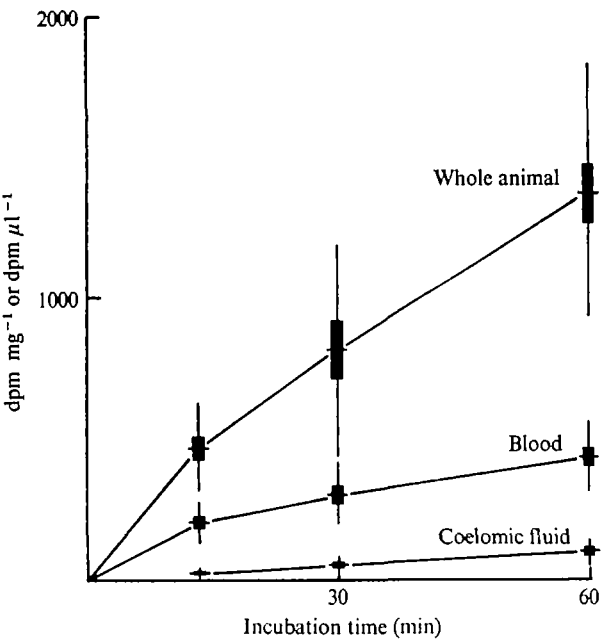

Fig. 3. Time-course of radioactivity uptake into blood, coelomic fluid and whole animal by incubation in 5·0 μM D-[6-^{3}H]glucose (specific activity: 318 dpm pmol^{-1}). $N = 44$.

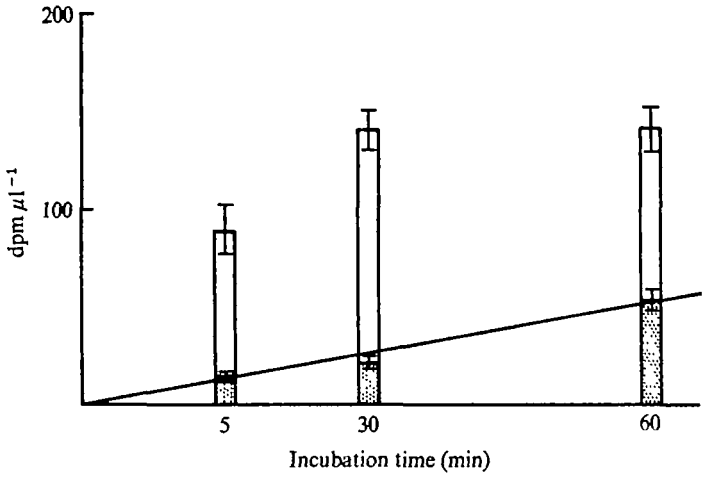

Fig. 4. Time-course of ^{14}C-activity entry into the coelomic space by incubation in 5·0 μM D-[U-^{14}C] glucose (specific activity: 523 dpm pmol^{-1}). The shaded parts of the columns represent ^{14}C-activity present as organic material, and unshaded areas represent $H^{14}CO^-_3$. The straight line was fitted by eye. Vertical bars show S.E.M. $N = 30$.

ethanol-extractable activity is found within the two extracellular compartments after 60 min incubation. In spite of the inaccuracy of this estimate, it is most likely that the major part of the labelled material under these conditions is intracellular.

In another experiment, animals were exposed to 5·0 μM D-[U-^{14}C]glucose and the time-course of ^{14}C-activity entry into the coelomic fluid was investigated (Fig. 4). The coelomic fluid was analysed for $H^{14}CO_3^-$ which may be formed metabolically from exogenous D-[U-^{14}C]glucose; the rest of the radioactivity must be attributed to labelled organic compounds. To identify the latter, samples of coelomic fluid and blood from an additional experiment were subjected to silica-gel thin-layer chromatography. The preparative procedure used resulted in elimination of volatile compounds (such as the final oxidation products of radioactive D-glucose) before the samples were applied to the thin-layer plate. As seen from Fig. 5, the only non-volatile labelled substance demonstrated was D-glucose. It can therefore be concluded that the compounds originating from exogenous D-glucose and entering the extracellular fluid during integumentary D-glucose uptake are the products of complete metabolic degradation (water and bicarbonate), and D-glucose proper.

Radioactivity in animal extracts

The results of thin-layer chromatography of animal extracts, obtained after incubation for different periods in 5·0 μM D-glucose (^3H- or ^{14}C-labelled) are shown in Fig. 6. Two major activity peaks are present, one coinciding with the standard D-glucose spot ($R_{st} = 1$), the other at the origin. The ratio of the activities of these peaks is almost constant during 240 min incubation. When the material in the origin-peak was eluted, exposed to alkaline phosphatase at pH 8·9 (Tris–HCl buffer) and rechromatographed, the activity followed the movement of D-glucose. Furthermore, when animal extracts were treated with the same enzyme prior to chromatography, the origin-peak did not appear. It is concluded that the labelled material remaining at the origin of the separation path is a D-glucose phosphate. The smaller peaks in the chromatogram have not yet been identified.

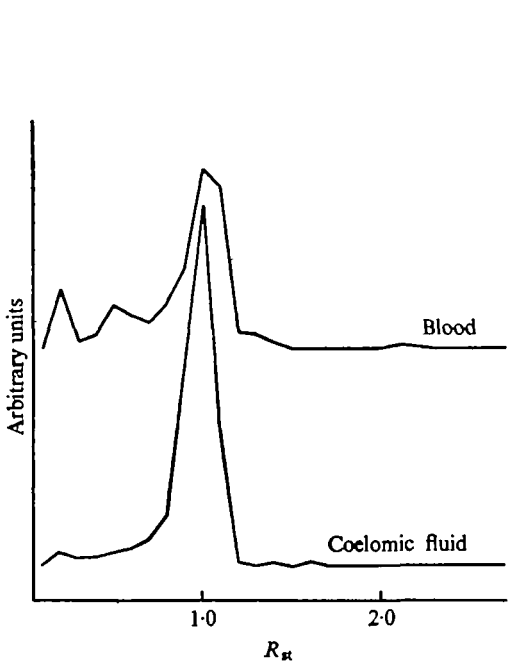

Fig. 5

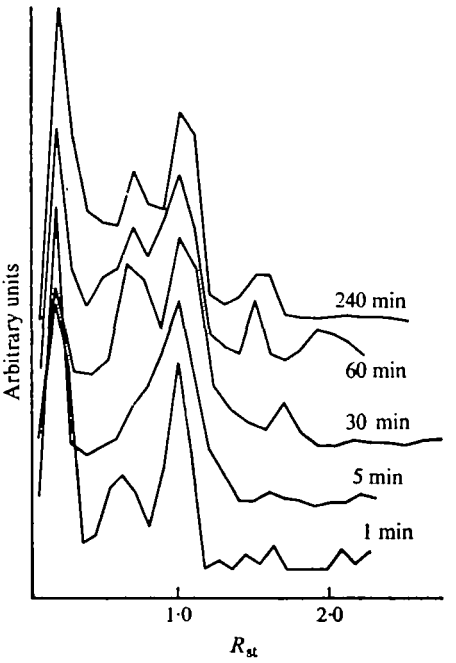

Fig. 6

Fig. 5. Thin-layer chromatogram of blood and coelomic fluid, obtained after 30 min incubation in 5·0 μM D-[6-^{3}H]glucose. Samples from 5 animals were pooled. In this and similar figures, the abscissa is calibrated in relative R_F-values (R_{st}), based on the location of a standard D-glucose spot (R_{st} = 1·0).

Fig. 6. Thin-layer chromatograms of animal extracts, obtained after incubation in 5·0 μM D-[6-^{3}H]glucose (1–60 min incubation) and 5·0 μM D-[U-^{14}C]glucose (240 min incubation). *Note:* the curves are not drawn to the same vertical scale.

To further elucidate the internal distribution of labelled material during incubation, one group of animals was exposed to 5·0 μM D-[6-^{3}H]glucose for 30 min. Another group received an injection into the coelomic cavity of a small volume of 50% sea water containing D-[6-^{3}H]glucose with the same specific activity as in the radioactive medium, and subsequently the animals were kept in unlabelled medium for the same period of time. Thin-layer radiochromatograms of ethanol extracts from both animal groups are shown in Fig. 7. Only in animals incubated in labelled medium was a significant part of the labelled D-glucose converted to D-glucose phosphate.

The concentration of D-glucose in the coelomic fluid of animals taken from the same collection was found by the hexokinase (Boehringer) method to be 308 ± 38 μM (mean $\pm$ S.E.M.; $N = 10$). When labelled D-glucose enters the extracellular fluid either by injection or through trans-integumentary uptake, the specific activity is considerably reduced because of 'dilution' with relatively large amounts of unlabelled D-glucose. The results depicted in Fig. 7 can only be explained by assuming that all, or at least most, of the D-glucose taken up through the animal surface does not pass through an extracellular compartment before being phosphorylated: this phosphorylation, as well as further metabolic conversion, must have occurred in the epidermal

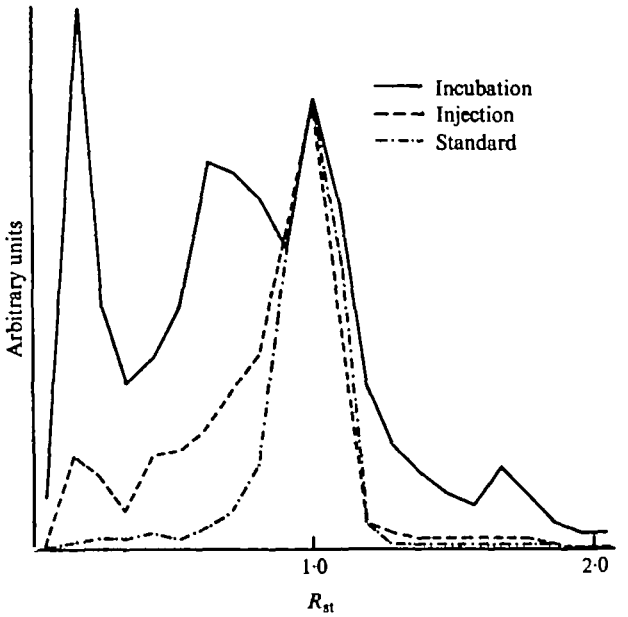

Fig. 7. Thin-layer chromatograms of animal extracts after incubation for 30 min in 5·0 μM D-[6-^{3}H]glucose, or 30 min after the injection of D-[6-^{3}H]glucose into the coelomic cavity; the D-[6-^{3}H]glucose chromatogram is shown for comparison.

cells.* Oxidation products of exogenous D-glucose (bicarbonate and water) are given off from the epidermal cells to the extracellular fluid and, conceivably, to the medium. Other cells may take up D-[6-^{3}H]glucose, but only from the extracellular fluid, which would supposedly be a relatively slow process because of the low specific activity of the extracellular D-glucose.

During this study, no attempt was made to exclude the possibility that part of the tritiated water was formed by isotope exchange from organic intermediates in D-[6-^{3}H]-glucose metabolism. Such exchange is quite commonly encountered with tritiated organic compounds (Feinendegen, 1967). However, whenever it was necessary to avoid ambiguous interpretations, ^{14}C-labelled D-glucose was employed in the experiments.

C. Characteristics of the rate-limiting step in integumentary D-*glucose uptake*

Concentration dependence

A study was made of integumentary D-glucose influx as a function of concentration in the incubation medium. Influx values were determined as the slopes of linear uptake curves similar to that in Fig. 1, but based on incubation of 1, 2, 3 and 4 min duration. As a rule, the kind of relationship found (Fig. 8) is interpreted as resulting from the simultaneous action of two rate-determining transfer systems, one saturable and one non-saturable in the concentration range investigated (1–100 μM). Assuming

* Ferguson (1967, 1968, 1970) and Little & Gupta (1969) have provided autoradiographic evidence for a preferential incorporation of labelled material into the epidermis subsequent to incubation of some marine invertebrates in a medium containing radioactive DOM. These observations are compatible with the present results on *Nereis*.

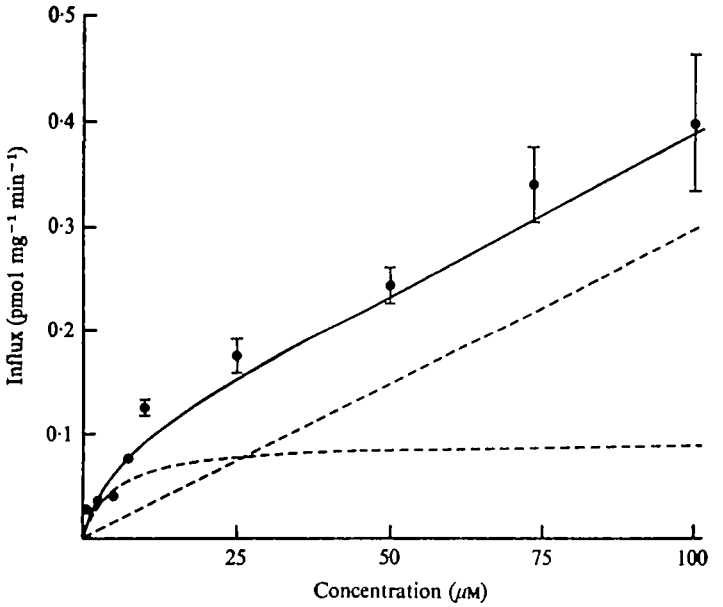

Fig. 8. Concentration dependence of D-glucose influx as determined from incubations in D-[6-^{3}H]glucose. The continuous curve was obtained by weighted non-linear regression analysis according to equation (1). The interrupted curves represent the saturable and non-saturable components, respectively. $N = 322$.

that the saturable system conforms to Michaelis–Menten kinetics, the following relationship holds:

$$J_{\text{in}} = \frac{(J_{\text{in}})_{\max}}{1 + K_t/C} + BC. \qquad (1)$$

J_{in} represents D-glucose influx (pmol mg^{-1} min^{-1}), C is the concentration of D-glucose in the medium, K_t the medium concentration at which influx via the saturable system is half its maximal value, and BC influx via the non-saturable system. $(J_{\text{in}})_{\max}$ and B are constants.

A curve following the above expression was fitted to the experimental data. The values of the three constants corresponding to the best fit were as follows: $(J_{\text{in}})_{\max} = 0{\cdot}095$ pmol mg^{-1} min^{-1}; $K_t = 5{\cdot}2$ μM; $B = 3{\cdot}0 \times 10^{-3}$ pmol mg^{-1} min^{-1} per μM. The two influx components, as calculated from these figures, are shown as broken curves in Fig. 8. The concentration dependence of D-glucose influx seems to be adequately explained by the operation of saturable and linear systems with the characteristics mentioned. The need for postulating two independent systems would be amply demonstrated if the magnitude of the influx via one system could be changed without interfering with the other system; this will be discussed in another paper (Gomme & Ahearn, in preparation).

The existence of a saturable influx component suggests that integumentary uptake of exogenous D-glucose involves at least one facilitating transfer system. However, it cannot be decided on the basis of data presented so far whether the linear component represents simple diffusion or facilitated transfer via a system that saturates at substrate concentrations higher than those investigated. An attempt to distinguish

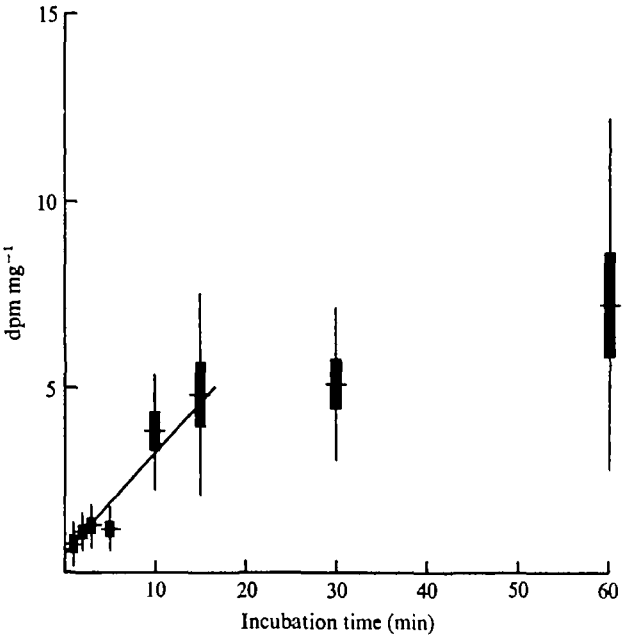

Fig. 9. Time-course of 3-*O*-[^{3}H]methyl-D-glucose uptake from a 5·0 μM solution (specific activity: 364 dpm pmol^{-1}). The line was obtained by linear regression analysis as in Fig. 1, using results from the incubation periods 1–15 min. The *y*-intercept and the slope is 0·5 ± 0·2 dpm mg^{-1} and 0·27 ± 0·07 dpm mg^{-1} min^{-1}, respectively (95 % limits). $N = 80$.

between these possibilities was made by studying the substrate specificity of integumentary D-glucose influx.

Specificity of D-*glucose influx, as determined from experiments with* 3-*O-methyl*-D-*glucose*

The time-course of uptake of the non-metabolizable glucose analogue, 3-*O*-[^{3}H]methyl-D-glucose, from a 5·0 μM solution is illustrated in Fig. 9. For a similar concentration of D-glucose, the two transport systems would carry 75 % and 25 %, respectively, of the total D-glucose influx (0·064 pmol mg^{-1} min^{-1}). The 3-*O*-methyl-D-glucose influx, as calculated from the initial slope of the curve, is $7{\cdot}5 \times 10^{-4}$ pmol mg^{-1} min^{-1}, or 1·2 % of the D-glucose influx. It was concluded that under the conditions of this experiment, 3-*O*-methyl-D-glucose was not significantly transported by any of the two systems responsible for D-glucose uptake.

If the linear D-glucose influx component represented simple diffusion across the rate-limiting barrier identified as the outward-facing part of the integument on the basis of above results, this degree of substrate discrimination would not seem possible. Applying principles summarized by Stein (1967) it can be estimated that the diffusion constant for 3-*O*-methyl-D-glucose in an aqueous medium must be almost identical with that for D-glucose. Similarly, for the passage through a lipid (cell) membrane, the diffusion permeabilities for the two compounds should deviate by less than a factor of two. It follows that the linear ('non-saturable') D-glucose influx component represents – as does the non-linear one – a facilitated transfer, that may show saturation at very high (and exceedingly unnatural) substrate concentrations. Both transport mechanisms involved must be located in the outward-facing membrane of the epidermal cells.

It is implicit in this interpretation that the uptake of D-glucose by simple diffusion is negligible in comparison with the facilitated uptake. The *maximal* diffusional D-glucose influx can be equated with the 3-*O*-methyl-D-glucose influx, and the corresponding maximal diffusion permeability is $1{\cdot}5 \times 10^{-4}$ pmol mg^{-1} min^{-1} per μM.

Influx by way of the intercellular spaces of the epidermis can occur by simple diffusion only. Consequently, the *maximal* intercellular D-glucose influx from a 5·0 μM solution must be $7{\cdot}5 \times 10^{-4}$ pmol mg^{-1} min^{-1}, which is of the same order of magnitude as the rate of uptake of exogenous D-glucose into the coelomic fluid, as determined from the line in Fig. 4 ($1{\cdot}8 \times 10^{-3}$ pmol μl^{-1} min^{-1}). However, in the light of the results in Section B, this process alone cannot account for the total trans-integumentary uptake.

D. Loss of radioactivity from animals after incubation in media containing labelled D-*glucose*

Fig. 10 illustrates the release of radioactivity from animals after a 30 min exposure to 5·0 μM D-[6-^{3}H]glucose. After incubation in the radioactive medium, the animals were placed individually in 25 ml 50% sea water with D-glucose concentrations of zero or 5·0 μM, from which samples were taken at intervals. A control group of animals was incubated under the same conditions, and the ethanol-extractable activity present at the beginning of the washout period was determined. The results are expressed as the fraction of initial activity remaining in animals (A') versus time (t).

It was found that the time-course of radioactivity release could be adequately described by the following equation:

$$A' = a_1 \exp(-\alpha t) + a_2, \qquad (2)$$

in which $a_1 + a_2 = 1$. In this expression, a_2 represents the residual activity in the animal (fraction of initial activity) when the release rate ($\alpha(A' - a_2)$) has dropped to zero. Table 1 contains the values of the constants a_1, a_2 and α resulting from regression analysis according to equation (2). (When regression analysis was performed according to the more general equation $A' = a_1 \exp(-\alpha t) + a_2 \exp(-\beta t)$, β was found to be zero.) The results demonstrate that only a fraction ($a_1 \simeq 50\%$) of the ^{3}H-activity taken up by the animal during incubation is transferred to the medium by release processes under these conditions. The characteristics of activity washout seem largely independent of the presence of 5·0 μM D-glucose in the medium.

In another experiment, 7 animals were incubated for 30 min in 5·0 μM D-[U-^{14}C]-glucose (spec. act. 454 dpm $pmol^{-1}$) before a washout of 200 min duration. Initially, the washout medium contained 5·0 μM unlabelled D-glucose, and antibiotics were added as described in Section A. A stream of atmospheric air was passed through the medium and the liberated CO_2 collected for radioactivity determination. Non-volatile radioactive compounds in the final washout media constituted $7{\cdot}5 \pm 1{\cdot}6\%$ (mean ± S.E.M.) (28 dpm mg^{-1}) of the total ^{14}C-activity released, the remainder being $H^{14}CO_3^-$. The non-volatile material represented $3{\cdot}2 \pm 0{\cdot}6\%$ of the total activity taken up as D-[U-^{14}C]glucose during incubation ($F = 0{\cdot}032$, see Appendix). When carrier D-glucose, and subsequently a large volume of 2,4-dinitrophenylhydrazine solution (saturated in 2 N-HCl) was added to an aliquot of the washout medium, a weakly labelled osazone precipitate was formed. This suggests (but does not demonstrate

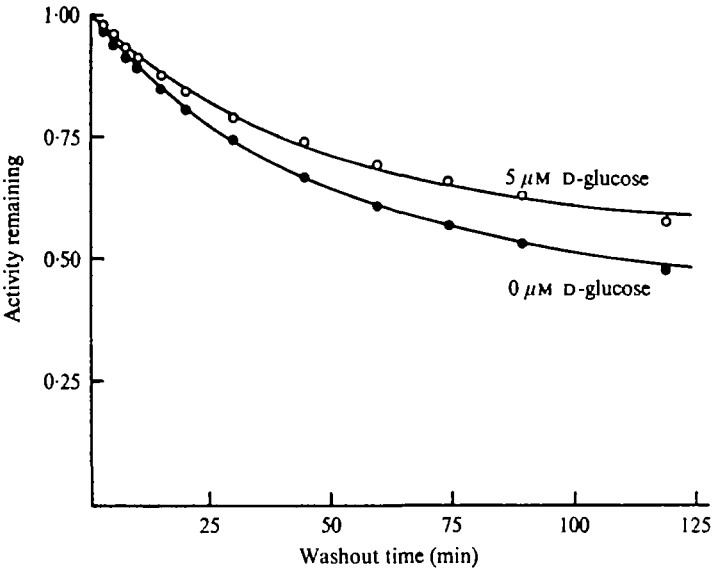

Fig. 10. Time-course of ^{3}H-activity washout from animals after 30 min incubation in 5·0 μM D-[6-^{3}H]glucose. The D-glucose concentrations in the washout media are shown for both curves. Five animals were used in each experiment. Non-linear regression analysis was performed according to equation (2) with uniform weighting of the experimental values.

Table 1. *Values of the constants a_1, a_2 and α, obtained from data in Fig. 10 by regression analysis according to equation* (2)

Washout medium	a_1	a_2	α (min^{-1})
No D-glucose	0·566	0·433	0·0203
5·0 μM D-glucose	0·456	0·544	0·0199
(average)			0·0201

conclusively) that at least part of the non-volatile labelled material is D-[U-^{14}C]-glucose.

Since the coelomic space is in contact with the outside through metanephridia, excretion should be considered as a factor in the release of radioactivity. The rate of excretory activity loss ($\mathcal{J}^*_{exc}$) is given by:

$$\mathcal{J}^*_{exc} = -uA_{co}, \tag{3}$$

in which u is the diuresis (μl min^{-1}) and A_{co} the coelomic fluid radioactivity (dpm μl^{-1}). If it is assumed, to simplify the calculations, that no reabsorption or secretion takes place in the nephridial tubules, we have during a washout experiment:

$$\mathcal{J}^*_{exo} = dQ^*_{co}/dt = -u(Q^*_{co}/V_{co}) = -\gamma Q^*_{co}. \tag{4}$$

Here, Q^*_{co} is the total radioactivity (dpm) present within the coelomic space V_{co}. The rate-constant of excretory activity loss ($\gamma = u/V_{co}$) can now be calculated and compared with the rate-constant found above (α, Table 1). For *N. diversicolor* acclimated to 50% sea water, the hourly urine volume was estimated by Smith (1970) to be 0·37% of body weight. The coelomic space is assumed to constitute 25% of body weight (cf. Section B). If W is taken to represent body weight, we have:

$$\gamma = u/V_{co} = (u/W)\,(V_{co}/W) = 0{\cdot}0037/(60 \times 0{\cdot}25) = 2{\cdot}5 \times 10^{-4}\ \text{min}^{-1}.$$

This value is 80 times smaller than the average α. It can therefore be concluded that the discharge of coelomic fluid through the excretory organs does not contribute significantly to the activity loss recorded in the experiment of Fig. 10. By using data from Fig. 5, the excretory loss of D-[U-^{14}C]glucose in the ^{14}C-washout experiment can be estimated separately from equation (4) as $2{\cdot}5 \times 10^{-4} \times 0{\cdot}25 \times 23 \times 200 = 0{\cdot}29$ dpm mg^{-1}, or $\simeq 1\%$ of the labelled non-volatile material given off. The release of labelled compounds in a washout experiment undoubtedly takes place from the body surface.

The small amount of ^{14}C-activity released could be D-[U-^{14}C]glucose from the incubation medium not removed from the surface by washing. Alternatively, it may represent organic ^{14}C-substances (possibly D-[U-^{14}C]glucose) from the epidermal cells, or D-[U-^{14}C]glucose lost from the extracellular fluid by diffusion through epidermal pathways. As determined from the intercept in Fig. 1, the amount of exogenous D-glucose associated with the surface of a 300 mg worm after rinsing corresponds to a volume of incubation medium not exceeding 0·8 μl. In the washout experiment with D-[U-^{14}C]glucose, the activity of the incubation medium was 2270 dpm μl^{-1}. The expected activity due to adhering D-[U-^{14}C]glucose would therefore be $\simeq (0{\cdot}8 \times 2270)/300 = 6{\cdot}1$ dpm mg^{-1}, or $\simeq 20\%$ of the total amount lost. A considerable fraction of the non-volatile labelled material may therefore originate from the epidermal cells or from the extracellular fluid.

The previously estimated maximal diffusion permeability of the epidermis ($1{\cdot}5 \times 10^{-4}$ pmol mg^{-1} min^{-1} per μM) may be used to define an upper limit to the intercellular loss of labelled D-glucose by ascribing all diffusional D-glucose exchange to the intercellular channels alone. After 30 min incubation in 5·0 μM D-[U-^{14}C]glucose, the coelomic fluid contains $25/523 = 0{\cdot}044$ pmol μl^{-1} = 0·044 μM exogenous (labelled) D-glucose (data from Fig. 4). Assuming that diffusion takes place from an extracellular compartment with a concentration of labelled D-glucose exceeding that of the coelomic fluid by less than a factor of ten (cf. Fig. 3), the intercellular diffusional outflux at the beginning of the washout period would be less than $1{\cdot}5 \times 10^{-4} \times 0{\cdot}044 = 6{\cdot}6 \times 10^{-5}$ pmol mg^{-1} min^{-1}. Provided an outflux of this magnitude took place throughout the experiment (200 min), the total activity lost as D-[U-^{14}C]glucose from the extracellular fluid would be $6{\cdot}6 \times 10^{-5} \times 200 \times 454 = 6{\cdot}0$ dpm mg^{-1}, corresponding to 20% of the labelled organic material in the final washout medium.

An upper limit to the loss of labelled D-glucose from the cells to the medium may be obtained from the present data by neglecting all other processes possibly contributing non-volatile ^{14}C-material to the medium.

From the experiments presented in Fig. 10 additional important information can be derived. The mono-exponential course of the curves can be explained as resulting entirely from the release of tritiated water, since this compound represents 87–95% of the radioactivity in the final washout medium. In *N. diversicolor* acclimated to 50% sea water, the rate constant for exchange of labelled water across the body wall was found to be 0·177 min^{-1} (Smith, 1970). This value is almost ten times larger than α, as presented in Table 1. This indicates that the metabolic formation of ^{3}HHO, rather than its transfer across the epidermal cell membrane, determines the rate of ^{3}H-washout. The rate of ^{3}HHO release may be described by an equation of the form:

$$\frac{dQ^*}{dt} = kf(t)Q^*, \tag{5}$$

in which $f(t)$ is the fraction of epidermal activity Q^* present as D-[6-^{3}H]glucose at time t and k is a rate-constant, characterizing the oxidation of D-[6-^{3}H]glucose, leading to formation of ^{3}HHO. The release of ^{3}HHO will proceed mono-exponentially as observed, provided $kf(t)$ is a constant. It thus appears that $f(t)$ is time-independent ($f(t) = f$), implying that labelled D-glucose represents a constant proportion of the total epidermal radioactivity throughout the washout experiment. This point will be considered further in the Discussion.

During incubation, an increasing outflux of labelled material should cause the curve depicting the time-course of radioactivity uptake to deviate from linearity. When constructing the curved line in Fig. 1 by methods given in the Appendix, account was taken of the backflux of ^{3}HHO to the medium. At 30 min, the 'theoretical' activity value is 16% lower than that obtained when assuming a linear relationship. For comparison, see also Fig. 3. The experimental data on radioactivity uptake, as shown in Fig. 1, did not depart noticeably from a straight line. However, to obtain unequivocal estimates of D-glucose influx by means of D-[6-^{3}H]glucose, it is advisable to use incubation periods well below 30 min. When influxes were determined during this study (Fig. 8), incubation periods did not exceed 4 min, and the linearity of uptake was checked in every case.

DISCUSSION

The integument of polychaetes consists of a single-layered epithelium, on which is situated a cuticular and epi-cuticular coating (Brökelmann & Fischer, 1966; Lawry, 1967; Storch & Welsh, 1970, 1972; Michel, 1972; Chien, Stephens & Healey, 1972). The structure of the integument of *N. diversicolor* is illustrated in Fig. 12, Plate 1. The outer face of the epidermal cells is furnished with microvilli which traverse the cuticle. In the apical region, adjacent epidermal cells are connected by adhering and occluding zonules and by septate desmosomes (terminology according to Berridge & Oschman, 1972). The basal cell surface is characterized by numerous irregular extensions which interdigitate with cells in the subepidermal tissue.

The integumentary exchange of D-glucose between animal and surroundings may take place across the epithelial cell membranes as well as through the intercellular spaces of the integument. In animals acclimated to 50% sea water, the present study has disclosed the outward-facing epidermal cell membrane as the rate-limiting barrier to the integumentary uptake of D-glucose, since most of the uptake was directly into the epidermal cells. This membrane probably contains two separate systems providing facilitated ('carrier-mediated') entry of D-glucose. One of these systems has a K_t (apparent Michaelis constant, transport constant) of 5·2 μM, which presumably is of the same order of magnitude as the interstitial D-glucose concentration in the sediment constituting the worms' natural habitat. The other one does not show saturation when animals are exposed to natural D-glucose concentrations. Both mechanisms have a marked specificity, as seen from the fact that they cannot accept 3-*O*-methyl-D-glucose as a substrate. The passage of exogenous D-glucose by simple diffusion across the cell membrane, as well as through the intercellular spaces, is negligible in comparison with the facilitated uptake.

Unless the membrane sites involved in facilitated transfer are located predominantly at the tip of the microvilli, exogenous D-glucose must pass through the cuticle prior

to translocation across the membrane. It is evident from the results presented in Section C that diffusion through the cuticle is not a limiting process in integumentary D-glucose uptake.

The results reported in Sections B and D suggest that exogenous D-glucose sequestered by the epidermal cells can be lost to the extracellular fluid and to the medium. As expected, the same applies to the final oxidation products of this compound, water and bicarbonate. After 30 min incubation in 5·0 μM D-[6-^{3}H]glucose, only half of the ethanol-extractable labelled material was available for release as D-[6-^{3}H]glucose and tritiated water. For the following reason, D-[6-^{3}H]glucose located in the extracellular space most likely represents the remaining 'unavailable' radioactive material. At the end of a 30 min incubation, only a minor fraction of the whole animal D-[6-^{3}H]glucose is present extracellularly, the major part being confined within the epidermal cells. On the other hand, during the washout period, when labelled D-glucose is no longer supplied from the medium, this substance must continue to enter the extracellular fluid as long as an epidermal store exists. However, due to the low specific activity of D-glucose in the extracellular space, the reverse process (uptake of extracellular D-[6-^{3}H]glucose into the epidermal cells and other cells in the body) proceeds slowly (cf. Section B). A large diffusional loss of labelled extracellular D-glucose to the outside can be ruled out on the basis of the low diffusion permeability of the integument (Section D). Furthermore, it has been demonstrated that, under these conditions, excretion does not cause a significant loss of activity from the extracellular fluid. Consequently, the loss of extracellular D-[6-^{3}H]glucose must be quite limited, and it is justified to consider this compound unavailable for release during the washout period. In contrast, tritiated water, received by the extracellular fluid from the epidermal cells, must be lost rapidly by diffusion due to the very high water permeability of the body surface (Smith, 1970). Therefore, the amount of tritiated water in the animal should be maintained at a low level during the washout experiment.

The hypothesis outlined above may be formally represented by the kinetic model of Fig. 11. As demonstrated in the Appendix, the model is in accordance with the experimental observations, provided the epidermal concentrations of tritiated water and D-[6-^{3}H]glucose each are assumed to be a constant fraction of the total epidermal radioactivity. During incubation, when the major part of the labelled material is found in the epidermal cells, labelled D-glucose seems to represent a constant fraction of the total non-volatile radioactivity (Fig. 6). During washout, labelled D-glucose may be considered a constant fraction of the epidermal radioactivity, as inferred from the washout experiments in Section D. The rate of formation of tritiated water during incubation and washout is probably proportional to the intracellular D-[6-^{3}H]glucose concentration, and the rate of loss seems to depend primarily on the rate of formation within the cells. As a first approximation, therefore, it appears plausible that stable intracellular activity ratios are maintained, and tentatively this hypothesis is considered in accordance with the observations.

On the basis of data presented in Section D, the following values from Fig. 11 can be ascertained as described in the Appendix: $f_1'k_{01}' = 1{\cdot}04 \times 10^{-3}$ min^{-1}; $f_1'k_{21}' = 3{\cdot}8 \times 10^{-3}$ min^{-1}; $f_1''k_{01}'' = 1{\cdot}53 \times 10^{-2}$ min^{-1}. In these calculations, no correction has been applied for the diffusional outflux of D-[6-^{3}H]glucose through the intercellular spaces or for the adherence of incubation medium to the animal surface, although both of

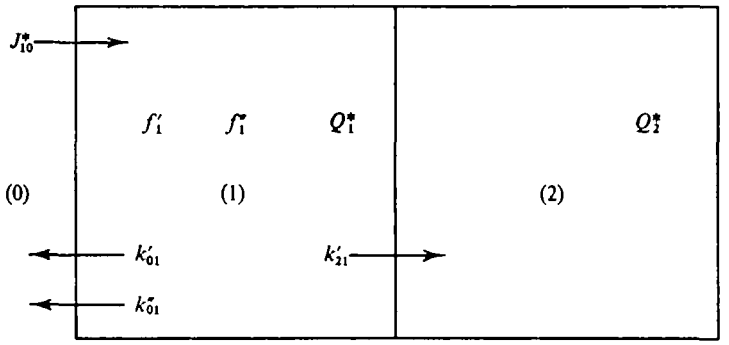

Fig. 11. A hypothetical model proposed to explain the kinetics of radioactivity washout. Compartments 0, 1 and 2 represent the medium, the epidermal cells, and the extracellular space, respectively. Q^*_1 and Q^*_2 are the total radioactivities (dpm) within compartments 1 and 2. f'_1 ($\neq 0$) and f''_1 ($\neq 0$) are the fractions of Q^*_1 present as D-[6-^{3}H] glucose and ^{3}HHO, respectively. J^*_{10}: radioactivity influx as D-[6-^{3}H]glucose (dpm mg^{-1} min^{-1}); k'_{01} and k'_{21}: rate constants (min^{-1}) of D-[6-^{3}H]glucose release from compartment 1; k''_{01}: rate-constant, corresponding to the loss of ^{3}HHO from the animal. Note that k''_{01} in this model encompasses ^{3}HHO initially released into the extracellular fluid, but subsequently given off to the medium! This simplification is considered permissible because of the high permeability of the body surface to water. For further explanation, see Appendix.

these may well be of significance. In order to obtain an estimate of *maximal* outflux of labelled D-glucose from the epidermal cells to the medium, all other processes potentially involved in the release of non-volatile labelled material have been neglected.

The results suggest that the D-glucose flux from the epidermal cells to the extracellular fluid is at least four times larger than the outflux of D-glucose across the apical cell border ($k'_{21}/k'_{01} = 3{\cdot}7$), possibly due to the apical and basolateral membranes having different transport properties. However, the outflux of D-glucose from the cells cannot be expressed in absolute values, since the intracellular specific activity of D-glucose is unknown. At present, it is therefore impossible – even in a crude manner – to evaluate the net fluxes across the epidermal cell membranes.

Due to the relatively high D-glucose concentration in the extracellular fluid, the net movement of D-glucose along the intercellular spaces of the integument must be outward. The maximal diffusion permeability estimated in Section C may be used to set an upper limit to the intercellular net loss of D-glucose. Assuming an extracellular concentration of 300 μM (cf. Section B), this upper limit works out to be 0·045 pmol mg^{-1} min^{-1}. The diffusional D-glucose loss through the interspaces is therefore of the same order of magnitude as, or lower than, the influx of D-glucose into the cells from a 5·0 μM solution (0·064 pmol mg^{-1} min^{-1}).

APPENDIX

We want to illustrate the kinetic consequences of the model presented in Fig. 11. The symbols to be used are those defined in the legend to that figure.

D-[6-^{3}H]glucose was shown to be rapidly metabolized subsequent to the uptake into the cell, leading to a rapid distribution of the radioactive label into a number of intermediates and end products (Section B). In accordance with the above discussion, it is assumed that ^{3}HHO and D-[6-^{3}H]glucose each represent a constant fraction of the

epidermal radioactivity throughout the experiment. We therefore have: $f_1' =$ constant; $f_1'' =$ constant; $f_1'+f_1'' < 1$.

Corresponding to an incubation experiment (characterized by $J_{10}^* \neq 0$), the model in Fig. 11 may be described in terms of the following set of equations:

$$\frac{dQ_1^*}{dt} = J_{10}^* - (f_1'k_{01}'+f_1'k_{21}'+f_1''k_{01}'')Q_1^* \tag{6}$$

$$\frac{dQ_2^*}{dt} = f_1'k_{21}'Q_1^*. \tag{7}$$

For $t = 0$, we have: $Q_1^* = Q_2^* = 0$.

The application of equations (6) and (7) implies that the rate of transfer of the *radioactive* compounds across the membranes is proportional to the intracellular radioactivity (fQ_1^*). For this requirement to be fulfilled irrespective of the type of transfer mechanism, the *total* intracellular concentrations of the respective solutes must be constant (steady-state assumption).

By introducing $C = f_1'k_{01}'+f_1'k_{21}'+f_1''k_{01}''$, and solving for Q_1^* and Q_2^*, we obtain:

$$Q_1^* = \frac{J_{10}^*}{C}(1-\exp(-Ct)), \tag{8}$$

and

$$Q_2^* = \frac{f_1'k_{21}'J_{10}^*}{C}\left[t-\frac{1}{C}(1-\exp(-Ct))\right]. \tag{9}$$

The total radioactivity present within the system (animal) is $Q_{tot}^* = Q_1^*+Q_2^*$. Combination of equations (8) and (9) gives:

$$Q_{tot}^* = J_{10}^* t\left[\frac{(C-f_1'k_{21}')(1-\exp(-Ct))+f_1'k_{21}'Ct}{C^2t}\right], \quad (t \neq 0). \tag{10}$$

The term in square brackets is always < 1, but for small values of t, it approximates to 1, so that $Q_{tot}^* \simeq J_{10}^* t$. Equation (10) was used to construct the curved line in Fig. 1.

Under the conditions of a washout experiment ($J_{10}^* = 0$), subsequent to an incubation of T min duration, equations (6) and (7) yield:

$$Q_1^* = \{Q_1^*\}_T \exp(-Ct), \tag{11}$$

and

$$Q_2^* = \frac{f_1'k_{21}'\{Q_1^*\}_T}{C}(1-\exp(-Ct))+\{Q_2^*\}_T. \tag{12}$$

Here, $\{Q_1^*\}_T$ and $\{Q_2^*\}_T$ are the radioactivities in compartments 1 and 2 at the end of incubation (beginning of washout, $t = 0$). These quantities can be determined by inserting T into equations (8) and (9). Using A', as defined in Section D, we have

$$A' = \frac{Q_{tot}^*}{\{Q_{tot}^*\}_T} = \frac{Q_1^*+Q_2^*}{\{Q_1^*\}_T+\{Q_2^*\}_T}, \tag{13}$$

and

$$A' = \frac{f_1'k_{01}'+f_1''k_{01}''}{C(1+D)}\exp(-Ct)+\frac{f_1'k_{21}'+CD}{C(1+D)}, \tag{14}$$

in which

$$D = \frac{f_1'k_{21}'T}{1-\exp(-CT)}-\frac{f_1'k_{21}'}{C}(= \text{constant}).$$

Equation (14) describes the time-course of radioactivity washout (D-[6-^{3}H]glucose + ^{3}HHO), as predicted by the model in Fig. 11. This expression is seen to have the same general form as the empirical equation (2). We therefore have

$$\alpha = C = f_1'k_{01}' + f_1'k_{21}' + f_1''k_{01}'', \tag{15}$$

$$a_1 = \frac{f_1'k_{01}' + f_1''k_{01}''}{C(1+D)}, \tag{16}$$

$$a_2 = \frac{f_1'k_{21}' + CD}{C(1+D)}. \tag{17}$$

From equations (15–17) it is possible to find the two quantities $(f_1'k_{01}' + f_1''k_{01}'')$ and $f_1'k_{21}'$, when using the experimental values of α, a_1 and a_2 (Table 1).

By considering

$$(J_{01}^*)_{glu} = \frac{dQ_0^*}{dt} = f_1'k_{01}'Q_1^* = f_1'k_{01}'\{Q_1^*\}_T \exp(-Ct), \tag{18}$$

which gives the outflux of labelled D-glucose across the outward-facing membrane during washout, and solving for Q_0^*, we obtain:

$$Q_0^* = \frac{f_1'k_{01}'\{Q_1^*\}_T}{C}(1 - \exp(-Ct)). \tag{19}$$

The *maximum* amount of radioactive D-glucose present in the washout medium at $t = 200$ min, expressed as a fraction of the activity taken up as D-glucose during incubation, was estimated in Section D as F:

$$F = \left(\frac{Q_0^*}{\{Q_{tot}^*\}_T}\right)_{t=200;\, T=30}. \tag{20}$$

Combining equations (19) and (20), and expressing $\{Q_1^*\}_T$ and $\{Q_{tot}^*\}_T$ by equations (8) and (10):

$$f_1'k_{01}' = \frac{F[(C - f_1'k_{21}')(1 - \exp(-Ct)) + f_1'k_{21}'Ct]}{1 - \exp(-Ct)}. \tag{21}$$

Since $(f_1'k_{01}' + f_1''k_{01}'')$is known, $f_1'k_{01}'$ can be determined.

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REFERENCES

AHEARN, G. A. & GOMME, J. (1972). Integumentary uptake, internal distribution, and metabolic degradation of exogenous glucose by *Nereis diversicolor* (Annelida, Polychaeta). *Am. Zool.* **12**, 208.

ANDERSON, J. W. & STEPHENS, G. C. (1969). Uptake of organic material by aquatic invertebrates. VI. Role of epiflora in apparent uptake of glycine by marine crustaceans. *Mar. Biol.* **4**, 243–9.

BERRIDGE, M. D. & OSCHMAN, J. L. (1972). *Transporting Epithelia*. London: Academic Press.

BRAY, G. A. (1970). Determination of radioactivity in aqueous samples. In *The Current Status of Liquid Scintillation Counting* (ed. E. D. Bransome). New York: Grune and Stratton.

BRÖKELMANN, J. & FISCHER, A. (1966). Über die Cuticula von *Platynereis dumerilii* (Polychaeta). *Z. Zellforsch. microsk. Anat.* **70**, 131–5.

CHIEN, P. K., STEPHENS, G. C. & HEALEY, P. L. (1972). The role of ultrastructural and physiological differentiation of epithelia in amino acid uptake by the bloodworm, *Glycera*. *Biol. Bull. mar. biol. Lab., Woods Hole* **142**, 219–35.

DALES, R. P. (1963). *Annelids*. London: Hutchinson.

ERNST, W. & GOERKE, H. (1969). Aufnahme und Umwandlung gelöster Glucose-^{14}C durch *Lanice conchilega* (Polychaeta, Terebellidae). *Veröff. Inst. Meeresforsch. Bremerh.* **11**, 313–26.

FEINENDEGEN, L. E. (1967). *Tritium-labelled Molecules in Biology and Medicine*. London: Academic Press.

FERGUSON, J. C. (1967). An autoradiographic study of the utilization of free exogenous amino acids by starfishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **133**, 317–29.

FERGUSON, J. C. (1968). An autoradiographic analysis of the uptake of exogenous glucose by three species of starfishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **138**, 14–25.

GOMME, J. & AHEARN, G. A. (1972). Transport of exogenous glucose across epidermal cell membranes of a polychaete worm. *Am. Zool.* **12**, 207.

GOMME, J. & AHEARN, G. A. (in preparation). Epidermal D-glucose transport in *Nereis diversicolor* and its relation to salt transport.

HALD, A. (1952). *Statistical Theory with Engineering Applications*. New York: Wiley and Sons.

HALE, L. J. (1955). *Biological Laboratory Data*. London: Methuen.

JOHANNES, R. E., COWARD, S. J. & WEBB, K. L. (1969). Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.* **29**, 283–8.

JOHANNES, R. E. & WEBB, K. L. (1970). Release of dissolved organic compounds by marine and freshwater invertebrates. In *Organic Matter in Natural Waters* (ed. D. Hood). Alaska: University of Alaska Press.

JØRGENSEN, C. B. (1966). *Biology of Suspension Feeding*. Oxford: Pergamon Press.

KROGH, A. (1930). Über die Bedeutung von gelösten organischen Substanzen bei der Ernährung der Wassertieren. *Zeitschr. vergl. Physiol.* **12**, 668–81.

KROGH, A. (1931). Dissolved substance as food of aquatic organisms. *Biol. Rev.* **6**, 412–42.

LAWRY, J. V. (1967). Structure and function of the parapodial cirri of the Polynoid Polychaete, Harmothoe. *Z. Zellforsch. mikrosk. Anat.* **82**, 345–61.

LITTLE, C. & GUPTA, B. L. (1969). Studies on Pogonophora. III. Uptake of nutrients. *J. exp. Biol.* **51**, 759–73.

MICHEL, C. (1969). Ultrastructure et histochemie de la cuticule pharyngienne chez *Eulalia viridis* Müller (Annélide Polychète Errante, Phyllodocidae). *Z. Zellforsch. mikrosk. Anat.* **98**, 54–73.

PÉQUIGNAT, E. (1970). Biologie des *Echinocardium cordatum* (Pennant) de la Baie de Seine. Nouvelles recherches sur la digestion et l'absorption cutanées chez les Echinides et les Stellerides. *Forma Functio* **2**, 121–68.

PÉQUIGNAT, E. (1972). Some new data on skin digestion and absorption in urchins and sea stars (*Asterias* and *Henricia*). *Mar. Biol.* **12**, 28–41.

PÜTTER, A. (1909). *Die Ernährung der Wassertiere und der Stoffhaushalt der Gewässer*. Jena: Fischer.

RAPKIN, E. (1969). *Sample Preparation for Liquid Scintillation Counting*. Plaisir, France: Intertechnique.

RASMUSSEN, E. (1973). Systematics and ecology of the Isefjord marine fauna (Denmark). *Ophelia* **11**, 1–507.

SMITH, R. I. (1970). Chloride regulation at low salinities by *Nereis diversicolor* (Annelida, Polychaeta). II. Apparent water permeability and water fluxes. *J. exp. Biol.* **53**, 93–100.

SOUTHWARD, A. J. & SOUTHWARD, E. C. (1970). Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates. Experiments on three species of Pogonophora. *Sarsia* **45**, 69–96.

STEIN, W. D. (1967). *The Movement of Molecules across Cell Membranes*, chapter 3. New York: Academic Press.

STEPHENS, G. C. (1962). Uptake of organic material by aquatic invertebrates. I. Uptake of glucose by the solitary coral *Fungia scutaria*. *Biol. Bull. mar. biol. Lab., Woods Hole*, **123**, 648–59.

STEPHENS, G. C. (1963). Uptake of organic material by aquatic invertebrates. II. Accumulation of amino acids by the bamboo worm, *Clymenella torquata*. *Comp. Biochem. Physiol.* **10**, 191–202.

STEPHENS, G. C. (1964). Uptake of organic material by aquatic invertebrates. III. Uptake of glycine by brackish-water annelids. *Comp. Biochem. Physiol.* **29**, 283–8.

STEPHENS, G. C. (1968). Dissolved organic matter as a potential source of nutrition for marine organisms. *Am. Zool.* **8**, 95–106.

STEPHENS, G. C. (1972). Amino acid accumulation and assimilation in marine organisms. In *Nitrogen Metabolism and the Environment* (ed. J. W. Campbell and L. Goldstein). London: Academic Press.

STORCH, V. & WELSH, U. (1970). Über die Feinstruktur der Polychaeten-Epidermis (Annelida). *Z. Morph. Ökol. Tiere* **66**, 310–22.

STORCH, V. & WELSH, U. (1972). Ultrastructure and histochemistry of the integument of air-breathing polychaetes from mangrove swamps of Sumatra. *Mar. Biol.* **17**, 137–44.

STRICKLAND, J. D. H. & PARSONS, T. R. (1968). *A Practical Handbook of Seawater Analysis*. Ottawa: Fisheries Research Board of Canada.

TESTERMAN, J. K. (1972). Accumulation of free fatty acids from sea water by marine invertebrates. *Biol. Bull. mar. biol. Lab., Woods Hole* **142**, 160–77.

EXPLANATION OF PLATE

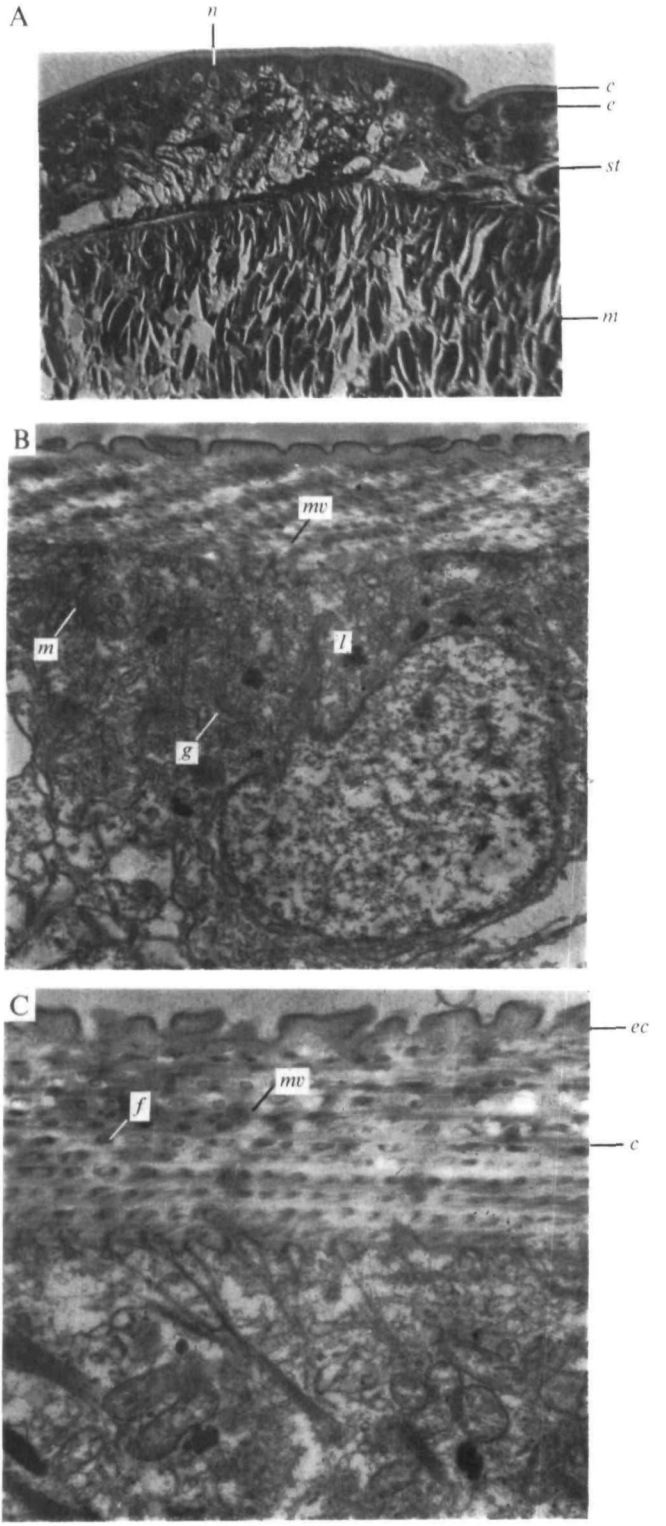
PLATE 1

Fig. 12. Pieces of body wall of *Nereis diversicolor*, acclimated to 50% sea water, were fixed for 1 h in 50% sea water with 1% OsO_4 and embedded in epon. Sections (1 μm) for light microscopy were stained with toluidine; ultra-thin sections for electron microscopy were stained with uranyl acetate.

(A) General view of the integument and underlying muscle tissue (*m*) from the dorsal part of a parapodium in the mid-body region (phase-interference microscopy). Note the cuticle (c) and the flat epidermal cell layer (*e*) with visible nuclei (*n*). *st*, subepidermal tissue (× 770).

(B) Part of an epidermal cell and the adjacent cuticle. Note the microvilli (*mv*) at the apical cell border. *g*, Golgi complex; *l*, lysosomes (?); *m*, mitochondria × 26 800).

(C) Cuticle, and the apical part of an epidermal cell. Note the microvilli (*mv*) with associated intracellular fibrils. *ec*, epicuticle; *c*, cuticle proper; *cf*, collagen fibres in cuticle (× 58 200).



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