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ABSENCE OF CELL COMMUNICATION FOR FLUORESCEIN AND DANSYLATED AMINO ACIDS IN AN ELECTROTONIC COUPLED CELL SYSTEM

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SUMMARY

Quantitative evaluation of the diffusion process of sodium fluorescein and dansylated amino acids in the salivary gland of the larvae of *Drosophila hydei* reveals that the differences in specific permeability between the junctional and nonjunctional membranes, as found for small ions, do not apply to the fluorescent probes. There are no significant differences between the permeability properties for the different dansylated amino acids tested, and the same properties are found for sodium fluorescein.

INTRODUCTION

Impalement of a wide variety of cell systems with glass micro-electrodes under microscopic control and subsequent injection of electrolytes revealed that small ions are allowed to move rather easily through the junctional membranes from one cell interior to the next. This phenomenon is called passive electrical cell communication [1–3]. Furthermore, qualitative observations suggest that the low-resistance junctions responsible for the intercellular ionic continuity also allow the transfer of a variety of tracer molecules such as fluorescein [3–13], Procion Yellow [7, 9, 10, 14–17] and some other dyes and fluorescent probes with a range of molecular weight varying between 300 and 69 000 [1]. It is claimed that all tracers move strictly from cell to cell, whereas no detectable leak of them to the bathing fluid could be observed. However, failure of intercellular continuity to fluorescein was shown by Slack and Palmer [18] in cleavage and early blastula stages of *Xenopus laevis* embryos, while restriction of flow of Procion Yellow is shown in external horizontal cells in the dogfish retina [17]. The intersegmental synapses of the median giant fibre and lateral giant axons of earthworms appear to be impermeable to Procion Yellow [16]. Quantitative analysis of the flow of Procion Yellow between neighbouring cells in sheep and calf Purkinje fibres reveals that the specialized contact areas present account for a

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considerable, yet not absolute, hindrance to the diffusion of this particle [19]. Other experiments show that fluorescein does not move from cell to cell in starfish and fish embryos, despite the presence of electrical coupling [20, 21].

Because of the fact that, in most of the studies mentioned, the evaluation of the intracellular continuity is carried out only in a qualitative way, and because of the importance of this phenomenon with respect to (development of) theories of growth control and differentiation, a quantitative study was set up: it was tested whether fluorescein, as well as dansylated amino acids, diffuse freely from one cell to another in the salivary gland of the larvae of *Drosophila hydei* whose cells are electrically coupled [22]. Exchange of the fluorescent probes can indeed be demonstrated, but permeation through the low-resistance junctions is not preferred because the differences in specific permeability between the junctional and nonjunctional membranes of these cells, as found for small ions, appear not to apply to the fluorescent probes.

MATERIALS AND METHODS

Object

Salivary glands of larvae of a wild type stock of *Drosophila hydei* (end third instar) were used for the present experiments. These glands have clear and accurately known communicative properties for small ions [22]. The culture conditions, the preparations of the glands and the composition of the extracellular medium were as previously described [22].

Injection and detection of fluorescein and dansylated amino acids

The fluorescent probes were dissolved in distilled water in concentrations of about 10 mM dependent on solubility. These solutions were injected hydraulically into the most distal cell, using micropipettes of about 1 μm tip diameter. Electrotonic spread measurements revealed that micropipettes with such diameters did not damage the gland cells with regard to electrical coupling. For the hydraulic injection, the micropipette is clamped into a closed metal chamber filled with the same solution as the micropipette. The chamber is then slightly heated by means of an adjustable electrical current. The enclosed solution expands and some of the fluorescent probe will be driven out of the micropipette, giving rise to a seeming concentration of about 1 mM in the injected cell as estimated from the amount of fluorescence. This corresponds to an injected quantity equal to about 10 % of the cell volume. Actually, this quantity will be much lower because of the considerable increase in quantum yield in the cell. After the fluorescent probe is injected into the cell, the micropipette is removed while the extracellular medium is renewed because of the possibility of some leakage out of the pipette into the extracellular medium. Normally the injection time is about 15–30 s.

Intercytoplasmic exchange of fluorescent probes is observed by using blue light for excitation (Leitz-Ortholux Microscope, Mercuri arc lamp, 75 W). Selection of primary (Leitz filters: BG 38 and UG 1) and secondary (TK 400/K400 and K460) filters guarantees an optimal ratio between detected secondary radiation and background radiation. The distribution of the fluorescence intensity in the different cells is photographed at different times using highly sensitive films (Kodak Tri-X: 29 DIN). The exposure times are about 30 s. To avoid errors due to differences between films,

a series is always photographed on one film. On each film a test series is photographed in order to estimate the relation between the density of the film and the concentration of the fluorescent probe. Every substance is checked to see that the fluorescence intensity does not diminish during a period of 30 min.

The density of the film is estimated with a densitometer. Using the test series, the relative concentration in the different cells is estimated from the density.

Fluorescent probes

The permeability behaviour of all dansylated derivatives of amino acids normally found in proteins was tested in a qualitative way. From these qualitative observations it seemed that the dansylated amino acids could be divided into four groups with more or less different permeability properties. From each group, one representative probe was tested in a quantitative way.

Dansyl-L-isoleucine cyclohexylamine salt	(mol. wt. 463.64)
Dansyl-L-glutamine	(mol. wt. 379.44)
Dansyl-L-serine cyclohexylamine salt	(mol. wt. 437.56)
Dansyl-methionine cyclohexylamine salt	(mol. wt. 481.68)

Moreover, the quantitative analysis is carried out for fluorescein sodium (mol. wt. 376.28).

All dansylated amino acids and the fluorescein sodium were purchased from British Drug Houses, Poole, England.

Control on electrical cell coupling

Electrotonic cell coupling measurements similar to those mentioned above revealed that micropipettes with tip diameters of about 1 μm did not damage the gland cells with regard to electrical coupling.

In order to get an impression about the influence of the radiation and the probes upon the viability of the cells, electrical cell coupling measurements were carried out on salivary glands incubated in control medium. These glands were then transferred to a medium containing one of the fluorescent probes (concentration 1 mM) and radiated with blue light with high intensity for 15 min. It appears that neither the probes nor the incubation and radiation affected the amount of electrotonic cell coupling.

As a final check, and in order to make sure that the cells are not decoupled during the injection procedure by the large-sized pipettes or changes in ion strength or tonicity, electrical cell coupling was measured, in a number of cases, before and about 5–10 min after the injection of the substances. During this period the glands were subject to blue radiation, as during all the experiments. No significant changes (systematic and at random) in electrical coupling, membrane potential, membrane resistances, transparency or cell volume were found.

THEORY AND ANALYSIS

Model assumptions

For the quantitative interpretation of the results, the salivary gland will be thought of as a core which is closed at the distal side by the half of a sphere. The diffusion in the bathing fluid, the luminal fluid and in the cytoplasm is supposed to be fast

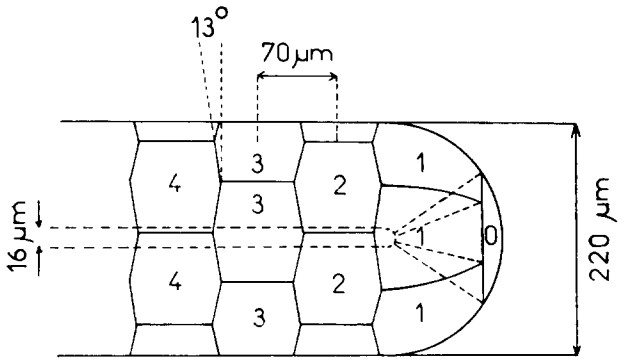

Fig. 1. Model of the configuration of the cells in the distal part of the gland. Each cell has been given an order number.

compared to the diffusion through the membranes. From observations, it was found that the cells in the distal part have almost equal volumes and that the cells on the basal side show a hexagonal pattern [22]. It is assumed that the ratio of the surface of the membrane on the basal side of the cell to the surface of the membrane on the luminal side is equal for each cell.

The configuration of the cells in the distal part of the gland is supposed to be like that shown in Fig. 1. The cells are lying in rings and have been given order numbers. Each ring contains 6 cells except the ring with order number 0, which consists of 1 cell. The validity of this model with respect to the interpretation of experiments on electrical cell coupling has already been shown in an earlier report [22]. Some typical dimensions of the cells and of the gland are indicated in Fig. 1.

For the interpretation of the performed experiments with respect to the amount of cell communication and to the implications for growth control and cell differentiation, it should be emphasized that only the effective permeabilities of the junctional and nonjunctional membranes are of relevance. For this reason the analysis will be carried out in terms of effective permeability of the membrane. These effective permeabilities are equal to the (average) effective permeability of a (projected) unit surface of the membrane considered, times the projected surface of that membrane. It is supposed that the effective permeabilities of a unit surface of the junctional membrane are equal for all distal cells. The same is supposed for the nonjunctional membranes of the distal cells.

In reality the shapes of the membranes are quite different from those shown in Fig. 1, in which membrane foldings and microvilli are neglected. Moreover, no attention is paid to the question as to which proportion of the junctional membrane area is actually involved in cell-to-cell transfer. These problems are important in membrane biology, but with respect to the interpretation of experiments on cell communication and its implications for growth control and cell differentiation these imperfections are not essential.

Symbols and definitions (alphabetically)

α the ratio between unbound fluorescent molecules and total amount of fluorescent molecules in a cell.

$c_n(t)$	concentration of fluorescent molecules in a cell with order number n at time t.
C	concentration of fluorescent molecules in the cell with order number 0 at time $t = 0$.
Δ_1	a measure for the accuracy of αP_i.
Δ_2	a measure for the accuracy of P_u/P_i.
D	Q_u/Q_i.
M	highest order number.
n	order number of a cell.
$N_n(t)$	number of fluorescent molecules in a cell with order number n at time t.
P_i	effective permeability of a unit surface of the (projected) membrane between cells with different order numbers.
P_u	effective permeability of a unit surface of the (projected) nonjunctional membrane of a cell.
Q_i	$S_{1,2}\ \alpha P_i/V$.
Q_u	$S_2{}^b\ \alpha P_u/V$.
r	the radius of the lumen.
R	the radius of the gland.
$S^b{}_n$	the (projected) surface of the nonjunctional membrane of a cell with order number n.
$S_{n,n+1}$	the (projected) surface of the membrane between two cells with order numbers n and $n+1$.
t	time after injection of fluorescent molecules in the most distal cell.
τ	$Q_i t$.
V	volume of a cell.

Diffusion equations

The projected surfaces can be calculated by using the indicated dimensions in Fig. 1 and considering all cells to have equal volumes. The following values are obtained: $S_0{}^b = 12\,000\ \mu m^2$; $S_1{}^b = 11\,500\ \mu m^2$; $S_n{}^b = 8000\ \mu m^2$ $(n \geqslant 2)$; $S_{0,1} = 3500\ \mu m^2$; $S_{n,n+1} = 3200\ \mu m^2$ $(n \geqslant 2)$. A possible binding of fluorescent molecules in the cytoplasm would take place according to the equilibrium equation:

[unbound molecules] × [binding molecules] = constant × [bound molecules]

If we assume that the number of binding molecules will hardly change, we may write:

[unbound fluorescent molecules] = α[total amount fluorescent molecules]

If P_i denotes the permeability of a unit surface of the projected membrane between cells with different order numbers and P_u denotes the permeability of a unit surface of the projected nonjunctional membrane of a cell, the diffusion process will be described by the aquations:

$$\frac{dN_0}{dt} = 6S_{0,1}\alpha P_i\{c_1 - c_0\} - S_0^b \alpha P_u c_0 \tag{1a}$$

$$\frac{dN_1}{dt} = S_{0,1}\alpha P_i\{c_0 - c_1\} + 2S_{1,2}\alpha P_i\{c_2 - c_1\} - S_1^b \alpha P_u c_1 \tag{1b}$$

$$\frac{dN_n}{dt} = 2\,S_{n-1,n}\,\alpha P_i\,\{c_{n-1}-c_n\} + 2S_{n,n+1}\,\alpha P_i\,\{c_{n+1}-c_n\} - S_n^b\,\alpha P_u\,c_n \tag{1c}$$

$(2 \leqslant n \leqslant M-1)$

$$\frac{dN_M}{dt} = 2S_{M-1,M}\,\alpha P_i\,\{c_{M-1}-c_M\} - S_M^b\,\alpha P_u\,c_M \tag{1d}$$

We define $Q_i = S_{1,2}\alpha P_i/V$, $Q_u = S_2^b\alpha P_u/V$, $D = Q_u/Q_i$ and $\tau = Q_i t$. Since $c_n = N_n/V$, $S_0^b/S_2^b = 1.5$, $S_1^b/S_2^b = 1.4$ and $S_{0,1}/S_{1,2} = 1.1$ the Eqns 1a, 1b, 1c and 1d can be written as:

$$\frac{dc_0}{d\tau} = -(6.6+1.5\,D)c_0+6.6\,c_1 \tag{2a}$$

$$\frac{dc_1}{d\tau} = 1.1\,c_0-(3.1+1.4\,D)c_1+2\,c_2 \tag{2b}$$

$$\frac{dc_n}{d\tau} = 2\,c_{n-1}-(4+D)c_n+2\,c_{n+1} \quad (2 \leqslant n \leqslant M-1) \tag{2c}$$

$$\frac{dc_M}{d\tau} = 2\,c_{M-1}-(2+D)c_M \tag{2d}$$

Estimation of the parameters Q_i and D

By defining the vector:

$$\vec{u} = (c_0, c_1, \ldots\ldots, c_M) \tag{3}$$

the set of coupled differential equations 2a, 2b, 2c, and 2d can be taken together in the vector equation:

$$\frac{d\vec{u}}{d\tau} = B\vec{u} \tag{4}$$

in which B is an M by M matrix with the configuration:

$$B = \begin{bmatrix} -6.6-1.5\,D & 6.6 & & & & \\ 1.1 & -3.1-1.4\,D & 2 & & & \\ & 2 & -4-D & 2 & & \\ & & 2 & -4-D & 2 & \\ & \emptyset & \ddots & \ddots & \ddots & \\ & & 2 & -4-D & 2 \\ & & & 2 & -2-D \end{bmatrix} \tag{5}$$

The formal solution of Eqn 4 is given by:

$$\vec{u}(\tau) = \exp(B\tau)\vec{u}(0) \tag{6}$$

in which $\vec{u}(0)$ represents the values of the concentrations at time $t = 0$. Because the injection time is short compared to the time in which the diffusion process takes place, we assume:

$$\vec{u}(0) = (C, 0, 0, \ldots\ldots, 0) \tag{7}$$

If Q_i, D and C are known $\vec{u}(t)$ can be calculated by diagonalization of the matrix B (see for instance ref. 23).

In order to estimate the values of Q_i, D and C from experimental results, we carried out the minimization procedure described by Hooke and Jeeves [24]: if $c_n(t_j)$ is an experimentally found value for the concentration in ring n at time t_j and $c_n^{\text{theor}}(C, Q_i, D, t_j)$ denotes the theoretical value, then C, Q_i and D are calculated by minimization of the expression:

$$G = \sum_n \sum_j \left(\frac{c_n(t_j)}{c_n^{\text{theor}}(C, Q_i, D, t_j)} - 1 \right)^2 \tag{8}$$

(We do not claim that this method is the optimal one, but still it is simple and effective).

In this way the values for C, Q_i and D are found whereas G gives information about the reliability of the estimated values. From the values of Q_i and D, the parameters αP_i and P_u/P_i can be calculated.

RESULTS

The values found for the permeabilities of fluorescein and the dansylated amino acids are given in Table 1. One of the best fitting results is given in Fig. 2 (Table I⋆), whereas Fig. 3 shows the diffusion process evaluated in Fig. 4 (Table I⋆⋆). All calculations are carried out under the assumption that $M = 5$.

From the averaged values found for αP_i and P_u/P_i of each group, it appears that there are no significant differences between the parameters of different groups. If we use all values listed in Table I, it follows that the averaged value of αP_i is $150 \cdot 10^{-6}$ mm $\cdot$ s^{-1} (median value $140 \cdot 10^{-6}$ mm $\cdot$ s^{-1}) and that the averaged value of P_u/P_i is 1.1 (median value 1.0). Thus it may be concluded that the junctional and non-junctional membrane parts have about equal permeability properties with respect

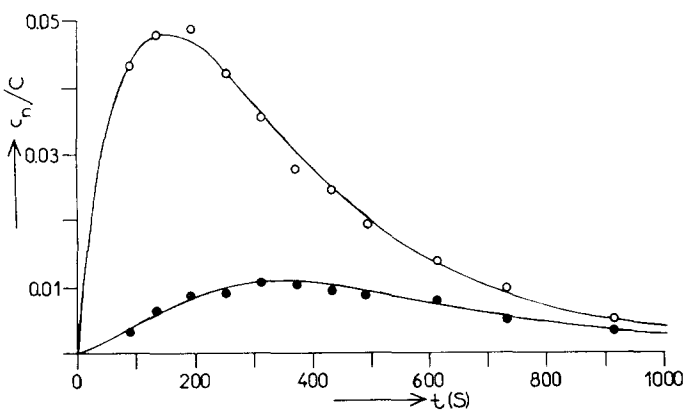

Fig. 2. Results of the measurements on the diffusion process of serine in the gland. The measurement series marked ⋆ in Table I is used. Measured concentrations in the cells with order number 1 (○), and order number 2 (●). The lines represent the best fitting curves obtained by minimization of G (Eqn 8).

TABLE I

Results of the quantitative evaluation of the measurement series. The series marked ⋆ is used in Figs 2 and 3. The series marked ⋆⋆ is used in Fig. 4. For the meaning of Δ_1 and Δ_2, see Discussion.

Fluorescent probe	Number of measuring points	αP_i (10^{-6} mm · s^{-1})	Δ_1 (10^{-6} mm · s^{-1})	P_u/P_i	Δ_2
Fluorescein	12	150	15	0.25	0.25
	20	80	40	0.25	0.25
	18	140	30	0.40	0.15
	18	140	60	0.90	0.50
	10	30	15	5.00	1.00
	18	130	70	1.00	0.30
	20	280	140	0.60	0.20
	14	70	30	2.10	0.50
	16	170	40	0.60	0.20
	14	70	30	1.70	0.90
	14	140	30	1.00	0.50
Iso-leucine	18	100	20	1.40	0.20
	18	120	20	1.10	0.20
	33	200	70	0.65	0.40
	27	460	200	0.25	0.15
	18	180	60	1.30	0.30
Glutamine acid	27	200	60	0.25	0.25
	16	140	40	1.10	0.30
	14	100	30	0.90	0.40
	14	225	50	0.85	0.40
	14	180	60	1.10	0.60
Serine	12	125	15	0.80	0.10
	14	110	30	1.60	0.40
	⋆22	110	10	1.00	0.10
	25	130	30	0.85	0.40
Methionine	⋆⋆38	100	30	1.10	0.40
	12	125	30	1.40	0.80
	20	150	80	1.20	0.40
	6	180	10	1.40	0.50

to the molecules tested, whereas for small ions it was found that for this object P_u/P_i equals about 0.01 [22].

The permeability coefficient found agrees with values obtained for the diffusion of amino acids through black lipid membranes (Egberink, personal communication) if it is supposed that $\alpha = 1$ and that the mean length of the unfolded membrane is increased by a factor 5. The latter assumption seems to be a reasonable one [4]. If the results are to be interpreted with respect to the amount of cell communication, the ratio $S_n^b P_u / S_{n,n+1} P_i$ should be considered. This ratio is also of the order of magnitude 1, indicating that the transport from cell to cell is not much different from the leakage of molecules to the outside of the cells.

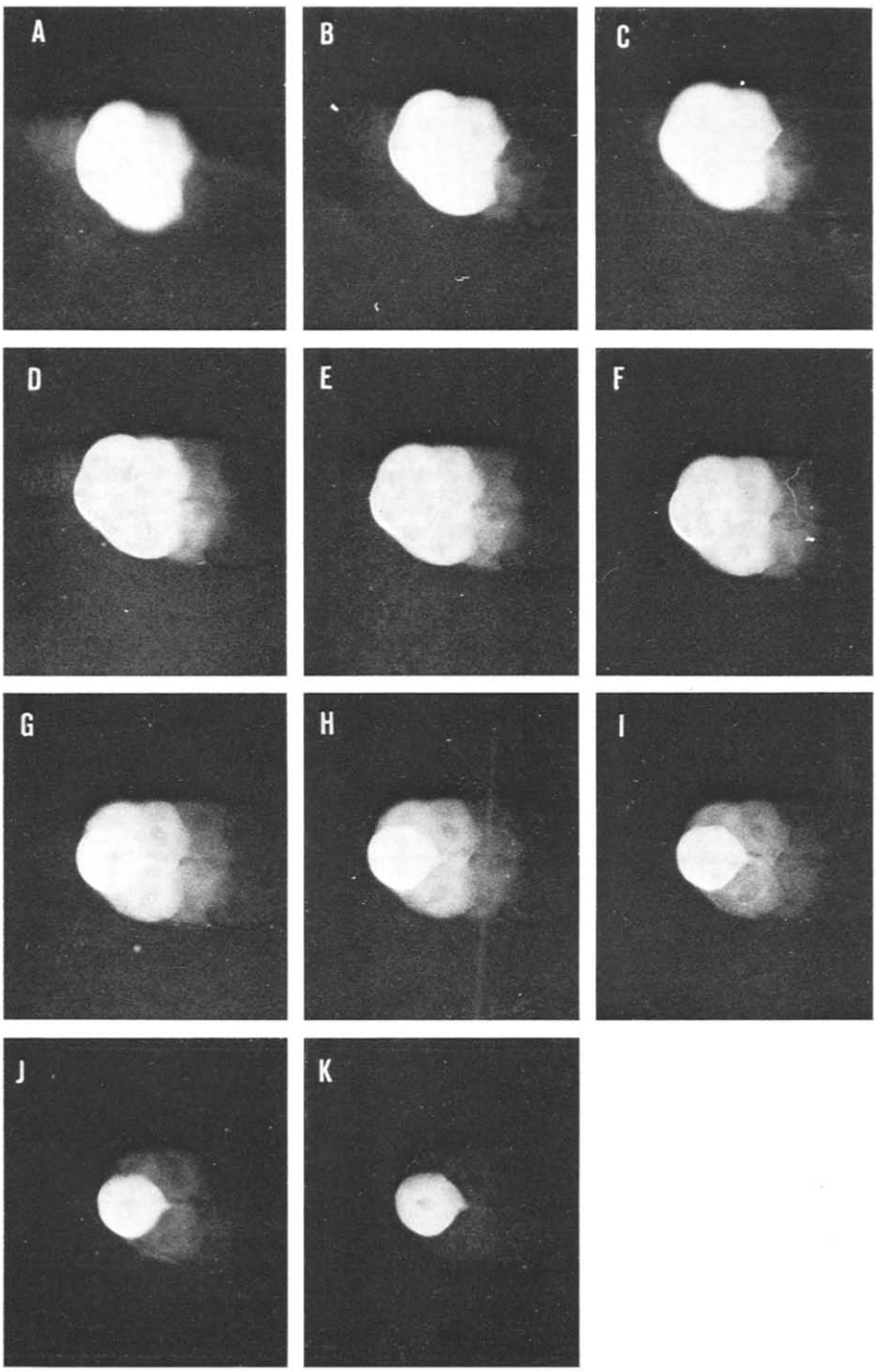

Fig. 3. Photographs of the diffusion process of serine evaluated in Fig. 2. (a) t = 90 s; (b) t = 135 s (c) t = 195 s; (d) t = 255 s; (e) t = 315 s; (f) t = 375 s; (g) t = 435 s; (h) t = 495 s; (i) t = 615 s. (j) t = 735 s; (k) t = 915 s.

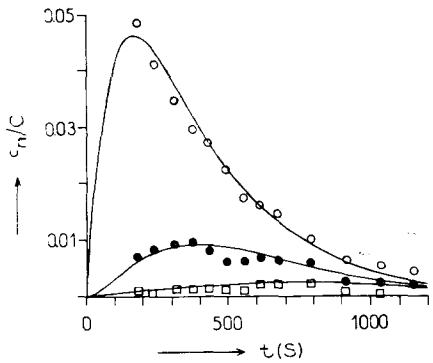

Fig. 4. Results of the measurements on the diffusion process of methionine in the gland. The measurement series marked ** in Table I is used. Measured concentrations in the cells with order number 1 (○). order number 2 (●) and order number 3 (□). The lines represent the best fitting curves obtained by minimization of G (Eqn 8).

DISCUSSION OF THE MODEL DESCRIPTION

Accuracy of the estimated parameters

The minimum value G_{min}, obtained by minimization of Eqn. 8 for every measurement series, was used in order to get an impression about the accuracy of the estimated parameters Q_i and D. For different values of $C^\star$ around C, $Q_i^\star$ around Q_i and $D^\star$ around D, the value of

$$G^\star = \sum_n \sum_j \left(\frac{c_n(C^\star, Q_i^\star, D^\star, t_j)}{c_n^{theor}(C, Q_i, D, t_j)} - 1\right)^2$$

was calculated. From the results the ranges of Q_i and D for which $G^\star \leqslant G_{min}$ were estimated. From these ranges, the corresponding maximal variations Δ_1 and Δ_2 of αP_i and P_u/P_i respectively were calculated: they are indicated in Table I. Thus $\alpha P_i \pm \Delta_1$ and $P_u/P_i \pm \Delta_2$ give upper and lower bounds for all values for which $G^\star \leqslant G_{min}$. Δ_1 and Δ_2 give an impression of the sensitivity of the fitting procedure and of the accuracy of the estimated parameters as far as this accuracy is limited by non-systematic errors.

We want to emphasize that no real statistical justification for the accuracy attributed to the parameters is obtained in this way. Such a justification is hard to obtain here because of the complexity of Eqn 6.

The values found for P_u/P_i extend over a wide range. There are a number of factors which may be responsible for this variability. The ages of the larvae used in the experiments varied between 155 and 165 h. This will introduce errors in the morphological quantities, and thus in αP_i and αP_u of the order of 20 %. Furthermore, in an earlier report [22] it has already been shown that a difference of 10 h in age between salivary glands results in a difference in P_u/P_i for small ions of a factor 2.5. Moreover, the model is a simplification in which no attention is paid to biological variability. For salivary glands with an age between 155 and 165 h the value of P_u/P_i for small ions varies by about a factor 10, which agrees with the variability in results listed in Table I.

Model assumptions

In order to restrict the computer time, it was supposed that $M = 5$. The concentrations calculated from the median values of αP_i and P_u/P_i for $M = 5$ and $M = 20$, respectively, differ less than 1 % in the cells with order numbers 1, 2 and 3 during a test period of 1200 s. Thus the choice of $M = 5$ does not influence the results obtained.

It was assumed that the concentration of fluorescent molecules in the bathing fluid was negligible. In order to get an impression about the correctness of this assumption, the diffusion of molecules out of a sphere was studied. The diffusion coefficient of the molecules in the bathing fluid and in the sphere interior was taken as equal to that in water, i.e. $5 \cdot 10^{-4}$ mm$^2 \cdot$ s^{-1} (corresponding to a molecular weight of 350 [25]) whereas, for the effective permeability of the outer surface, the median value from Table I ($140 \cdot 10^{-6}$ mm $\cdot$ s^{-1}) was chosen. The volume of the sphere was taken as equal to the volume of the most distal cell of the gland.

The computations of the diffusion process due to a concentration C at $t = 0$ in the sphere (concentration outside the sphere at $t = 0$ equal to zero) were carried out using a numerical scheme described previously [26]. In Fig. 5 the results of the calculations are given at two locations, namely at distances of 8 μm and 30 μm from the outer surface in the bathing fluid. Together with these findings the results of the calculations of the diffusion process in the salivary gland with effective permeabilities equal to $140 \cdot 10^{-6}$ mm s^{-1} are given for the cells with order number 3.

It is hardly possible to detect the concentration in the cells with order number 3 (see also Figs 3 and 4). This leads to the conclusion that the concentration outside the gland may indeed be neglected. It is hardly possible to detect the concentration in the bathing fluid. Thus the fact that no leakage is seen does not imply that the non-junctional membranes are much more impermeable than the junctional membranes. Furthermore, measurements during which salivary glands were immersed for 20 min in control medium containing sodium fluorescein (1 mM) show a cytoplasmic staining of the gland cells. This staining is quenched within 5 min when the glands are rinsed with control medium. From the calculations of the diffusion out of the sphere it also became evident that the concentrations inside the sphere are hardly dependent on the site within the sphere.

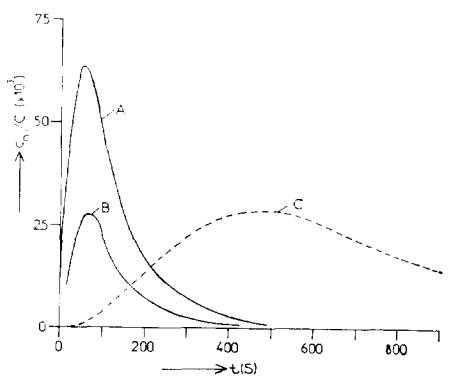

Fig. 5. Results obtained for the diffusion out of a sphere. Concentration at a distance of (A) 8 μm from the outer surface in the bathing fluid and (B) 30 μm. (C) Concentration in the third order cells.

From the measurements mentioned above it follows also that the assumption that the diffusion in the cytoplasm and in the bathing fluid is very fast compared to the diffusion through the membranes is indeed correct.

The photographs show the average concentration during the exposure time of about 30 s. Fig. 2, however, shows clearly that this will not give rise to important errors in the experimental values for the concentrations, because the changes in concentrations are negligible in a period of 30 s (see also Figs 2 and 4). Also, the injection time is short compared to the time in which diffusion occurs.

In our theoretical analysis the transport of the dyes through the junctional and nonjunctional membranes is described by means of simple diffusion equations (Fick's law). From the results (Figs 3 and 4) it appears that there exists a fair agreement between the experimental findings and the theoretical results. In fact we do not have any experimental evidence (like systematic differences between the theoretical and experimental curves) indicating that Fick's law in its simple form is not obeyed.

The exact kind of transport mechanism and the possibility of special pathways are not necessarily of importance here. The conclusion may be formulated as follows. It is found experimentally that the transport processes investigated behave similarly to those in a cell system which can be described by simple diffusion equations and in which the transport through junctional membranes is not highly preferred to transport through nonjunctional membranes. It follows then that cell communication which would provide for easy intercytoplasmic exchange of large molecules involved in growth control and cell differentiation, can not be effective here. Self-evidently, this conclusion is subject to the condition that the tracers give information as to how these processes take place.

GENERAL DISCUSSION

The quantitative analysis of the diffusion patterns of fluorescein and dansylated amino acids reveals that the junctional membranes in salivary gland cells of *Drosophila hydei* account for a substantial barrier to diffusion of these large molecules. Despite the fact that the qualitative analysis of the diffusion of fluorescein from one cell to another suggests intercellular continuity to large molecules, because the leakage of the fluorescent probes to the bathing fluid is hardly detectable (see discussion of model descriptions), the effective permeabilities of the junctional and nonjunctional membrane are found to be almost equal.

The set of fluorescent probes tested includes molecules with different configuration and electrical charge. These differences, however, do not find expression in terms of different permeability properties. This may be partly due to a masking effect of the dansyl group on the amino acid properties and partly to the spread in the values of one single probe. It may be concluded that the behaviour of fluorescein, at least within this gland epithelium, approximates closely to the behaviour of the tested molecules.

The seeming intercellular continuity to large molecules like fluorescein was at the basis of speculations concerning a possible function of cell-to-cell diffusion with respect to cell differentiation and regulation of tissue growth by permitting diffusional transfer of regulatory message molecules [2].

Our results and also results of others [16–21] do not confirm the existance of low-resistance junctions providing for intercytoplasmic exchange of large molecules. For this reason we would like to propose a role for the widespread phenomenon of intercellular ionic continuity with respect to growth control and cell differentiation. It is generally accepted that enzymes require certain optimal environmental conditions for maximal activity. Low-resistance junctions may contribute to optimal functioning of the cell machinery by permitting a balancing of ionic concentrations within the cell system. Changes in membrane properties of a part of the cell system, for instance due to cell injury, will lead to transport of ions into this particular part. The providing cells will maintain their equilibrium situation by an increased activity, whereas in the other cells restoration of the ionic composition of the cytoplasm will reduce recovery processes. Electrical cell coupling should in other words tend to buffer the cell system ionically.

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