J. Exp. Biol. (1972), **56**, 639–645
With 4 text-figures
Printed in Great Britain

THE MOVEMENT OF GLUCOSE AND GLYCINE THROUGH THE TISSUES OF *CORYMORPHA PALMA* TORREY (COELENTERATA, HYDROZOA)

BY G. CHAPMAN* AND R. L. PARDY

Department of Zoology, University of California, Los Angeles, California 90024

(*Received* 4 *October* 1971)

INTRODUCTION

While the uptake of small organic molecules by the tissues of certain coelenterates has been studied (Stephens, 1961, 1962) and the movement of water into and out of *Hydra* has been traced using tritiated water (Lilly, 1955) there is no general understanding of the rate at which materials can diffuse through the mesogloea or pass through the cell layers – a point emphasized by Mackie & Mackie (1967). A study of the rate of diffusion using ^{14}C-labelled substrates would be useful in providing an understanding of the part played by the mesogloea in the movement of molecules between the two cell layers of the coelenterate body. Advantage was taken of the unusually large size and of the structure of *Corymorpha palma*, a solitary hydroid, to make measurements of the rate of movement of glucose and glycine through the isolated mesogloea and through living cell layers. The results of a few experiments using fructose, and some using killed cell layers, are also included.

METHOD

Measurements were made in a perspex cell (Fig. 1) consisting of two parts held together by brass screws. The half-cells retained a sheet of tissue between them in such a way as to divide the central chamber into two half-cells. Each half-cell had two small ports for the introduction of the solutions and through which samples were taken. Uniformly labelled substrates (New England Nuclear Corporation) were prepared in either distilled water or in Millipore-filtered sea water at a specific activity of 1 μCi per ml. Unlabelled substrate was used to make up the total concentration to 0·1 M. After the membrane had been positioned, one half-cell was filled with the labelled solution, the other with sea water or distilled water. The volume of each half-cell was 0·2 ml. At intervals of time, samples of 0·020 ml were removed from each side, mixed in a vial with 1·0 ml of NCS Solubilizer (Amersham-Searle) and 5·0 ml of a fluor consisting of 50 g of 2,5-diphenyloxazole, 0·625 g 1,4-bis-2,5-phenyloxazolyl benzene in 350 ml of toluene and 150 ml of methanol. The samples were counted for 1 min in a Beckman LS 233 Liquid Scintillation Systems counter using the wide-energy window for ^{14}C. All data from experiments were normalized and expressed as per-

* Permanent address: Department of Biology, Queen Elizabeth College, Campden Hill, London, W8 7AH.

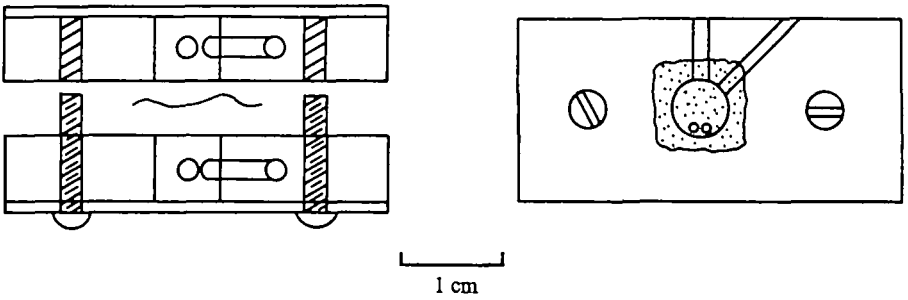

Fig. 1. Perspex diffusion cell. Left, top view, showing position of membrane relative to the chamber before assembly; right, front view, after assembly.

centages. The experiments were all made at room temperature, which ranged from 18 to 22 °C, and the cell was continuously shaken on a reciprocating shaker except while samples were being taken. Two glass beads of 1 mm diameter were placed in each half-cell to assist in the mixing of the contents.

PREPARATION OF THE MATERIAL

Large specimens of *Corymorpha palma* may reach a length of 15 cm. They were collected at low water in the harbour at Newport Beach, California. Each consists of a hydranth supported by a stalk which is anchored to the substratum of muddy sand by many adhesive frustules. The stalk may measure 0·8 cm in diameter and, unlike that of most hydroids, is enclosed in a perisarc for only about one-fifth of its length. The outer cell layer of ectoderm is separated by the mesogloea from the endoderm which makes up the bulk of the stalk and consists mainly of large vacuolated cells ranging in diameter up to 200 μm and which resemble plant parenchyma. The enteron of the hydranth continues into the stalk, not as a single cavity but branching into a series of some 16 longitudinal canals lying just inside the mesogloea. These canals are line by endodermal cells which are distinguishable, when seen in transverse section, from the great bulk of the parenchymatous cells.

Sheets of mesogloea were obtained by cutting off the hydranth and the basal portion, opening the cylinder by a longitudinal cut and immersing the preparation in distilled water for a few minutes. This treatment rapidly lysed the cells, the remains of which were brushed off with a fine paint brush. This procedure produced a sheet of mesogloea which took up stable dimensions after the muscles attached to it in life had been removed. Specimens of mesogloea prepared in this way were examined after staining with aqueous toluidine blue and were seen to be free from cells. Any weak places or tears could be detected by viewing against a black background using side illumination. Samples of mesogloea were always freshly prepared for permeability experiments, although the material could be left in distilled water for several days without appearing to undergo any degradation or losing its strength and resistance to damage when handled.

Portions of the body-wall with the cell layers intact were prepared in a similar way except that the preparation was carried out in sea water cooled to between 0 and 5 °C. Even at this temperature, and in some cases after anaesthesia with magnesium chloride, the action of cutting the cell layers caused slow contraction of the muscle cells which

made it difficult to prepare a sheet of body-wall and place it in the experimental chamber. However, if this operation was performed quickly enough the body-wall minus the bulk of the parenchymatous cells could be mounted in the diffusion apparatus. Following this manipulation the integrity of the cell layers was checked by examination under the stereo-microscope. After mounting the composite membrane the half-cells were flushed out with five changes of sea water during a 30 min period. The sea water was filtered through a 0·45 μm Millipore filter before use.

After preliminary tests a series of experiments was performed in which samples from each side of the membrane were taken (at 10 min intervals) from the beginning of the experiment. Two samples of the original stock solution were also taken as standards, and the membrane of mesogloea or body-wall was examined for defects before removal from the cell. The membrane was then taken out, washed rapidly in 20 ml of distilled water or sea water, drained on a filter paper and counted with the other samples.

The thickness of the mesogloea was measured directly with a micrometer screw-caliper by placing the material between two cover-glasses, the combined thickness of which had been measured in the same way. The thickness of the mesogloea was approximately 15 μm. The volume of the membrane lying between the two half-cells was therefore approximately 0·4 mm^3 (which is one-fiftieth of the volume of each sample removed).

RESULTS

Glucose

The results of the experiments on the transfer of ^{14}C-labelled glucose across the mesogloea are presented graphically in Fig. 2A. The movement of glucose from one side of the membrane to the other is demonstrated by the fall in radioactivity of the original solution and the gain in radioactivity of the other side. This movement is sufficiently rapid to result in equilibrium being attained in about 40 min. Similar results were obtained with labelled fructose.

Glycine

Generally similar results were obtained for the diffusion of glycine across the mesogloea and are shown in Fig. 2B.

The residual radioactivity of the mesogloea in all experiments was of the same order of magnitude as that which would be contained in its own volume of the equilibrated solution, showing that no absorption had occurred.

Transfer across mesogloea and cell layers

It can be clearly seen, from comparison of Fig. 3 with Fig. 2, that the presence of cell layers on the mesogloea severely curtails the diffusion of glycine, as one might expect. There is, however, somewhat more variation in the experiments involving cell layers than in those made with mesogloea alone, and this is possibly due to the fact that the cell layers are injured at the edges of the opening when the halves of the diffusion chambers are tightened sufficiently to prevent slipping of the membrane. This is inherent in the design of the apparatus but does not obscure the fact that the cell layers are relatively impermeable, as compared with the mesogloea, to the metabolites used in the experiments even at the high concentration employed (0·1 M).

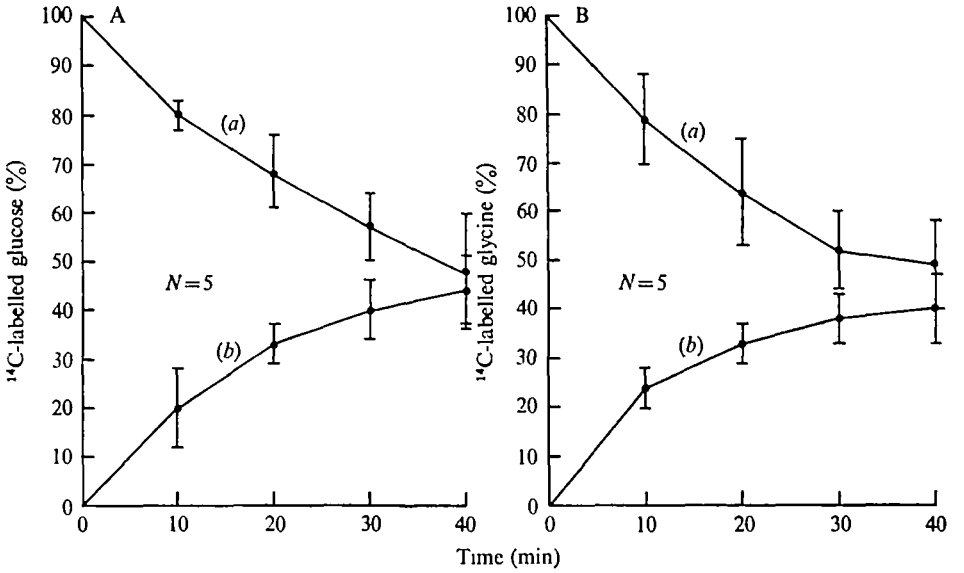

Fig. 2. A, Movement of ^{14}C-labelled glucose across the isolated mesogloea of *Corymorpha palma*. Loss of ^{14}C-labelled glucose from half-cell (*a*) separated by mesogloea from other half-cell (*b*), which shows gain in radioactivity. Ordinate, ^{14}C-labelled glucose in samples as percentage of initial sample. Numbers of observations are given and standard deviations are indicated by vertical lines. B, Movement of ^{14}C-labelled glycine across isolated mesogloea.

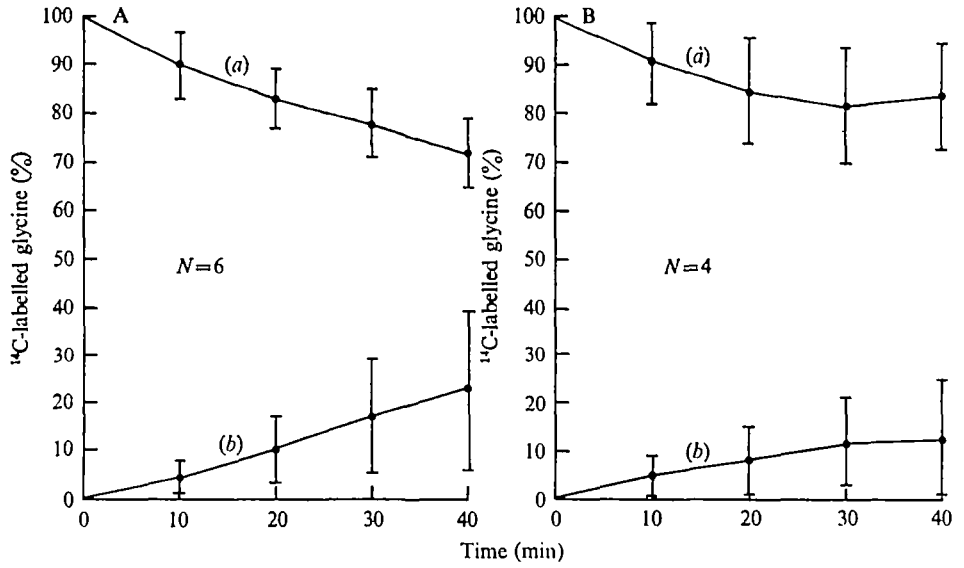

Fig. 3. A, Movement of ^{14}C-labelled glycine across the mesogloea + cell layers of *Corymorpha palma*. Loss from half-cell (*a*) on ectodermal side and gain in half-cell (*b*) on endodermal side. B, Loss from half-cell (*a*) on endodermal side and gain in half-cell (*b*) on ectodermal side.

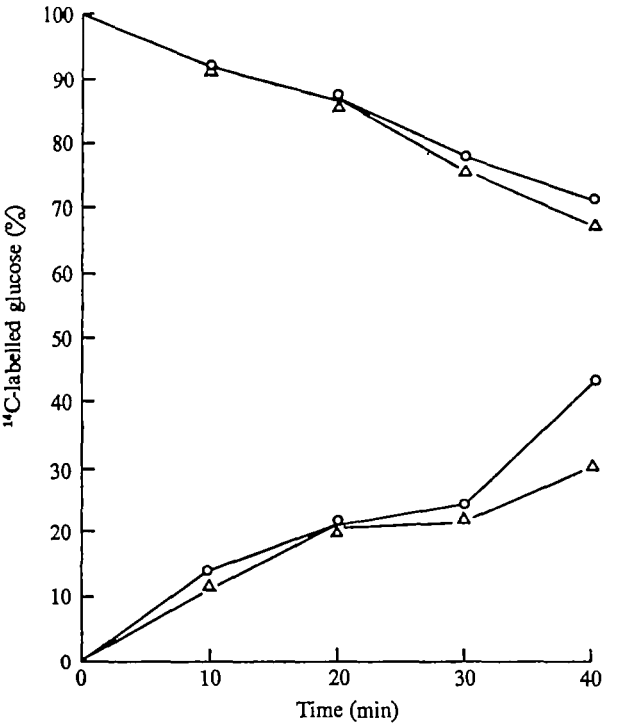

Fig. 4. Movement of ^{14}C-labelled glucose across a Millipore filter (pore size 0·45 μm), O; and across dialysis tubing, △.

Experiments were performed to see whether the rate of movement of molecules was different in the direction ectoderm → endoderm from that in the reverse direction, endoderm → ectoderm. As will be seen from comparison of Fig. 3A with Fig. 3B, little difference was demonstrated.

The cells of the body-wall were killed by filling the cell with 10% formaldehyde in sea water after a diffusion experiment on the living material had been performed. Following fixation the membrane was washed in sea water for 30 min and the diffusion experiment was repeated. The results from such experiments were not clear-cut but suggested that the rate of diffusion lay between that found for the isolated mesogloea in distilled water and that found for the intact body wall in sea water.

Millipore filter and dialysis tubing

In order to provide a comparison with common laboratory materials the rates of movement of ^{14}C-labelled glucose across a Millipore filter and across dialysis tubing were measured. Although much thicker than mesogloea (15 μm), the thickness of the Millipore filter being 150 μm and that of the dialysis tubing being 30 μm, both materials provided a comparable barrier to diffusion (Fig. 4).

Diffusion coefficients of glucose and glycine in mesogloea

Calculation of the diffusion coefficients of glucose and glycine in mesogloea enables a comparison to be made between the hindrance which this material offers compared with that of water or other materials. While the experiments which we performed did

not result in the steady-state condition that is desirable when making such measurements, values obtained by averaging over a short time can be used to obtain an approximate value for the diffusion coefficient from Fick's second law expressed in the form $dm/dt = -DA\,(dc/dx)$. In this equation dm/dt is the rate of transfer of solute across the membrane in moles sec^{-1}, $-dc/dx$ is the concentration gradient in moles cm^{-4}, A is the area of the membrane in cm^2 and D is the diffusion coefficient which has the dimensions $cm^2\ sec^{-1}$. The customary assumption is made that the rate at which labelled molecules pass across the membrane can be taken as a measure of the rate of transfer of the solute.

Taking the first 10 min interval it can be seen from Fig. 2 that the concentration of glucose in the half-cell falls from 0·1 M to 0·08 M. The volume of the half-cell being 0·2 ml, the amount transferred during the period is $(0{\cdot}02 \times 0{\cdot}2)/1000$ moles. Accordingly

$$\frac{dm}{dt} = \frac{0{\cdot}02 \times 0{\cdot}2}{1000 \times 600} \text{ moles sec}^{-1}.$$

Since the average concentration difference between the half-cells is 0·08 M, and since the thickness of the membrane is 15 μm (15×10^{-4} cm),

$$-\frac{dc}{dx} = \frac{0{\cdot}08 \times 10^4}{1000 \times 15} \text{ moles cm}^{-4}.$$

The area of the membrane is 0·33 cm^2. Substituting these numerical values in Fick's equation,

$$\frac{0{\cdot}02 \times 0{\cdot}2}{1000 \times 600} = D \times 0{\cdot}33 \times \frac{0{\cdot}08 \times 10^4}{1000 \times 15},$$

from which

$$D = 3{\cdot}8 \times 10^{-7}\ \text{cm}^2\ \text{sec}^{-1}.$$

Using numerical values from the two subsequent periods of 10 m in one obtains values for the diffusion coefficient of $3{\cdot}8 \times 10^{-7}$ and $6{\cdot}6 \times 10^{-7}$ respectively.

Published figures (Höber, 1945; Clark, 1952) for the diffusion coefficient of glucose in water are of the order of $0{\cdot}6 \times 10^{-5}$, or some 16 times greater than our values for diffusion through mesogloea. Three factors probably account for this. (i) Some matrix, probably polysaccharide, is present with the fibres of the mesogloea and retards diffusion of solute molecules. (ii) The fibrous structure results in a reduction in the area of the matrix through which diffusing molecules can move and also (iii) causes an increase in path length. There appears to be no way in which the effects of these factors can be separated using the present data.

From comparable figures for three 10 m in periods, values obtained for the diffusion coefficient of glycine in mesogloea are 4·1, 5·3 and $7{\cdot}8 \times 10^{-7}$ $cm^2\ sec^{-1}$ as compared with published values (*Handbook of Chemistry and Physics*) for the diffusion coefficient in water of $1{\cdot}06 \times 10^{-5}$ $cm^2\ sec^{-1}$. The hindrance to the diffusion of glycine presented by the mesogloea would therefore appear to be similar to that for glucose.

The first two 10 m periods yield values of approximately 12×10^{-7} for the diffusion coefficient of glucose across both Millipore filter (150 μm) and dialysis tubing (30 μm).

DISCUSSION

While the results of the experiments recounted here cannot be regarded as giving more than an indication of the rates of diffusion of small organic molecules in coelenterate tissue we believe that they are the first direct measurements which have been made and that they could lead to further work of a quantitative nature. The results indicate that the mesogloea acts as somewhat more of a barrier to diffusion than does the same amount of water but does not appear to constitute a significant barrier for small molecules. As would be expected, the cell layers do provide a greater barrier to free diffusion. Published work on the structure of the mesogloea of hydroids (Hausman & Burnett, 1969; Lentz, 1966) suggests that the fibrous elements present are clearly separated from the interstitial material. The results of a study of the mesogloea of *Corymorpha* (unpublished) indicate that the mesogloea is of the same nature and at the molecular level presents no membrane-like barrier to molecular movement. In spite of its relatively high permeability to molecules the material has considerable structural strength; it does not fall apart when left in distilled water, and it can withstand a pressure of as much as 60 cm of water applied to the contents of one of the half-cells. These features suggest that the diffusion of molecules in mesogloea, especially in the more gelatinous mesogloea of medusae and siphonophores, may take place at rates only a little less than their rates of diffusion in water. However, proof of this must await experiment.

SUMMARY

1. The rate of diffusion of ^{14}C-labelled glucose and glycine across isolated mesogloea and across the mesogloea and cell layers of the large solitary hydroid, *Corymorpha* was measured.

2. Diffusion coefficients of glucose and glycine in mesogloea were found to be about 16 times lower than their values in water and of the same order as their values in dialysis tubing and Millipore filters.

REFERENCES

Clark, W.M. (1952). *Topics in Physical Chemistry*. Baltimore: Williams and Wilkins.

Hausman, R. E. & Burnett, A. L. (1969). The mesogloea of *Hydra*. I. Physical and histochemical properties. *J. exp. Zool.* **171**, 7–13.

Höber, R. (1945). *Physical Chemistry of Cells and Tissues*. Philadelphia: Blakiston.

Lentz, T. L. (1966). *The Cell Biology of Hydra*. New York: John Wiley.

Lilly, S. J. (1955). Osmoregulation and ionic regulation in *Hydra*. *J. exp. Biol.* **32**, 423–39.

Mackie, G. O. & Mackie, G. V. (1967). Mesogloeal ultrastructure and reversible opacity in a transparent siphonophore. *Vie Milieu*. Ser. 1–A, **18**, 47–71.

Stephens, G. C. (1961). Uptake of amino acids by marine invertebrates. *Limnol. Oceanog.* **6**, 175–81.

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.