Transport Cell for Examining Solute Transfer across Biological Membranes

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Abstract □ A transport cell was designed for examining *in vitro* solute transfer across biological membranes. When using the intestine as a model membrane, a primary advantage of the method is that there is no need to evert the intestine. This eliminates the influence of eversion on the structural and functional integrity of the intestine and thus its possible influence on solute transfer. Preliminary studies using salicylamide were performed to quantitate various parameters of the apparatus. Clearance values for the mucosal-to-serosal transfer of salicylamide across the everted rat intestine were calculated per unit of membrane surface area. The clearance per area values obtained agree reasonably well with those reported in the literature using different methods. The reproducibility of the transport data agrees favorably with other methods. The utility of the present method is explored.

Keyphrases □ Cell, transport—for examining solute transfer across biological membranes, clearance values, salicylamide □ Biological membranes—transport cell for examining solute transfer □ Solute transfer, *in vitro*—transport cell for biological membranes, clearance values, salicylamide

A number of preparations are available for studying *in vitro* drug transport across biological membranes. Methods utilizing intestinal membranes were recently reviewed (1). Probably the most widely used *in vitro* preparation in drug transport studies is the Crane and Wilson (2) modification of the everted intestinal sac technique (3), with various minor alterations on this basic approach. Although this preparation has a number of advantages over other *in vitro* techniques, it may be criticized on the basis that relatively little is known about the influence of the eversion process on the structural and functional properties of the membrane and the resulting influence on drug transport, and appropriate experiments are not easily designed to answer these questions. A method is reported here for examining drug transfer which obviates the need for membrane eversion. The technique should provide a useful approach for examining directional drug transfer as well as the influence of eversion on drug transport.

EXPERIMENTAL

Transport Cell and Preparation of Intestine—The apparatus may be accurately tooled from Plexiglas (Figs. 1 and 2). The entire apparatus is self-contained and may be placed into a water bath to maintain a constant temperature. The edges of the inner face of one-half of the cell, where contact is made when the unit is closed, are lined with flexible plastic adhesive[1] for a watertight seal. One end of each of the four screws is embedded in one-half of the cell. These screws pass through holes in the opposite half of the cell and are tightened with wing-nuts once the membrane is placed into position.

Male, Sprague–Dawley rats[2], weighing approximately 250 g., were starved 18–22 hr. prior to the experiment with water allowed *ad libitum*. The animals were anesthetized with ether, and a midline abdominal incision was made. The intestine was severed at the ileocecal and pyloric junctions, and the lumen was washed with normal saline and placed into a dish of normal saline at room temperature.

In the studies reported here, the intestine was everted by attaching the proximal part *via* a ligature to a glass rod and gently sleeving the intestine over itself. The first 15 cm. of the proximal intestine was discarded. The intestine was then cut lengthwise with scissors along the point of attachment of adhering tissue. An approximate 6-cm. length of flat intestine was placed over the "window" of one-half of the cell containing embedded pins whose points were sharpened. To prevent the intestine from falling through the window, a solid piece of Plexiglas was placed into the window to provide a solid surface for positioning the membrane. The edges of the membrane were then forced over the pins to secure it in place. During this process the intestine was handled only at the edges with the use of forceps. The other half of the cell was then placed in position, the intestine was sandwiched, and wing-nuts attached to the screws were tightened. The plastic adhesive along the inner cell surface prevented the membrane from being damaged during this process. The Plexiglas insert was then removed, and the cell was placed into a water bath to maintain the drug and buffer solutions at 37 ± 1°. Twenty milliliters of drug in the buffer solution was placed into one compartment of the cell (bathing the mucosal surface), and 20 ml. of buffer solution devoid of drug was placed into

Table I—Comparison of the Mucosal-to-Serosal Salicylamide Clearance Value Obtained in This Study to Those Reported in the Literature

Reference	This Study	*Reference 6*	*Reference 8*	*Reference 9*
Method	Transport cell	Everted gut	Everted gut	Everted gut
Mucosal solution, buffer and pH	Bicarbonate, pH 7.4	Phosphate, pH 6.0	Phosphate, pH 6.6	Bicarbonate, pH 7.4
Solution volume, ml.:				
Mucosal	20	80	140	100
Serosal	20	2	15	2
Mucosal surface area[a], cm.[2]	1.79	13	13	13
Mucosal salicylamide concentration, mg./ml.	4.0	2.0	1.0	1.0
Mucosal-to-serosal salicylamide clearance, ml./hr.	0.287	3.87	5.24	3.42
Clearance per surface area, ml./hr./cm.[2]	0.160	0.298	0.403	0.263

[a] The surface area represents the gross dimensions and not necessarily the effective area available for drug transfer. The area reported for this study represents a measurable area corresponding to the window dimensions. The other values are calculated based upon a membrane segment of 10-cm. length with a circumference of 1.3 cm., assuming a simple cylinder.

[1] Silicone Seal, Canadian General Electric Co., Toronto, Ontario, Canada.

[2] Canadian Breeding Laboratories, Montreal, Quebec, Canada.

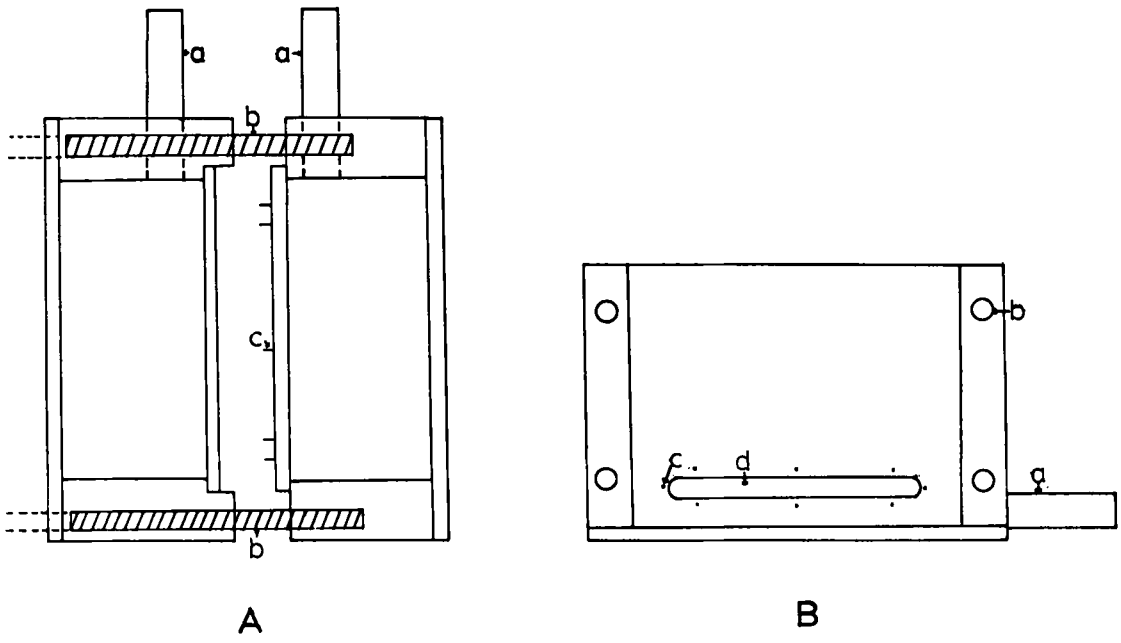

Figure 1—*Diagrammatic sketch of the transport cell. (A) Top view. Key: a, tube outlet to vacuum for removing entire volume of fluid in compartment; b, connecting screws for closing unit; and c, pins for securing intestinal membrane. Inside compartment dimensions are: height, 5.2 cm.; length, 6.0 cm.; width, 2.9 cm. (B) Front view of inside surface of one-half of cell. Key: a, tube outlet to vacuum; b, receiving holes for connecting screws (0.4-cm. diameter); c, receiving holes for pins; and d, membrane window (length 4.85 cm.; width, 0.37 cm.). The bottom edge of the window is approximately 0.5 cm. above the inside bottom surface of the compartment.*

the other compartment (bathing the serosal surface). Solutions in both compartments were gassed with 95% O_2–5% CO_2 *via* thin polyethylene tubing, and underwater magnetic stir plates provided the stirring of small magnetic stirrers in the transport cell. The compartment into which drug was diffusing was easily sampled by pipet or, alternatively, by total removal of the volume in that compartment by vacuum *via* tubing connected to the vacuum outlet tubes.

This procedure was repeated using a second transport cell of dimensions identical to the first for another 6 cm. of intestine which was just distal to the first segment.

Transport Experiments—Salicylamide[3] was the test compound used for examining mucosal-to-serosal transfer to quantitate various parameters of the transport cell. The mucosal drug solution contained 4 mg./ml. salicylamide in a modified Kreb's sodium bicarbonate buffer, pH 7.4 (4). The serosal solution was buffer devoid of drug. Two-milliliter samples of the serosal solution were taken every 10 min. during the 2-hr. experiment, and each sample was immediately replaced by 2 ml. of fresh buffer. Corrections were made for the quantity of salicylamide removed by this sampling technique. Because of the large concentration of drug in the mucosal solution, sink conditions were maintained and a constant rate of transport was obtained. The drug was assayed spectrophotometrically[4] by the method of Trinder (5) as modified by Feldman and Gibaldi (6). All chemicals were of reagent grade.

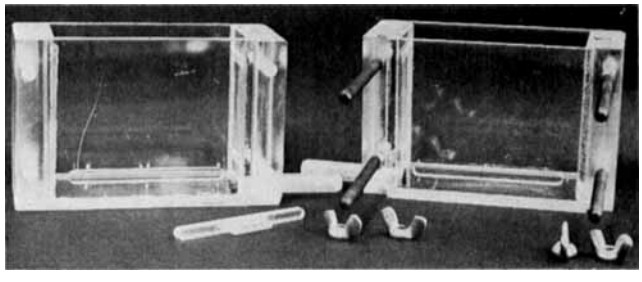

Figure 2—*Photograph of the transport cell separated into both units.*

[3] Reagent grade, Lot No. 2278020, British Drug House, Toronto, Ontario, Canada.

[4] Zeiss PMQ-II spectrophotometer, Oberkochen, Germany.

RESULTS AND DISCUSSION

Figure 3 is a representative plot of the cumulative amount of salicylamide transferred as a function of time from the mucosal to the serosal solution. The data points of the straightline portion of the curve (*i.e.*, transfer after steady state has been reached) were fitted by least-squares regression. An alternative approach to plotting transfer data, which was used previously (7), is illustrated in Fig. 3; one graphs the rate of transfer per unit of time as a function of the midpoint of that time interval. This graphical method

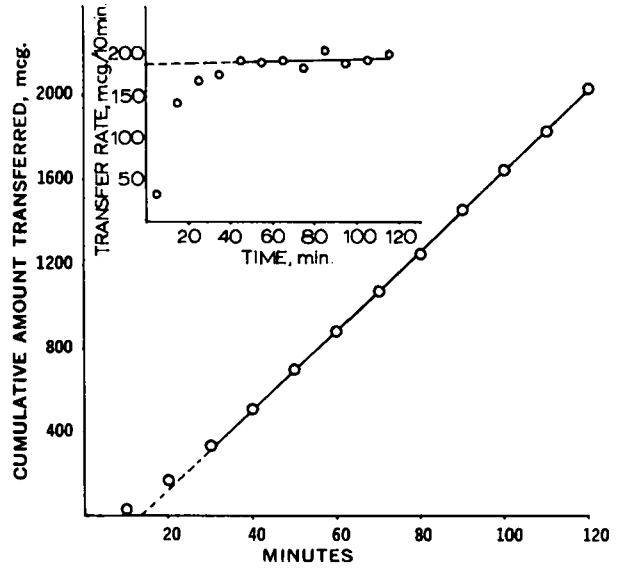

Figure 3—*Representative plot of the cumulative amount of salicylamide transferred across the everted rat intestine from the mucosal to the serosal solution as a function of time. Data were taken from one experiment. The inset graph illustrates an alternative method of plotting transport data. The rate of transfer per 10 min. as a function of the midpoint of the time interval was plotted for the same data as in the cumulative plot.*

provides a valuable assessment of variation in transfer rate in a given experiment. As may be seen, there was relatively little fluctuation in the rates of transfer over the time period of the experiment. Since there were no significant differences ($p > 0.05$) in transfer rates between segments obtained from the same animal, as determined by a paired Student's t test, rates of transfer from all segments were lumped together and averages were determined from all the data. The steady-state transfer rates were determined from a least-squares fit of cumulative amount transferred plots. The steady-state transfer rates were transformed into a clearance value (milliliters per hour) by dividing the rate of transfer by the initial mucosal drug concentration. In seven individual experiments, the arithmetic mean ± standard deviation is 0.287 ± 0.034 ml./hr. The range of values in this series of experiments is 0.223–0.315 ml./hr., with a percent coefficient of variation of approximately 12. Based upon the type of variation normally encountered in transport studies utilizing biological membranes, these values appear quite reproducible. For example, the percent coefficient of variation for salicylamide transfer across the everted rat intestine may be calculated from several literature reports to be approximately 8 (6) or 10 (8).

A comparison of the mucosal-to-serosal salicylamide clearance values obtained in this study to other literature values was attempted (Table I). Several other pertinent parameters of those systems cited are included in the table. The values to be compared are those in the last line of the table: clearance per unit surface area. The surface area of the membrane in the transport cell used in this study is a constant and is calculated from the dimensions of the window. This area is based only on the gross measurable dimensions of the window and, as with the other preparations reported in Table I, is not necessarily the effective membrane surface area available for transfer. A number of assumptions were made to estimate the surface area of the other preparations listed in Table I. The length of all segments listed in those reports (6, 8, 9) is 10 cm. From our own experiments, the circumference of the rat intestine (*i.e.*, the width of the flat membrane once the intestine is cut lengthwise) was estimated to be approximately 1.3 cm. The luminal diameter varies from animal to animal and depends upon the portion of the intestine being used. Other workers showed the intestinal circumference of the rat to be approximately 1.7–1.8 cm. (10). Nonetheless, the clearance values per square centimeter listed in Table I are in relatively good agreement. A number of reasons might explain the fact that the smallest clearance per area value is found in the present study. As mentioned, there is likely to be considerable variation in the diameter of the intestinal lumen from animal to animal, which alters the calculated value of the membrane surface area of the other preparations reported here. In addition, the serosal fluid in the everted gut method has a tendency to distend the intestine laterally. This distension might result in an increased luminal diameter or a more complete exposure of the villi to the mucosal drug solution. Either effect would increase the area available for transfer. Finally, in the other reports cited, there is some type of lengthwise distension of the gut segment during the experiment other than the eversion process itself.

From the preliminary data presented here, it appears that the method reported provides reproducible transport data in reasonably good agreement with previous reports using different methods. The apparatus is easily used and has a number of advantages to recommend it over other *in vitro* techniques. In addition to the fact that there is no need to evert the intestine (one is able to examine the influence of eversion on directional drug transport), relatively small volumes of solution may be used in these studies. This latter point might prove useful when small quantities or expensive materials are to be examined. The primary disadvantage of the transport cell is the small surface area of the membrane available for drug diffusion. This creates a problem when one is performing classical transport studies where amounts or concentrations of drug are determined by analytical methods. Although this transport cell was originally designed for a different approach to studying drug transport, the membrane area available for diffusion will limit the analytical method to be used. If sensitive analyses are available, there should be little difficulty in obtaining valid data. When using experimental animals that have intestines of widths different from the rat, the apparatus may be easily modified by altering the height of the window. The utility of the apparatus described here for *in vitro* drug transport studies will be the subject of future reports.

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