

Effects on blood sugar levels in Charles River rats were observed for several of the rhodanine derivatives<sup>3</sup>. 5-(2-Pyrrolylmethylene)rhodanine (22) (150 mg/kg) increased blood glucose significantly at 1, 2, and 4 hr after oral dosing, and 5-(2-thienylmethylene)rhodanine (150 mg/kg) increased blood glucose at 4 hr after oral dosing. No effect on blood glucose was observed for 5-(4-pyridylmethylene)rhodanine (150 mg/kg) or for 5-(2-pyridylethylidene)rhodanine (150 mg/kg). Increases in blood glucose at 4 hr after oral administration were also found for 2,2'-dithio-bis[3-(2-furyl)acrylic acid] (22) (100 mg/kg) and 2,2'-dithio-bis-(3-phenylacrylic acid) (23) (75 mg/kg). Significant effects on blood glucose levels may be caused either by suitable 5-substituted rhodanines or their hydrolysis products, the dithioacrylic acids.

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# Effects of Polyelectrolytes on Drug Transport II: Permeation

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**Abstract** □ The permeation rate of salicylate across a dialysis membrane was studied in the presence of various types of carboxymethylcellulose. The presence of the polymer in the salicylate solution increased the permeation rate of salicylate. The data were analyzed with a diffusional model in which the viscosity and charge effects were evaluated separately.

**Keyphrases** □ Salicylate—permeation rate across a dialysis membrane in presence of various types of carboxymethylcellulose □ Permeation rate—salicylate across a dialysis membrane in presence of various types of carboxymethylcellulose □ Drug transport—permeation rate of salicylate across a dialysis membrane in presence of various types of carboxymethylcellulose □ Carboxymethylcellulose, various types—effect on permeation rate of salicylate across a dialysis membrane □ Polyelectrolytes—various types of carboxymethylcellulose, effect on permeation rate of salicylate across a dialysis membrane

Previously (1), it was observed that the presence of the polyelectrolyte carboxymethylcellulose decreased the aqueous diffusivity of a small solute (salicylate) only

moderately and that salicylate, which was present as a co-ion of the polymer, was transported out of the polymer solution and into an aqueous environment more rapidly than if the polymer were not present. This report describes further studies on this enhanced transport phenomenon involving an *in vitro* permeation apparatus.

## EXPERIMENTAL

**Permeation Cell**—A permeation cell was constructed from plastic<sup>1</sup> and consisted of two symmetrical parts clamped together with a dialysis membrane at the plane of symmetry. The diffusion cell was described previously (2). Each compartment holds about 125 ml of liquid, and the area of the membrane available for permeation is 16.8 cm<sup>2</sup>. The cell was immersed in a water bath for temperature control, and each compartment was stirred with a stirring rod tipped with flattened rubber tubing and rotated at 2000 rpm.

<sup>1</sup> Lucite, Rohm & Haas, Philadelphia, Pa.

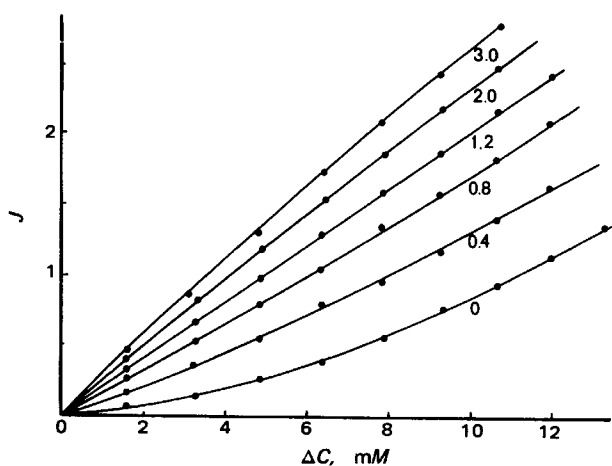


Figure 1—Permeation flux (nanomoles per square centimeter per second) of salicylate versus salicylate concentration difference. Numbers adjacent to curves represent percent (w/v) carboxymethylcellulose sodium (V).

The cellulose membrane, cut from dialysis tubing<sup>2</sup>, was soaked at least 2 days in water containing a preservative (1% sodium azide) under refrigeration. It was then washed with distilled water and kept wet during the assembling of the apparatus. The thickness of the wet membrane was 46.6  $\mu\text{m}$  as measured with a micrometer. With this thickness, the lag time to reach steady-state flux was short and could not be detected.

The water content of the wet membrane was determined by the weight difference between a membrane dried with filter paper and a membrane dried under vacuum. The weight fraction of water in a wet membrane was 0.568, which corresponds to a volume fraction of water of 0.667. The figures agree with reported values (3, 4).

**Materials**—Various types of pharmaceutical grade carboxymethylcellulose sodium<sup>3</sup> were used as obtained without further purification. Two types with a 1.2 degree of substitution were used; one<sup>4</sup> was a medium high viscosity grade (I) and the other<sup>5</sup> was a medium grade (II). Three types had a degree of substitution of 0.7; the first<sup>6</sup> was a medium high viscosity grade (III), the second<sup>7</sup> was a medium grade (IV), and the third<sup>8</sup> was a low grade (V). Hydroxyethylcellulose<sup>9</sup> (VI) was used as the nonionic polymer. The physical properties of these polymers have been reported (1) or are available from the manufacturer (5). Reagent grade sodium salicylate<sup>10</sup> was used as supplied.

**Procedures**—The permeation cell and the solutions were equilibrated with the water bath (37°) prior to an experiment. To start an experiment, 125 ml of the solution containing sodium salicylate (1.6 mM) and the polymer was transferred to one side of the cell (Compartment 1). In most cases, the other side (Compartment 2) was filled with 125 ml of distilled water. The appearance of salicylate in Compartment 2 was monitored by a flow system consisting of a peristaltic pump<sup>11</sup>, polyethylene tubing, and a flowcell. The latter had a 1-mm light path and was mounted in a recording spectrophotometer<sup>12</sup> (202 nm). Data obtained in this manner indicated a linear relationship between absorbance and time, and such experiments were carried out for about 3–7 min.

After this initial phase of the experiment, 4 ml of solution was removed from Compartment 1 and replaced with 4 ml of 50 mM sodium salicylate solution containing the same concentration of polymer as the original solution. Again, the absorbance increase with time was measured. By continuing in this manner, the concentration difference of salicylate across the membrane could be increased stepwise from 1.6 to 12 mM without changing the solutions. The salicylate concentration in Compartment 2 increased, of course, but it never achieved a concentration of over 0.2 mM.

Table I—Permeability and Apparent Partition Coefficient for Transport of Salicylate across Cellophane Membrane from Polymer Solutions to Water (37°)

Polymer	Percent (w/v)	$P \pm SE$ $\times 10^4$ , cm/sec	$K$
I	0.4	$1.89 \pm 0.02$	1.23
	0.8	$1.98 \pm 0.03$	1.49
	1.2	$2.06 \pm 0.04$	1.89
	1.6	$2.18 \pm 0.03$	2.63
	2.0	$2.06 \pm 0.01$	2.98
II	0.2	$1.76 \pm 0.03$	1.03
	0.5	$1.86 \pm 0.03$	1.16
	1.0	$2.07 \pm 0.03$	1.52
	1.5	$2.19 \pm 0.02$	1.98
III	0.4	$1.83 \pm 0.02$	1.18
	0.8	$2.03 \pm 0.01$	1.55
	1.2	$2.22 \pm 0.02$	2.18
	1.6	$2.13 \pm 0.03$	2.50
IV	2.0	$1.96 \pm 0.13$	2.59
	0.3	$1.72 \pm 0.03$	1.02
	0.6	$1.91 \pm 0.02$	1.23
	1.0	$1.90 \pm 0.01$	1.35
V	1.5	$2.14 \pm 0.02$	1.90
	2.0	$2.34 \pm 0.04$	2.84
	0.4	$1.68 \pm 0.05$	0.98
	0.8	$1.81 \pm 0.02$	1.12
VI	1.2	$2.07 \pm 0.01$	1.39
	2.0	$2.32 \pm 0.02$	1.83
	3.0	$2.59 \pm 0.03$	2.62
	0.42	$1.65 \pm 0.02$	— <sup>a</sup>
	0.84	$1.54 \pm 0.04$	—
	1.26	$1.30 \pm 0.02$	—
	1.68	$1.25 \pm 0.05$	—

<sup>a</sup> With  $K$  assumed to be unity.

## RESULTS AND DISCUSSION

**Permeability**—The absorbance–time charts obtained as described were read by a digitizer, and the data were transferred directly into a calculator<sup>13</sup>. Because the absorbance is linearly proportional to the concentration in the range studied, the flux per unit area,  $J$ , could be readily calculated from the least-squares slopes of the absorbance–time data and the absorptivity together with the following relationship:

$$J = \frac{V_2 dC_2}{A dt} \quad (\text{Eq. 1})$$

where  $V_2$  and  $C_2$  are the volume and concentration, respectively, in Compartment 2;  $A$  is the area of the membrane; and  $t$  is the time. Since each flux determination required only about 5 min and the absorbance–time data were linear, the fluxes obtained may be considered to be initial transport rates. The mass balance and sequential additions also were programmed in the calculator so that flux–concentration difference lines were constructed by the calculator plotter directly. Typical data are shown in Fig. 1.

The experimental permeability,  $P$ , defined as:

$$P = \frac{J}{\Delta C} \quad (\text{Eq. 2})$$

where  $\Delta C$  is the concentration difference across the membrane, was determined directly from the slope of the linear region on the flux–concentration difference plots. Although a more extensive analysis can be carried out utilizing the phenomenological equations of Kedem and Katchalsky (6, 7), the use of the more simple form of transport expression, Eq. 2, results in not more than 2% error, which is in the range of the experimental precision (2). The curvature at lower ionic concentrations due to either salicylate or polyelectrolyte is apparently the result of residual carboxyl groups on the membrane (4). The permeability of salicylate at various concentrations of polymer was obtained as the least-squares slope of the linear portion ( $r > 0.99$ ) of the flux–concentration difference curves to exclude this residual charge effect. Permeabilities obtained in this manner along with the standard errors are given in Table I. The salicylate permeability without polymer was  $1.77 \times 10^{-4}$  cm/sec.

Salicylate permeability across the dialysis membrane was increased when carboxymethylcellulose sodium was in Compartment 1. Since any effect of the charged membrane was eliminated by using only the linear regions of the flux–concentration difference curves, the results were

<sup>2</sup> Visking dialysis membrane, Union Carbide Corp., Chicago, Ill.

<sup>3</sup> Cellulose Gum, Hercules, Inc., Wilmington, Del.

<sup>4</sup> Type 12M31P.

<sup>5</sup> Type 12M8P.

<sup>6</sup> Type 7M27SF.

<sup>7</sup> Type 7MXF.

<sup>8</sup> Type 7LXF.

<sup>9</sup> Type KR.

<sup>10</sup> Mallinckrodt Chemical Works, St. Louis, Mo.

<sup>11</sup> Proportioning pump model I, Technicon Instrument Co.

<sup>12</sup> Cary 15, Cary Instruments, Monrovia, Calif.

<sup>13</sup> HP9100, Hewlett-Packard Co., Palo Alto, Calif.

**Table II—Permeability of Salicylate under Various Polyelectrolyte Gradient Conditions**

Compartment 1	Compartment 2	Polymer, % w/v	$P \pm SE \times 10^4$ , cm/sec
I	Water	0.4	$1.89 \pm 0.02$
I	I	0.4	$1.70 \pm 0.02$
Water	I	0.4	$1.52 \pm 0.03$
III	Water	0.4	$1.83 \pm 0.02$
III	III	0.4	$1.56 \pm 0.02$
Water	III	0.4	$1.55 \pm 0.03$
IV	Water	0.3	$1.72 \pm 0.03$
IV	IV	0.3	$1.56 \pm 0.05$
IV	Water	0.6	$1.91 \pm 0.02$
IV	IV	0.6	$1.47 \pm 0.02$
IV	Water	1.0	$1.90 \pm 0.01$
IV	IV	1.0	$1.37 \pm 0.08$

probably due, at least in part, to the interaction of the charges between the co-ion and the polyelectrolyte (1). The polyelectrolyte concentration gradient at the interface of Compartment 1 and the membrane gives rise to an electrical field, which creates a driving force for the transport of ions in this vicinity. This effect would be in addition to the normal diffusional movement.

To explore this charge effect further, three types of experiments were performed: (a) polyelectrolyte was added to Compartment 2, i.e., it is present in both compartments; (b) a nonionic polymer was used in place of the polyelectrolyte in Compartment 1; and (c) a swamping electrolyte was added to nullify the charge interaction.

The experimental results for the case where polyelectrolyte was present on both sides of the membrane are given in Table II. Salicylate permeability decreased when the concentration of carboxymethylcellulose in Compartment 2 was the same as in Compartment 1. Although this type of experiment effectively eliminates the net polyelectrolyte gradient that should reduce the permeation rate, it also may increase the diffusional resistance at the interface between the membrane and Compartment 2. The further reduction in permeability when salicylate diffuses against the polyelectrolyte gradient, i.e., with water in Compartment 1, suggests that this latter effect is only of secondary importance.

When the nonionic polymer hydroxyethylcellulose was used in Compartment 1 in place of the polyelectrolyte, the permeability decreased with an increase in the polymer concentration. These results are shown as the last entries in Table I. Because there is no possibility for charge interaction under these conditions, the effect is due to the increase in diffusional resistance, which will be discussed later.

When a "swamping" electrolyte (0.2 N NaF) was added to the solutions in both compartments, the permeability decreased as the polyelectrolyte concentration increased (Fig. 2). This high ionic strength effectively eliminated the ionic interactions between salicylate and the polymer. At zero polymer concentration, the permeabilities do not coincide in Fig. 2 because of the widely differing ionic strength.

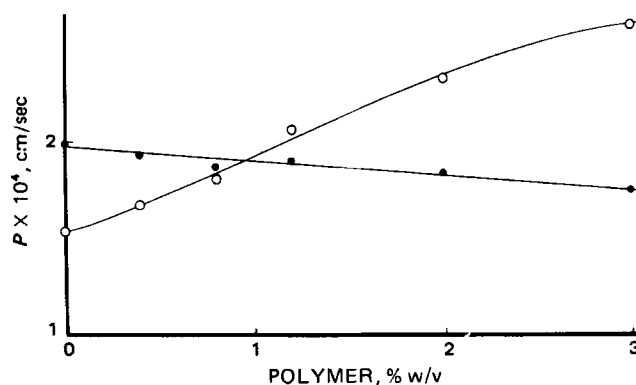
**Permeation Model**—Where the polyelectrolyte gradient exists, the enhancement of salicylate transport can be interpreted in terms of an apparent partition coefficient. Based on the stagnant or unstirred liquid diffusion layer hypothesis, the model shown in Fig. 3 was used for data analysis. The  $h$  is the thickness of the diffusion barrier;  $D$  is the diffusivity; subscripts 1, 2, and  $m$  distinguish the properties in the two compartments and the membrane; and  $K$  is the apparent partition coefficient of salicylate between the solution in Compartment 1 and the water in the membrane.

From the application of Fick's first law to the case of three barriers in series, as shown in the model of Fig. 3, with an apparent partition coefficient between the first and second layers only, the following relationship can be derived:

$$\frac{1}{P} = \frac{h_1}{D_1} + \left[ \frac{h_m}{\psi_m D_m} + \frac{h_2}{D_2} \right] \frac{1}{K} \quad (\text{Eq. 3})$$

where  $\psi_m$  is the volume fraction of water in the membrane and  $D_m$  is an apparent diffusivity because it includes any tortuosity effects in the membrane.

To calculate  $K$  from experimental permeation data, the values for  $D_m$  and the  $h$ 's must first be determined as follows. For salicylate transport with only water in the two compartments,  $D_m$  can be determined as a function of  $h_w$  ( $=h_1, h_2$ ), where the subscript  $w$  indicates water only, from Eq. 3 with  $K = 1$ . Similarly, with hydroxyethylcellulose in Compartment 1,  $h_1$  can be calculated as a function of  $h_w$  ( $=h_2$ ). For this calculation, it is necessary to know  $D_1$ , the diffusion coefficient of salicylate in the



**Figure 2—Effect of strong electrolyte on permeability of salicylate (centimeters per second) as a function of carboxymethylcellulose sodium (V) concentration. Key: ●, 0.2 N NaF in both compartments; and ○, no sodium fluoride.**

polymer solution. Experimentally, it was found (1, 2) that it is described by the linear relationship:

$$D_1 = D_0(1 - 3.8V_p) \quad (\text{Eq. 4})$$

where  $D_0$  is the diffusivity in pure water and  $V_p$  is the volume fraction of polymer. Assuming a value for  $h_w$ , one can calculate  $h_1$  as the viscosity of solution in Compartment 1 changes. The simplest relationship between  $h_1$  and viscosity  $\eta$  experimentally was:

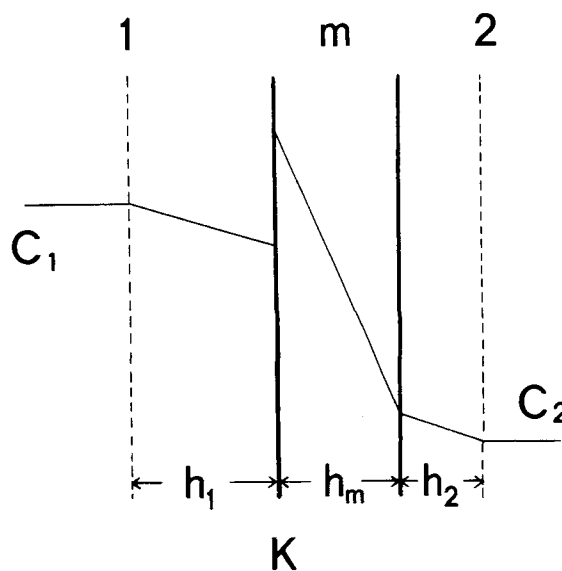
$$\ln h_1 = a + b \ln \eta \quad (\text{Eq. 5})$$

where  $a$  and  $b$  are constants (Fig. 4). If the assumed value of  $h_w$  is correct, the value of  $h_1$  in Eq. 5 extrapolated to  $\eta = 1$  cps (viscosity of water) should be equal to the assumed  $h_w$ . A value of  $h_w$  near  $82 \mu\text{m}$  satisfied this criterion. This value corresponds to  $a = -4.800 \pm 0.086$  (SE) and  $b = 0.189 \pm 0.016$ , with a correlation coefficient of 0.993. From Eq. 3, a calculated value for  $D_m$  of  $1.69 \times 10^{-6}$  cm<sup>2</sup>/sec corresponds to this  $h_w$ .

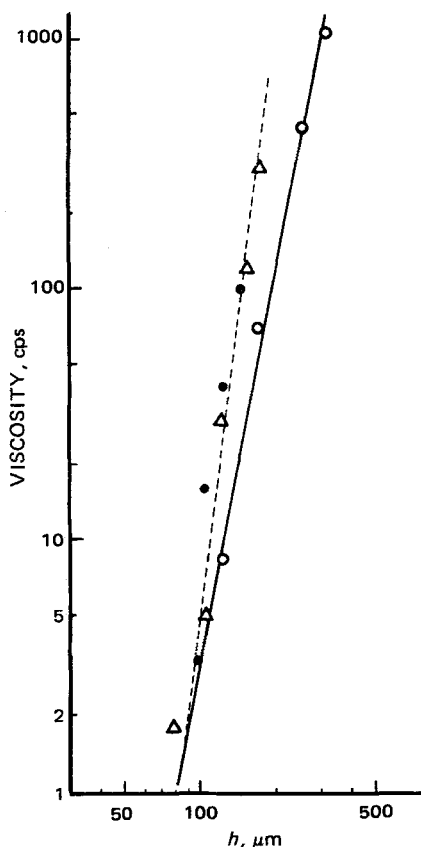
To check the validity of this value for  $h_w$ , a similar computation was carried out for salicylate transport in the presence of a polyelectrolyte gradient but with a swamping electrolyte of 0.2 M NaF on both sides of the membrane to nullify the charge effect. A value of  $81.9 \mu\text{m}$  was obtained. A plot of data according to Eq. 5 is given in Fig. 4.

Knowing  $D_m$ ,  $h_w$ ,  $\eta$ , the constants in Eq. 5, and  $D_1$  from Eq. 4, one can calculate the apparent partition coefficient  $K$  from Eq. 3. The values of  $K$  calculated in this manner are included in Table I and shown in Fig. 5.

The presence of carboxymethylcellulose sodium in Compartment 1 facilitates the salicylate transport across the membrane by providing a driving force in addition to diffusion. This driving force arises from the electrical interaction between the charge of the co-ion and the electrical



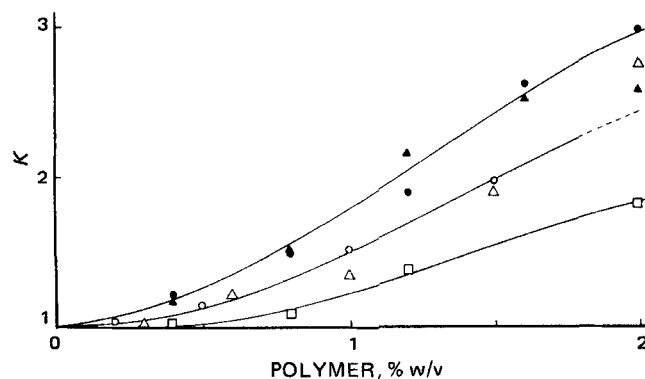
**Figure 3—Diffusion layer model.**



**Figure 4**—Relationship between viscosity and hypothetical diffusion layer thickness. Key: ○, hydroxyethylcellulose (VI); ●, carboxymethylcellulose sodium (V); and Δ, carboxymethylcellulose sodium (IV).

field of the polyelectrolyte gradient. While perhaps not reflecting the most fundamental parameter involved, *i.e.*, the force exerted on the co-ion, the use of Eq. 3 to evaluate the experimental data results in a measure of this co-ion enhancement in terms of an apparent partition coefficient of the permeant as a function of polyelectrolyte concentration.

When carboxymethylcellulose sodium was present in Compartment I, the salicylate permeation rate was increased. If this increase arose from the co-ionic charge interaction, one would expect the chain length and the degree of substitution to affect this permeation enhancement. The calculated apparent partition coefficients are plotted in Fig. 5 as a function of polymer concentration for various polyelectrolytes. The longer



**Figure 5**—Apparent partition coefficient versus concentration of carboxymethylcellulose sodium. Key (types): ●, I; ○, II; ▲, III; Δ, IV; and □, V.

the chain length (higher viscosity grades), the greater is the charge effect between the co-ion and the polyelectrolyte, which may be summarized for the various grades as III > IV > V and I > II. Regarding the degree of substitution, however, no conclusion could be reached because the curves involving polymers having about the same chain length but different degrees of substitution on the carboxymethylcellulose are rather close to each other, *e.g.*, II and IV and I and III; therefore, only two lines are included on Fig. 5 for these data.

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