WATER FLOW THROUGH FROG GASTRIC MUCOSA*

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INTRODUCTION

The transport of water through biological membranes can be attributed to several causes. Gradients of chemical activity caused by the application of hydrostatic pressure or differences in solute concentration across the membrane may produce a net water flow, called *passive* transport (1). In the absence of such gradients a net flow may also occur which is defined as *active* transport, and is presumed to arise from metabolic processes occurring in the membrane. Pappenheimer, Renkin, and Borrero (2) and Koefoed-Johnsen and Ussing (3) have independently shown that passive flows can be interpreted to give a model of the membrane in terms of pore structure.

In the present study two types of experiments have been performed on passive water transport across the isolated gastric mucosa of the frog. First, the unidirectional diffusion flow of water in either direction has been measured using tritiated water as a tracer. Second, an osmotic gradient has been applied across the membrane and a passive net flow has been measured. At the same time an active net flow in the same direction as HCl secretion has also been observed.

In addition to the water transport studies, separate measurements have been made of the restriction offered by the membrane to diffusion of various sized molecules. The combined results of these experiments are interpreted in terms of a polydisperse population of pores in the membrane.

Apparatus and Methods

1. *Rana pipiens*, and occasionally *rana clamitans*, were kept at room temperature without food up to 3 weeks. The frogs were pithed and the stomach was removed and opened along the lesser curvature. The mucosal layer was gently separated from the muscularis, washed off with a modified Krebs-Henseleit solution (whose composition is given in Table I), and then mounted between the two lucite chambers described below.

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The dimensions of the exposed membrane were: area 1.6 cm.^{2}; average net weight 128 mg.; average dry weight 13.4 mg.; average thickness (calculated from weight and area, assuming a density of 1.0), 0.08 cm. All experiments were run at 25 ± 1°C.

2. Two different lucite chambers were used in making the measurements: on the serosal side of the membrane, a large one (A in Fig. 1) with a capacity of about 15 ml.; and on the mucosal side, a small one (B in Fig. 1) with a capacity of either 0.8 or

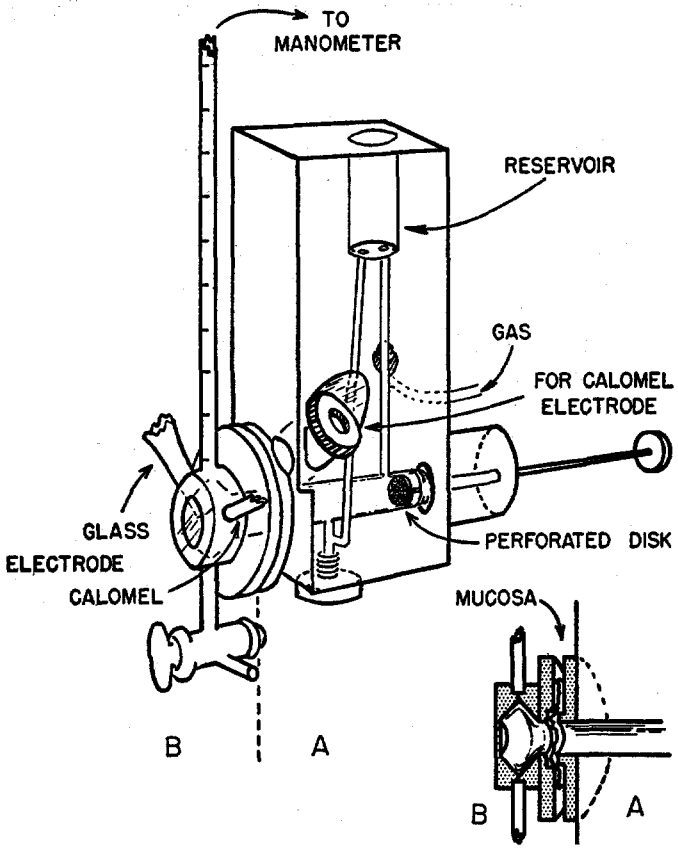

FIG. 1. Lucite chambers for frog gastric mucosa.

2.4 ml., designed for the accurate direct measurement of net volume flow. Fig. 2 is a schematic illustrating the usual assembly. In some experiments, however, the chambers were reversed with respect to the membrane, so that measurements on the serosal side could be made with small quantities of solution.

In the large chamber the solution is oxygenated and stirred by a stream of gas (oxygen, or 95 per cent oxygen and 5 per cent carbon dioxide) as shown in Fig. 1. The solution in the small chamber is oxygenated before it is introduced and is stirred by a small, glass-enclosed iron wire inside the chamber, driven by a rotating magnet on the outside. The potential difference across the membrane is measured by Beckman

calomel electrodes (No. 270) fitting into a holder in the large chamber and connected to the interior of the small chamber by a column of saline agar. For pH measurement a Beckman glass electrode (No. 290) is sealed into the small chamber and can be introduced into the reservoir solution in the large chamber at the top.

The inside of the small, or volume, chamber is beveled out to facilitate filling without bubbles. The chambers are fitted with flanges with a small circular ridge (or mating depression) to make a tight seal when the frog gastric mucosa is placed between the chambers and the flanges are clamped together with pinch clamps (A. H. Thomas Co. No. 3241).

A plug screwed into the back of the large chamber holds a lucite rod which ends in a perforated disc with a diameter slightly smaller than the inside of the chamber. The other end of the lucite rod, extending to the outside, bears a stop. When the lucite rod

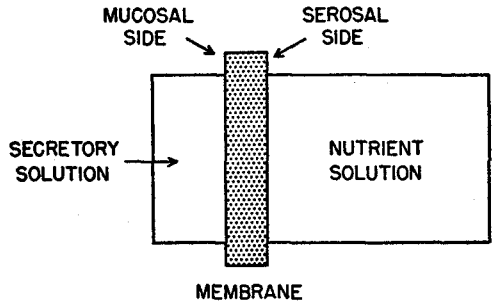

FIG. 2. Schematic assembly of frog gastric mucosa chambers.

is advanced to the stop and clamped, the disc fixes the location of the membrane for volume readings.

A graduated 0.2 ml. pipette is sealed vertically into the top of the volume chamber. This is used to obtain the volume reading, with the membrane fixed in the standard position against the disc by application of a standard pressure of 37 mm. Hg (as measured by a manometer) to the solution in the 0.2 ml. pipette. Except for the short period required to make the measurement, both chambers were open to the atmosphere.

For each observation three readings were taken; the first was discarded because it was found to be variable, and the second two averaged. In order to check that this procedure gave accurate volume measurements, a set of 11 experiments was carried out, both on parafilm membranes and frog gastric mucosae inactivated by NaCNS. The results of these experiments, each involving multiple readings, showed that volumes measured under these conditions were reproducible within a standard deviation[1] of ± 2 μl.

3. Tritium[2] served as the tracer to measure the unidirectional diffusion of water

[1] All errors will be given in terms of standard deviation unless otherwise stated.

[2] Tritium was obtained from the Atomic Energy Commission at Oak Ridge, as was the Cl^{36}. Of the other isotopes used, Na^{24} was obtained from the Atomic Energy Commission at Brookhaven, and C^{14}-labelled sucrose was purchased commercially.

(4). Samples were taken from both chambers at hourly intervals and appropriately diluted for measurement of tracer concentration. Tritiated water (THO) was determined in a proportional counter using the method of Robinson (5). The initial concentration of tritium on the labelled side was from 0.3 to 3.3 μc./ml. The accuracy of the tritium determinations, including dilution and pipetting errors, is 2 per cent.

4. C^{14} urea, C^{14} sucrose, raffinose, inulin, hemoglobin, Na^{24}, and Cl^{36} were used in diffusion experiments to measure the restriction to flow of molecules and ions of graded size. The concentrations used in all cases were so low that osmotic effects resulting from the addition of the test molecule could be ignored. As a control, samples were taken from both chambers during the course of the experiment.

(*a*) C^{14} urea,[3] uniformly labelled, was added to the serosal side at a concentration of 0.08 mM/liter. For these experiments the frogs were pretreated with antibiotics (100 mg. sulfaguanidine, 5 mg. terramycin, 25,000 units of penicillin) daily for 2 days as recommended by Dintzis and Hastings (6) and Kornberg and Davies (7) to eliminate the urease activity associated with the bacterial flora of the intestinal tract.

(*b*) C^{14} sucrose, uniformly labelled, was added to the serosal side at a concentration of 2.9 mM/liter. Both C^{14} urea and C^{14} sucrose were measured in a Robinson windowless proportional flow counter (8), using the method of Hunter and Commerford (9). No corrections for self-absorption were necessary since the same amount of solute was present in all planchets used in each experiment. The accuracy, as determined from the differences in duplicate samples, was $\pm$6.9 per cent for urea and $\pm$1.1 per cent for sucrose.

(*c*) Raffinose was added to the mucosal side at a concentration of 33.6 mM/liter. Samples were hydrolyzed and measured as fructose by the modified resorcinol-thiourea method of Lowry (10) which was accurate to 6.3 per cent under our conditions.

(*d*) Inulin, added to the serosal side at a concentration of 4 mM/liter, was also measured as fructose by the resorcinol-thiourea method with an accuracy of 7.7 per cent. The inulin was purified by recrystallization but it cannot be concluded that the preparation is monodisperse, so that results obtained with this test substance must be accepted with caution.

(*e*) Hemoglobin was prepared by hemolyzing human red cells with distilled water, acidifying to pH 5.7 with 0.1 N HCl, and filtering off the precipitated ghosts (11). The filtrate was dialyzed for a minimum of 3 days against large volumes of nutrient solution with several changes. Glucose was then added to a final concentration of 11.1 mM/liter in the dialyzed solution, which was used directly on the mucosal side. Hemoglobin concentration was determined on a Beckman spectrophotometer model B at a wave length of 416 mμ. The membrane is so little permeable to hemoglobin that final concentrations of 6×10^{-5} mM/liter were found on the serosal side. Although the method of measurement is accurate at moderate concentrations, at very low concentrations a small absolute error becomes a large percentage error. Consequently, the accuracy under our conditions may be estimated as $\pm$130 per cent.

(*f*) In the case of the ions Na^{24} and Cl^{36}, it was necessary to compensate for the potential difference across the membrane. From Hogben's (12) data, it appears that

[3] The labelled urea was kindly supplied by Dr. A. B. Hastings.

within the limits of biological variation, the average of flows in either direction across the membrane approximates the flow with zero potential difference. Accordingly, experiments were carried out to measure the flux in both directions, and the results were averaged. In the case of Na^{24}, 36 μc., and in the case of Cl^{36}, 7 to 65 μc. were added to the large chamber. Gamma rays from Na^{24} were measured in solution in a scintillation counter to an accuracy of ± 2.2 per cent. Samples of Cl^{36} were neutralized and counted by the technique employed for C^{14} with an accuracy of ± 1.8 per cent.

Experimental Procedures

The usual experimental procedure was to place a frog gastric mucosa between the two chambers, using the secretory and nutrient solutions whose composition is given in Table I. Net flow was determined by measurements of volume in the small chamber at 30 minute intervals for a period of 1 to 1½ hours. Then an osmotic gradient was set up across the membrane by adding one of the modified solutions, described below.

TABLE I

Physiological Solutions

Compound	Nutrient solution	Secretory solution
	mM/liter	*mM/liter*
NaCl	84.6	102.1
KCl	3.2	4.3
$CaCl_2$	1.8	1.8
KH_2PO_4	0.8	—
$MgSO_4 \cdot 7H_2O$	0.8	0.8
$NaHCO_3$	17.8	—
Glucose	11.1	11.1

This gradient was allowed to remain across the mucosa for 1½ hours. Replacement with ordinary nutrient and secretory solutions reestablished original conditions. Finally, flow was observed in the presence both of NaCNS (to inhibit metabolic activity) and an osmotic gradient.

Measurements made for the first half-hour period following the establishment of initial conditions, or any subsequent change in conditions as described above, were routinely discarded. The purpose of this was to eliminate transient effects between consecutive steady state conditions. Control measurements on membrane swelling or shrinking showed that this process was essentially complete within the first half-hour.

Osmotic gradients were produced across the mucosa by adding glucose to the secretory solution or by reducing the electrolyte concentration of this solution. The addition of glucose to a total concentration of 222 mM/liter in the secretory solution did not appear to damage the membrane. By contrast the serosal side was quite sensitive to changes in osmolarity, however produced, as indicated by a fall in potential difference and cessation of acid and water secretion. Consequently, reversal of the osmotic pressure gradient was effected by diluting the secretory solution. However, when no salts were present in the secretory solution, the membrane ap-

peared to be less viable, so that results under these conditions are to be accepted with reserve. The detailed composition and calculated osmolarities of the solutions used are given in Table II.

NaCNS was used to inhibit acid and net water secretion. In agreement with the work of Crane, Davies, and Longmuir (13) and Davenport (14), it was found that a concentration of 12 mM NaCNS/liter on the nutrient side was sufficient to inhibit the spontaneous secretion of acid by the frog gastric mucosa. At the same time, the spontaneous water flow, normally observed to occur from the nutrient to the secretory side, is abolished but the potential difference across the mucosa remains unchanged. Removal of the NaCNS results in renewed acid secretion and net water flow, usually up to the previous levels, and this reversibility was taken as evidence that NaCNS does not damage the membrane.

TABLE II

Solutions for Osmotic Flow Experiments and Calculated Osmolarities

Solution	Modification	Calculated osmolarity*
		m.osM/liter
A Nutrient		219
B Secretory		215
C Secretory	+100 mM glucose/liter	315
D Secretory	+211 mM glucose/liter	426
E Secretory	−73 mM NaCl/liter	79
F Glucose (11.1 mM/liter)		11.1

* After correction for osmotic coefficients (29).

RESULTS

Net Water Flow.—

In the absence of an applied osmotic gradient, a net flow of solution was always observed from the serosal to the mucosal side of the membrane. This net flow, called spontaneous net flow, is apparently greater in summer than winter frogs; the results that follow have been obtained with summer frogs. In forty-seven experimental half-hour periods on twenty-five different membranes, this spontaneous net flow was found to be 9 ± 1.9 μl./(1.6 cm.^{2} membrane 30 minutes) and seemed to be independent of H^+ secretion as shown in Table III. This is equivalent to a flow of 11.3 μl./cm.^{2} hr.

Under the influence of an osmotic gradient, the net solution flow was observed to change in the direction predicted for a semipermeable membrane, as shown in Table IV. The solution flow due to osmotic pressure and the spontaneous net flow are additive, so that

$$\text{Net flow}_{\text{observed}} = \text{Net flow}_{\text{osmotic}} + \text{Net flow}_{\text{spontaneous}} \quad (1)$$

Consequently, to obtain the Net $\text{flow}_{\text{osmotic}}$, Net $\text{flow}_{\text{spontaneous}}$ in the periods before and after the period of osmotically induced flow was averaged and sub-

TABLE III

Net Solution Flow and H^+ Production by Frog Gastric Mocosa

Experiment	Net Solution flow (μl/1.6 cm.² membrane, 30 min.)			H^+ flow
	Period 1	Period 2	Average	
				*μeq./hr.**
1	8		8	1.98
2	7		7	0
3	11		11	2.84
4	12	8	10	0
5	12	10	11	1.51
6	14	12	13	0.99
7	10	9	9.5	2.39
8	10	8	9	1.44
9	11	8	9.5	0.16
10	14	4	9	1.73
11	8	10	9	2.15
12	12	13	12.5	0
13	8	8	8	0.30
14	7	10	8.5	0.73
15	10	10	10	0
16	5	9	7	0
17	9	8	8.5	1.91
18	11	6	8.5	0.74
19	8	4	6	0.18
20	7	5	6	1.30
21	11	14	12.5	0.60
22	10	9	9.5	1.67
23	10	8	9	1.26
24	7	8	7.5	1.36
25	7	6	6.5	1.14
Average	9.6	8.5	9.0	1.1
Standard deviation	±2.3	±2.6	±1.9	±0.9

* For the period 0.5 to 1.5 hours.

TABLE IV

*Osmotic Flow Data**

Secretory solution (Table II)	D	C	E	F
Osmotic gradient (m.os M/liter)	207	96	−140	−208
Net $flow_{observed}$	18.3 ± 0.8 [26]	12.1 ± 1.7 [8]	−3.5 ± 1.5 [2]	−10.6 ± 1.6 [16]
Net $flow_{spontaneous}$	7.0 ± 0.5 [46]	6.4 ± 0.5 [16]	7.0 ± 2.5 [5]	5.0 ± 0.6 [32]
Net $flow_{osmotic}$	11.3 ± 0.9 [calc.]	5.7 ± 1.8 [calc.]	−10.5 ± 2.9 [calc.]	−15.6 ± 1.7 [calc.]
Net $flow^{NaCNS}_{observed}$	13.8 ± 1.1 [15]	5.0 ± 0.6 [7]	−6.0 ± 2.1 [1]	−12.4 ± 1.5 [9]

* Flows are given in μl./1.6 cm.² membrane, 30 minutes. Number of periods in each case is shown in brackets. The error shown is the standard error of the mean of each set of measurements. The mean value for the Net $flow_{spontaneous}$ is different from that given in Table III, since the above values were averaged throughout the experiment, whereas the values in Table III were obtained in the first two experimental periods.

tracted from the Net $flow_{observed}$. When NaCNS is added to the nutrient side, the spontaneous net flow becomes zero and the Net $flow^{NaCNS}_{observed}$ = Net $flow_{osmotic}$ as can be seen from the agreement in the bottom two lines of Table IV.

Fig. 3 shows the net flows plotted against the osmotic gradient. It can be seen that the Net $flow^{NaCNS}_{observed}$ is directly proportional to osmotic pressure in the region from zero to 207 m.osM/liter. The slope of this line is 60 ± 7 μl./(osmol/liter) calculated by least squares. In view of our reservations about the viability of the membrane in the absence of salts in the secretory solution, the

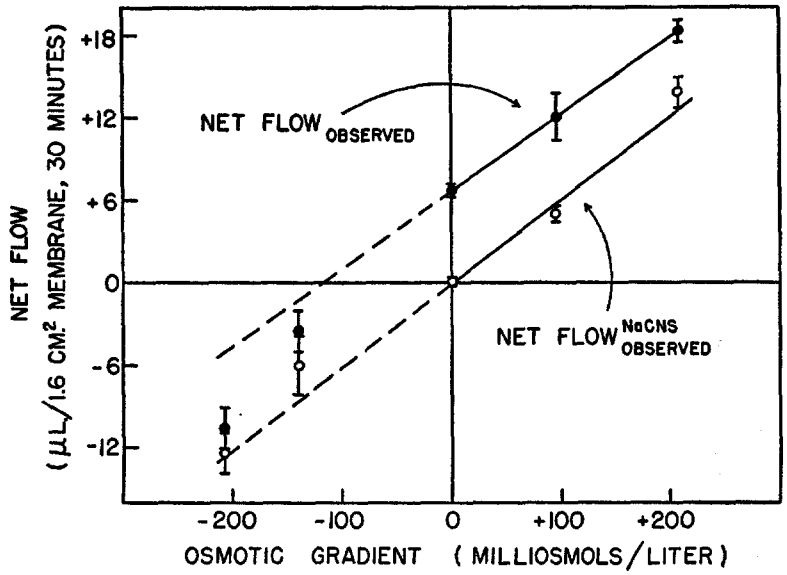

FIG. 3. Observed net flows under osmotic gradient.

region of negative osmotic gradient (dashed line) has not been included in this calculation.

The line for Net $flow_{observed}$, calculated in similar manner, has a slope of 57 ± 5 μl./(osmol/liter). Thus the agreement of the above two slopes is well within experimental error. It may be concluded that NaCNS does not affect the resistance of the membrane to osmotic flow.

Diffusion Experiments.—

Membranes may be characterized in terms of their permeability to any lipid-insoluble test substance[4] by the ratio

[4] Urea, which would appear to be the most lipid-soluble of the test substances, is negligibly soluble in lipid, as shown by the olive oil–water partition coefficient of 0.00015 given by Collander and Bärlund (15). This conclusion is borne out by the diffusion rate of urea across both the gastric mucosa and the hind-limb capillary (2).

$$\frac{\text{Total pore area available for diffusion}}{\text{Pore length}}$$

(16). This ratio, called $A/\Delta x$, is a virtual quantity since it represents that ratio of pore area to pore length which would be necessary to account for the observed diffusion rates through the membrane on the basis of Fick's law. As the pore area approaches molecular dimensions, free diffusion is increasingly restricted. Nonetheless, it is assumed that the diffusion coefficient in the pores of the membrane is equal to the value determined in aqueous solution, and the

TABLE V

Diffusion Data of Molecules and Ions of Graded Size

Molecular species	No. of experimental periods	Radius*	$D_{25}°$	$\frac{A}{\Delta x}$
		Å	(cm.²/sec. × 10^5)	cm.
Water	64	1.5	2.66 (17)	3.34 ± 0.57
Cl^{36}	14	1.9	1.94 (20)	0.69 ± 0.23
Na^{24}	14	2.6	1.31 (21)	0.23 ± 0.08
Urea	9	2.6	1.38 (22)	0.17 ± 0.08
Sucrose	9	4.7	0.52 (23)	0.17 ± 0.09
Raffinose	6	5.6	0.43 (23)	0.22 ± 0.12
Inulin	6	15.2	0.16 (24)	0.29 ± 0.05
Hemoglobin	6	30.8	0.08 (25, 26)	0.05 ± 0.03

* The radius used for H_2O has been obtained from x-ray data given by Morgan and Warren (18). The ionic radii have been obtained from conductance data by Gorin (19). The molecular radii are calculated from the Stokes-Einstein equation except for urea. In this case the radius is estimated as the radius of an equivalent sphere, because of the small size of the urea molecule. The Stokes-Einstein radius is given by: $r' = RT/6\pi\eta D N$; and the equivalent sphere radius is given by: $r = (3M/4\pi \rho N)^{1/3}$, in which M is the molecular weight, ρ is the density, N is Avogadro's number, R is the gas constant, T is the absolute temperature, and η is the viscosity of the solvent.

virtual quantity, $A/\Delta x$, is considered to include all the restrictions to free diffusion. It may be determined experimentally from Fick's law, as follows:

$$dn/dt = -(DA/\Delta x)(C_2 - C_1) \tag{2}$$

in which dn/dt is the number of moles of the test substance that crosses the membrane in unit time under the influence of a concentration difference, $(C_2 - C_1)$, across the membrane, expressed in mol/liter. D is the diffusion coefficient of the test substance. The diffusion of a test substance across the membrane from side 2 to side 1 is then given by:

$$(dn/dt)_{2\to1} = (DA/\Delta x)C_2 \tag{2 a}$$

In the case of water, one way diffusion in the absence of an applied osmotic gradient was measured with THO (4). In sixty-four experimental periods on seventeen membranes the total unidirectional flow by diffusion was found to

be 278 ± 47 μl./(1.6 cm.² hr.). This is equivalent to a flow of 174 μl./cm.² hr. (2.68 μM/cm.² sec.). Using this flow and the diffusion coefficient, D_w, given by Wang *et al.* (17), the ratio $A_w/\Delta x$ was calculated to be 3.34 ± 0.57 cm., as given in Table V.

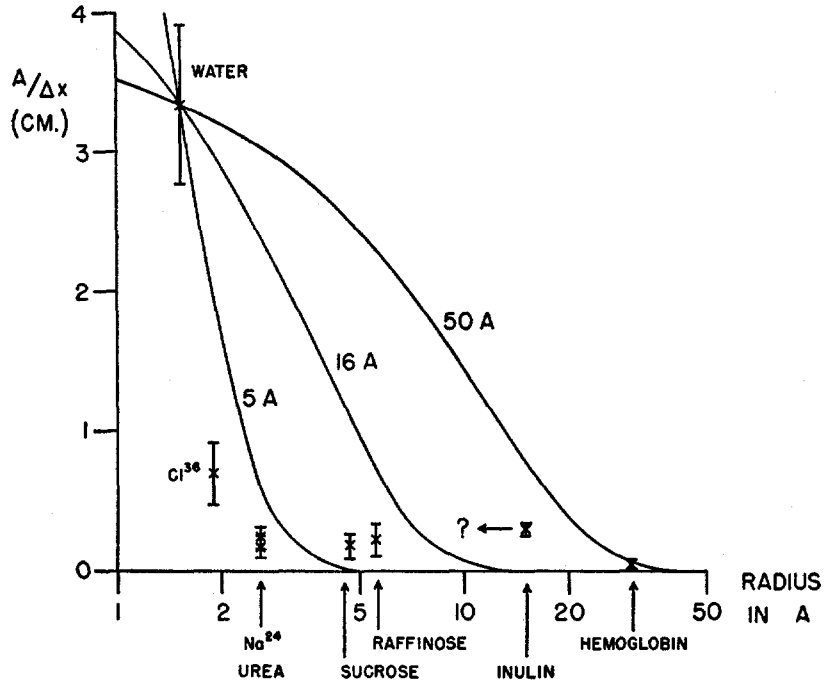

FIG. 4 *a*

FIG. 4. The restricted area/thickness ratio, $A/\Delta x$, for diffusion is plotted against the radius of the test molecule. The errors shown in $A/\Delta x$ are standard deviations. The error shown for the radius of inulin corresponds to the uncertainty about the homogeneity of the inulin preparation.

(*a*) The curves drawn are obtained from equation (4) for single pore radii of 5, 16, and 50 Å respectively.

In addition, a number of other substances were used for graded diffusion experiments. The diffusion of test substance in every case was so small that back diffusion could be ignored in the calculations. The results of these measurements are given in Table V. The calculated $A/\Delta x$ are given in column 5 of this table and are also shown in Fig. 4.

DISCUSSION

The above results show that an osmotically induced flow, comparable in volume to that spontaneously secreted by the frog, can be produced by doubling

the osmolarity of the fluid bathing the secretory surface of the mucosa. Previous efforts to measure osmotically induced flow through gastric mucosa have been unsuccessful. Davies and Terner (27) used hydrostatic pressures of the order of 0.01 atmosphere, much less than the effective equivalent pressures

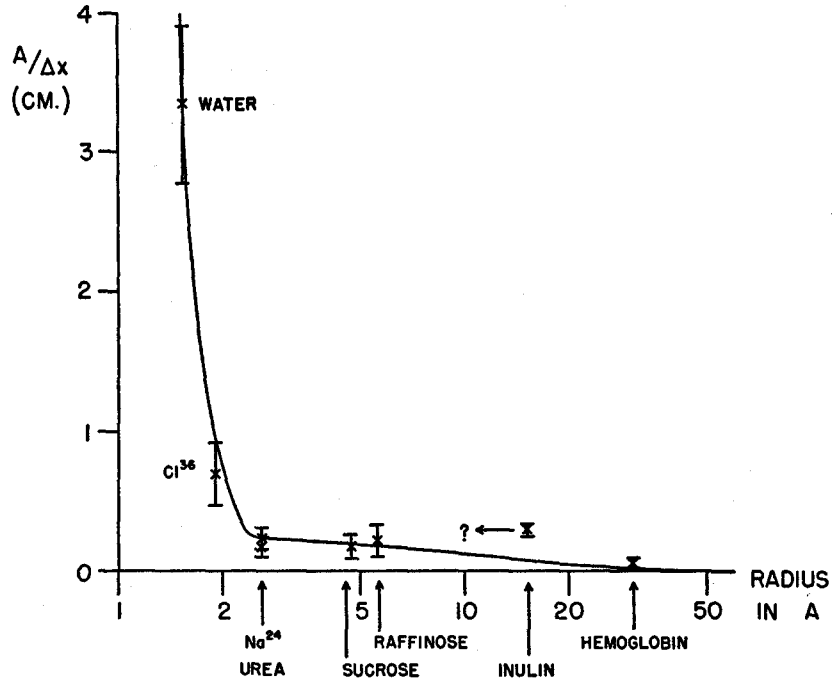

FIG. 4 *b*

(*b*) The curve drawn is obtained as described in the text on the basis of two discrete pore radii. The pore sizes are 2.5 and 60 Å, and the relative area/thickness contributions are 93 per cent for the 2.5 Å population, and 7 per cent for the 60 Å population.

used in the present experiments. Rehm and collaborators (28) in work on dog mucosa *in vivo* used osmotic gradients comparable to the present ones. However, the technique they employed to measure volume changes was not accurate enough to detect osmotic flows as small as those measured above.

Diffusion and Bulk Flow.—

In order to interpret the experimental results, it is necessary to describe flow through semipermeable membranes. Water transport through these membranes may occur as a result not only of diffusion, but also as a result of bulk flow through pores. This problem has been treated theoretically by Pappenheimer

(16) and Koefoed-Johnsen and Ussing (3) who arrive at somewhat different results. It is shown in the Appendix that these two formulations, in the limiting case represented in the present experiment, reduce to the same equation.

The assumptions involved in the equation given below which describes the net water flow due to the combined effect of diffusion and bulk flow are: (*a*) water flow occurs through circular pores of uniform radius perpendicular to the plane of the membrane; (*b*) the membrane is truly impermeable to the solute; (*c*) diffusion flow may be defined as flow according to Fick's law, with the diffusion coefficient within the pores equalling the diffusion coefficient in aqueous solution; and (*d*) bulk flow may be defined as flow according to Poiseuille's law, which is assumed to hold even for small pore radii with the viscosity within the pores equalling that in bulk solution. It is recognized that these assumptions may not be valid for a real membrane; nonetheless for clarity it seems desirable first to set out the equation based on these assumptions, and then to examine the assumptions critically in a subsequent section. The equation follows:

$$\Delta = \Delta_d + \Delta_f = \varphi \Delta\pi (A_w/\Delta x)(D_w \bar{V}_w/RT + r^2/8\eta_w) \tag{3}$$

in which

Δ = net flow in milliliters of water per unit time

Δ_d, Δ_f = net flow due to diffusion and bulk flow respectively

φ = practical osmotic coefficient, which is necessary when the van't Hoff equation as given below is used for non-ideal solutions. φ is defined in Harned and Owen's (29) equation 1-9-6.

$\Delta\pi$ = $RT\Delta C$, the van't Hoff equation

$A_w/\Delta x$ = $A/\Delta x$ for water, D_w = diffusion coefficient for water

$\bar{V}_w$ = partial molal volume for water

η_w = viscosity of water

The relative contributions of net diffusion and bulk flow may now be evaluated on the basis of equation (3). The contribution of net diffusion is given by:

$$\Delta_d = \varphi \Delta\pi (A_w/\Delta x)(D_w \bar{V}_w/RT) \tag{3 a}$$

which can be calculated for the maximum case, by using the highest glucose concentration of 222 mM/liter and the value of $A_w/\Delta x$ given in Table V. At this glucose concentration, the solution may be considered to be ideal, so that $\varphi = 1$, and hence $\Delta_d = 0.8$ μl./cm.^{2} hr. The measured osmotic net flow under this concentration gradient is 15.6 μl./cm.^{2} hr. The difference of 14.8 μl./cm.^{2} hr. between these two figures must be ascribed to bulk flow.

An examination of the assumptions involved in the evaluation of Δ_d reveals that the value of 0.8 μl./cm.^{2} hr. is indeed a maximum. Thus $\Delta\pi$ is the maximum value of the osmotic pressure across a leaky membrane. If the membrane offers any restriction to diffusion, the value of D_w will be decreased, so that D_w is a maximal value. Furthermore, in the experiments discussed above,

$A_w/\Delta x$ was measured by tracer diffusion in the presence and in the absence of osmotically induced flow, and was found to be independent of flow. Consequently, the bulk flow as calculated above represents a minimum estimate. For this conclusion we need not assume that Poiseuille's law holds for these small pore radii; the conclusion that osmotically induced flow cannot be described by diffusion alone is independent of the exact mechanism which governs bulk flow. This result is in opposition to the conclusions of Chinard (30) who states that osmotic pressure cannot give rise to bulk flow and that all flow resulting from activity gradients across a semipermeable membrane is the result of diffusion.

Fluid Flow and Membrane Structure.—

On the basis of the assumptions already made, equation (3) may also be used to solve for an effective pore radius in the frog gastric mucosa. These calculations based on the osmotic flow experiments give a preliminary value of 16 Å as the pore radius. However, the results of the molecular and ionic diffusion experiments do not agree with this figure. The data from these experiments can be evaluated on the basis of the restricted diffusion equation, given by Renkin (31, Equation 10). Renkin has shown experimentally that this equation is valid for the diffusion of molecules with radii from 2 to 30 Å through cellulose membranes, 0.005 to 0.008 cm. thick, with pore radii from 15 to 80 Å. Dividing the Renkin equation by Δx, we obtain:

$$A/\Delta x = (A_0/\Delta x)(1 - a/r)^2[1 - 2.104(a/r) + 2.09(a/r)^3 - 0.95(a/r)^5] \quad (4)$$

in which A is the pore area available for diffusion of molecules of radius a, A_0 is the total pore area, and r is the pore radius.

The restricted diffusion predicted by this equation on the basis of a single pore size is compared in Fig. 4 *a* with the results experimentally obtained. From this figure it can be seen that a 16 Å radius pore does not fit the data. Furthermore, it is apparent that no other single radius will fit the data, as can be seen from the curves that have been drawn for pore radii of 5, 16, and 50 Å.

Consequently, we have next examined the data on the basis of two distinct pore radii. It would also be possible to use pore length as a variable, or to choose an asymmetric distribution of pore radii to obtain a fit. However, the simplest approach is the choice of two discrete pore radii, and the more complicated procedures do not appear to be justified at present. The pores have been chosen to give the best fit to the data by eye, using equation (4), and the area/thickness values have been estimated by successive approximation so that the sum of the contributions from both pores passes through the experimental point for water. A further restriction that has been used in calculating the pore radii is the requirement that the radii chosen account for the osmotic flow as discussed below.

The pore sizes used for Fig. 4 *b* are 2.5 Å and 60 Å; the relative area/thick-

ness contributions are 93 per cent for the 2.5 Å population ($A_{w_s}/\Delta x$ = 3.09 cm.) and 7 per cent for the 60 Å population ($A_{w_l}/\Delta x$ = 0.25 cm.).

The osmotic flow may be calculated on the basis of three major assumptions already given: (1) theoretical osmotic pressure maintained across the leaky membrane; (2) extension of Poiseuille's law to pores of 2.5 Å radius, and (3) uniform, right circular pores. On this basis we may write:

$$\Delta_f = \Delta_s + \Delta_l$$

in which Δ_f = bulk flow in milliliters of water per unit time, of which Δ_s passes through small pores of 2.5 Å radius and Δ_l passes through large pores of 60 Å radius. Of the total net flow of 14.8 μl./cm.^{2} hr., we may calculate that 0.4 μl./cm.^{2} hr. passes through the small pores, [$(Ar^2/\Delta x) = 20 \times 10^{-16}$ cm.^{3}], and 14.4 μl./cm.^{2} hr. pass through the large pores, [$(Ar^2/\Delta x) = 900 \times 10^{-16}$ cm.^{3}].

Next let us turn to a detailed examination of the assumptions that have been made. From the data above, it appears that 97 per cent of the bulk flow passes through the large pores. Since the pore radius is taken as 60 Å and the osmotic pressure was caused by glucose with a 3.6 Å radius, the membrane can certainly not be considered as truly semipermeable. Staverman (32) has approached this problem from the viewpoint of irreversible thermodynamics without specifying the mechanism of transport. This treatment is preferable for our purposes to that of Grim (33) and Laidler and Shuler (34) which does not take account of bulk flow. For leaky membranes, Staverman finds that the experimental osmotic pressure observed, $\pi_{exp.}$, is equal to $\sigma\pi_{th.}$, in which $\pi_{th.}$ is the theoretical osmotic pressure. The quantity $(1 - \sigma)$ is to be obtained in an idealized ultrafiltration experiment as the ratio of solute concentration in a sample after flowing through the membrane to the solute concentration before. This would be equivalent to an experiment of the type described by Renkin (31) in which, however, the contribution by diffusion to the total solute flow is measured separately. After compensation for this contribution, we can obtain $(1-\sigma)$ experimentally as (A_s/A_w), in which A_s and A_w are the restricted areas of filtration available for the solute and water molecules respectively. Calculating this ratio according to equation (19) given by Renkin (31), we obtain σ = 0.1 for the large pores and 1.0 for the small pores. In view of the uncertainties involved in the calculation of σ, the value of 0.1 for large pores can only be taken as a first approximation. Furthermore, there is no theoretical treatment available to indicate how the separate σ's for the two sets of pores may be combined to give an effective osmotic pressure, either for the bulk solution, or in the immediate neighborhood of the individual pores. In view of these considerations, we may tentatively conclude that an appreciable fraction of the total bulk flow passes through the small pores and that consequently the pore radii assigned do not form a wholly self-consistent model.

This conclusion may be supported by an examination of the remaining as-

sumptions. There is little reason to believe that bulk flow through 2.5 Å pores obeys Poiseuille's law. Furthermore, the 2.5 Å figure is obtained from graded diffusion experiments which may reflect a narrow minimum aperture rather than a long pore of the uniform radius. Indeed, we would rather expect the pores to be irregular and tortuous, and that each of the two figures tentatively assigned for pore radius would represent the mean radius of a broad population of pores.

Many uncertainties are involved in the assignment of pore radii of molecular dimensions to biological membranes. Nonetheless it seems important to describe the membrane operationally in terms of the pore hypothesis, bearing in mind the tenuous ground on which we stand. With these reservations, the frog gastric mucosa may be represented operationally by an equivalent membrane composed of two groups of pores, with 93 per cent of the pore area contributed by pores of 2.5 Å radius and 7 per cent by pores of 60 Å radius. The tentative nature of this conclusion is a direct consequence of the fact that no adequate theory is yet available by which the behavior of membranes may be predicted on a molecular scale. Nonetheless the important *experimental* facts remain. They are the characterization of the frog gastric mucosa with respect to osmotically induced flow and with respect to molecular and ionic diffusion.

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APPENDIX

It is to be shown that the formulation of Koefoed-Johnsen and Ussing (3) and that of Pappenheimer (16) for water flow through pores in membranes reduce to the same equation in the limiting case represented in the present diffusion experiments. Koefoed-Johnsen and Ussing's final equation (reference 3, Equation 21) is

$$\ln (M_{in}/M_{out}) = \ln (a_{ws}/a_{wn})[1 + G_w/g'_w] \quad (1)$$

in which M_{in} is water flow towards the nutrient side and M_{out} is water flow towards the secretory side; a_{ws} and a_{wn}, activity of water on the secretory side and nutrient side of the membrane respectively; G_w and g'_w, the frictional force on a mole of water, moving at unit velocity, exerted by water and the membrane, respectively. It is assumed here that the membrane is impermeable to the solute giving rise to the above activity difference, that transports due to hydrostatic pressure differences and metabolic processes are zero, and that the solutions on both sides of the membrane are well stirred and of constant composition.

Assume for simplicity that the solution on the secretory side is pure water, and that the nutrient solution is moderately dilute. Introducing g, the rational osmotic coefficient of Bjerrum, equation (1) becomes

$$\ln (M_{in}/M_{out}) = -g[1 + G_w/g'_w] \ln N_{wn} \quad (2)$$

in which N_{wn} is the mol fraction of water on the nutrient side.

If Δ is the osmotic water flow toward the nutrient side (measured in units of $\left(\frac{\text{volume}}{\text{time}}\right)$

$$M_{in} = M_{out} + \Delta \tag{3}$$

In the present experiment, since $\Delta = 0.05\ M_{out} = \text{Net flow}^{\text{NaCNS}}_{\text{observed}}$

$$\ln [(M_{out} + \Delta)/M_{out}] \cong \Delta/M_{out}; \text{ also } \ln N_{wn} \cong -N_{sn}/N_{wn} \tag{4}$$

in which N_{sn} is the mol fraction of the solute on the nutrient side. Equation (2) becomes

$$\Delta/M_{out} = g(N_{sn}/N_{wn})(1 + G_w/g'_w) \tag{5}$$

Since Δ is small, M_{out} is within a few per cent of the unidirectional water flow, M, as measured by tracer water flow under our conditions.

$$M = (A_w/\Delta x)D_w \tag{6}$$

Koefoed-Johnsen and Ussing have used Poiseuille's law in the limiting case of large bulk flow to evaluate $g'_w = 8\eta\bar{V}_w/r^2$ in which $\bar{V}_w$ is the molal volume of water, η its viscosity, and r the radius of the cylindrical pores through which the flow is supposed to occur. By definition, $G_w = RT/D_w$. Using these values for M_{out}, G_w, and g'_w in (5), and solving for Δ,

$$\Delta = (gD_wN_{sn}A_w/N_{wn}\Delta x) + (gr^2N_{sn}RTA_w/8\eta\Delta xN_{wn}\bar{V}_w) \tag{7}$$

Since the solution is dilute, $N_{sn}/(N_{wn}\bar{V}_w)$ may be put equal to C_{sn}, the concentration in moles/liter of solute on the nutrient side. Putting $\pi = C_{sn}RT$

$$\Delta = (gD_w\bar{V}_w\pi A_w/RT\Delta x) + (gr^2\pi A_w/8\eta\Delta x) \tag{8}$$

$$= \Delta_1 + \Delta_2$$

These two contributions to Δ are identical with the following equation taken from equations (8) and (9) of Pappenheimer (16) for the case in which $g = 1$ (ideal solution with no hydrostatic pressure difference).

$$\Delta = (D_w\bar{V}_w\pi A_w/RT\Delta x) + (r^2\pi A_w/8\eta\Delta x) \tag{9}$$

We may ascribe Δ_1 to diffusion caused by the difference in water activity across the membrane, and Δ_2 to bulk flow caused by this difference. Clearly equation (1) must be used rather than (8) when Δ is *not* small compared to M. But neither (1) nor (8) can be assumed to hold in cases in which bulk flow does not obey Poiseuille's law, since g'_w has been evaluated from this law.

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