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THE MECHANISM OF WATER TRANSPORT BY THE GALL-BLADDER

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The gall-bladder concentrates bile by reabsorbing NaCl and water in isotonic proportions (Diamond, 1962*a*), and it has already been shown that the transport of NaCl is an active process (Diamond, 1962*b*). The present paper will be concerned with the kinetic mechanism and thermodynamic basis of water transport.

Many other organs of the body share with the gall-bladder the ability to transport water and salt together, and the absorbate or secretion is also isotonic in such epithelia as the kidney proximal tubule, intestine, pancreas, and ductless exocrine glands. Nevertheless, isotonicity is not a universal property of fluid transport, since the ducts of exocrine glands form a hypertonic absorbate, the salt gland of birds a hypertonic secretion, and the Malphigian tubules of stick insects a hypotonic secretion. The mechanism of salt transport has attracted more experimental interest because of its often spectacular specificity, but on a molar basis isotonic mammalian absorbates and secretions are more than 99 % water. Active salt transport can apparently be given a precise thermodynamic definition, and the carrier hypothesis may be an important step towards understanding its kinetic basis. However, little direct evidence exists concerning the kinetic mechanism of water transport, and there is no quantitative explanation of the nature of the coupling between salt and water transport which results in a characteristic osmolarity for each body fluid. There is also a more fundamental uncertainty over how to decide whether water transport is active or passive, since no thermodynamic treatment of water movement in the presence of active solute transport is available. A particular point in dispute is whether movement of water against osmotic gradients may mean that salt movement is providing the energy for water movement (e.g. Curran & Solomon, 1957) or proves that water is being actively transported (e.g. Fisher, 1955). Among the forces frequently invoked to explain water transport, only filtration, classical osmosis, and electro-osmosis can be put to simple experimental tests.

As will be shown in the first part of this paper, none of the three last-mentioned passive mechanisms can account for water transport in the

gall-bladder *in vitro*. The second part analyses active transport by means of irreversible thermodynamics, and derives three general criteria for distinguishing active from passive water transport. By any of these three criteria isotonic water transport in the gall-bladder may be quantitatively explained passively. The underlying kinetic mechanism is formally analogous to co-diffusion.

A preliminary account of some of this work has already been given (Diamond, 1961).

METHODS

Techniques are in general the same as those described previously (Diamond, 1962*a*, *b*). The composition of the experimental solutions has been given in the first of these papers. The species of fish used in all cases was the roach (*Rutilus rutilus*).

Volumetric capillary techniques. To observe the effect of variations in hydrostatic pressure, the cannula tied into the cystic duct of the gall-bladder was connected by a tight-fitting polythene sleeve to a horizontal piece of nylon capillary tubing, secured on a millimetre ruler whose height above the preparation could be varied (see Fig. 1). Since air bubbles were rigorously excluded from the system, a continuous column of Ringer's solution extended from the lumen of the gall-bladder to the meniscus in the horizontal capillary, and the position of the meniscus along the ruler was read to the nearest tenth of a millimetre every minute. The internal diameter of the capillary was determined as 0·59 mm from the weight of a column of mercury or distilled water of measured length (*ca.* 150 cm), the formula for

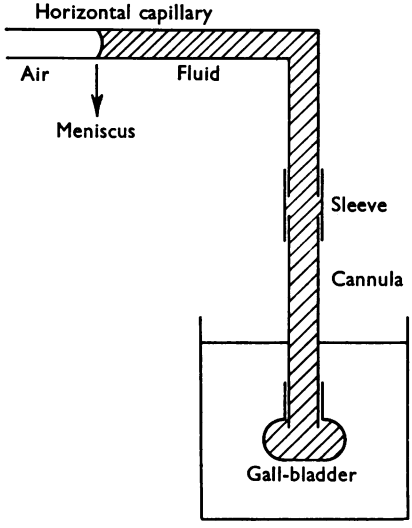

Fig. 1. Arrangement for determining the effect of hydrostatic pressure on water absorption by the gall-bladder. The gall-bladder cannula is connected by a polythene sleeve to a nylon capillary, all filled with a continuous column of fluid. When the volume of the gall-bladder becomes constant, the rate of movement of the meniscus in the horizontal part of the capillary is proportional to the rate of water absorption by the gall-bladder at constant hydrostatic pressure. The hydrostatic pressure may be varied by raising or lowering the horizontal part of the capillary. The ligatures securing the cannula in the cystic duct are not shown.

the volume of a right circular cylinder being used. Thus, a recession of the meniscus 1 cm towards the gall-bladder implied that 2·74 μl. of Ringer's solution had passed from the capillary into the lumen of the gall-bladder, since an air gap saturated with water vapour beyond the meniscus reduced evaporative losses at the meniscus to an undetectably low value. As a check on the constancy of diameter of the capillary tube, the length of a 2·4 cm column of mercury was measured at fourteen 10 cm intervals, and was found to be constant to $\pm$ 1·4 mm, or 6·%. The hydrostatic pressure in centimetres of Ringer's solution was taken as the height of the horizontal capillary above the surface of the solution bathing the gall-bladder.

Radioactive techniques. One-way fluxes were determined by adding tritiated water to the lumen of a gall-bladder and changing the outer solution at 10 min intervals, as described already (Diamond, 1962*b*). Since the external specific activity was only about 1 % of the luminal specific activity at the end of each 10 min period, the back-flux was negligible. Samples were counted to at least 10,000 counts by scintillation counting of a 1 ml. aqueous sample mixed with 12 ml. of redistilled dioxane and liquid phosphor SP-2. Corrections were made for counter dead-time and also for quenching, since salts decreased the counting rate by this liquid-phosphor method. The quenching factor ranged from 0·86 for distilled water (relative to Ringer's solution) to 1·00 for Ringer's solution, and was measured in each experiment for each kind of aqueous solution counted.

RESULTS

Experiments on water transport

Filtration

Since the fluid inside the gall-bladder normally represents a closed system at some hydrostatic pressure higher than that outside, water will leave the lumen by filtration at a rate depending upon the hydraulic conductivity of the preparation. To estimate quantitatively the contribution of this effect, the gall-bladder cannula was connected to a horizontal nylon capillary filled with Ringer's solution. The principle was that as the gall-bladder absorbs fluid the meniscus in the capillary should recede towards the preparation.

Figure 2 depicts the distance in millimetres which the meniscus has moved towards the gall-bladder in 1 min. This is proportional to the rate at which fluid is entering the gall-bladder (1 mm = 0·27 μl.), and if the volume of the gall-bladder remains constant, to the rate at which fluid is being absorbed. Just before $t = 0$ in Fig. 2 the pressure was raised from 4 to 19·5 cm solution. After 15–20 min fluid is entering the gall-bladder at a steady rate, but the first 15–20 min are occupied by a transient, during which fluid is entering more rapidly than in the steady state. If the pressure was suddenly lowered, the initial rate of fluid entrance was slower than in the steady state, and for large sudden drops of pressure the initial direction of meniscus movement was reversed, indicating net fluid movement from the gall-bladder lumen into the capillary. These transients mean that the volume of the gall-bladder increases with increasing pressure, as is of course to be expected. The luminal volume changes might be due either

to the elasticity of the connective tissue or else to changes in tonus of the musculature. The duration of the transients was not shortened in Ringer's solution containing adrenaline and atropine, which relax the musculature (Lieb & McWhorter, 1915).

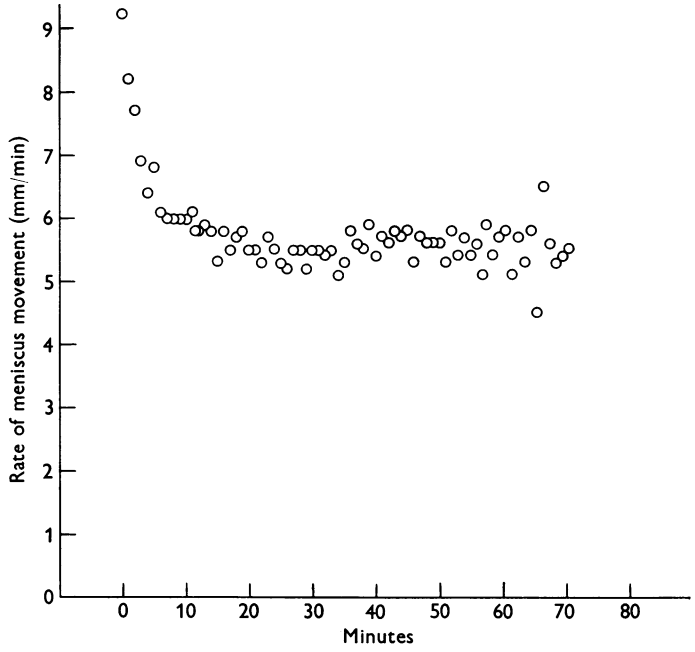

Fig. 2. Water absorption by the gall-bladder at constant hydrostatic pressure. A continuous column of fluid extended from the lumen of the gall-bladder to the meniscus in a horizontal capillary, as shown in Fig. 1. Ordinate, distance (mm) by which the meniscus has receded towards the gall-bladder in 1 min. Just before $t = 0$, the height of the horizontal part of the capillary (hence the hydrostatic pressure) was increased from 4 to 19·5 cm Ringer's solution. When the volume of the gall-bladder becomes constant after 15–20 min, the constant rate of meniscus movement is proportional to the rate at which the gall-bladder is absorbing fluid.

When the luminal volume reaches its steady-state level after a change in pressure, the rate at which fluid enters must equal the rate at which it is being absorbed. For two gall-bladders, after a steady-state rate of fluid transfer had been measured by this horizontal-capillary method, the capillary was then disconnected, the cannula plugged, and the absorption rate measured by the weighing method (Diamond, 1962*a*). In the first case the capillary method gave 13·3 μl./hr for 50 min at a pressure of 11·3 cm solution, after which the rate of weight loss for 45 min corresponded to 16·3 μl./hr; in the second case, the result was 49·5 μl./hr for 100 min at a pressure of 6·1 cm solution by the capillary method, followed

by 43·8 μl./hr for 1 hr by weighing. Thus, the two methods are in satisfactory agreement, and steady-state meniscus movement may be taken as a measure of fluid absorption.

In four experiments fluid absorption was measured consecutively at two different hydrostatic pressures from steady-state meniscus movements. The results were: 24·7 μl./hr at a pressure of 11·3 cm Ringer's solution, and 21·5 μl./hr at 17·8 cm; 54·4 μl./hr at 6·4 cm, and 57·8 μl./hr at 13·2 cm; 46·2 μl./hr at 14·2 cm, and 36·3 μl./hr at 7·7 cm; 69·3 μl./hr at 11·0 cm and 70·9 μl./hr at 19·5 cm. With the possible exception of the third experiment, there is no indication of an effect of pressure upon water movement between 6·4 and 19·5 cm solution. Since the normal pressure in the biliary tract is 10–15 cm (McMaster & Elman, 1926), and during the weighing experiments 4–15 cm, the hydraulic conductivity of the gall-bladder must be too low to produce detectable filtration *in vivo* or *in vitro*. That filtration cannot be the mechanism of water transport is also clear from work *in vivo* on anaesthetized dogs by Rous & McMaster (1921), who found that the gall-bladder could still concentrate bile when its lumen was connected to an empty rubber balloon, thus preventing the building up of intra-luminal pressure.

It was not possible to determine by this experimental method whether the gall-bladder can pump against a hydrostatic pressure gradient, since negative pressures siphon all the fluid out of the lumen. However, the small intestine, in which the effect of filtration can also be discounted (Fisher, 1955), has been shown to be able to absorb against a pressure difference (Wells, 1931).

Classical osmosis

If active transport of NaCl increases the osmolarity of the outer solution relative to the luminal solution, water might follow salt out of the lumen by classical osmosis at a rate depending upon the osmotic permeability of the gall-bladder to water.

Figure 3 illustrates weight changes in the same gall-bladder incubated successively with three different solutions. First, NaCl Ringer's solution is present on both sides, and the preparation loses weight at 40·0 mg/hr. Then the luminal solution is made hypertonic by adding 20 mM sucrose, which is known to be impermeant in this preparation (Diamond, 1962*a*), but fluid continues to leave the lumen against an osmotic gradient, though at a reduced rate (19·8 mg/hr). Finally, active transport is eliminated by cyanide-iodoacetate, and with the same luminal hypertonicity due to sucrose, fluid enters the gall-bladder in the direction of the osmotic gradient, as expected. Thus, although the gall-bladder behaves passively as a conventional osmometer, the pump can function against an osmotic gradient.

The effect of varying osmotic gradients on a normally pumping gall-

bladder is shown in Fig. 4. It is seen that the rate of water movement varies linearly with the osmotic gradient, no matter whether anisotonicity arises from the outside being hypertonic or hypotonic, or the lumen hypertonic. There is no net water movement when the lumen is hypertonic by about 35 m-osmolar. At that point the osmotic gradient driving water

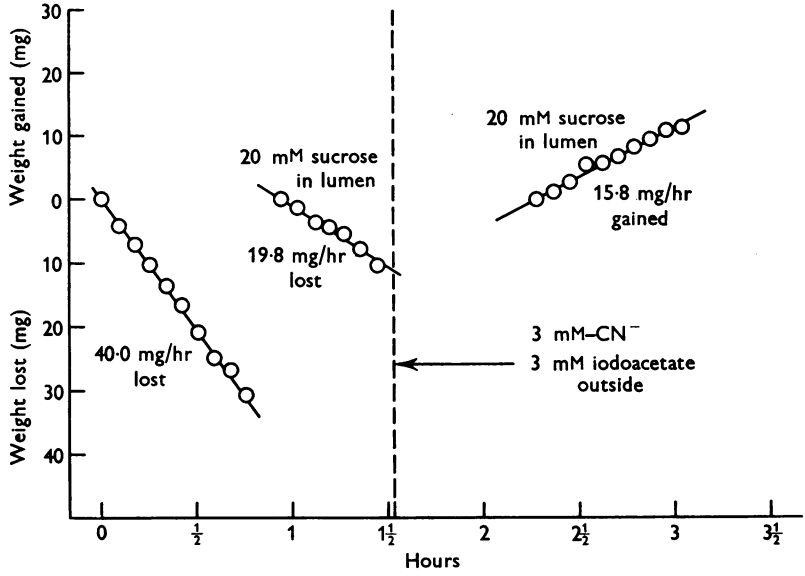

Fig. 3. Effect of an adverse osmotic gradient on water absorption by the gall-bladder. Ordinate, weight of a gall-bladder preparation (minus a constant term). Weight loss indicates that fluid is being absorbed from the lumen; weight gain, that fluid is entering the lumen. In the first series of measurements NaCl Ringer's solution bathed both sides of the gall-bladder. In the second, the luminal fluid was made hypertonic by addition of 20 mM sucrose. At $t = 1\frac{1}{2}$ hr, 0·9 ml. of a neutral isotonic solution of 103 mM-NaCN, 103 mM iodoacetic acid was added to 30 ml. of the outer solution, whose osmolarity therefore remained unchanged. In the third set of measurements the poisoned gall-bladder was then exposed to the same osmotic gradient that was tested on the normal gall-bladder in the second set—i.e. the luminal solution was made hypertonic by addition of 20 mM sucrose.

inwards must just balance the force of the pump transferring water outwards. For four normal gall-bladders the average adverse osmotic gradient required to reduce net water movements to zero was 40 ± 4 m-osmolar. Water movement against lesser osmotic gradients was also observed in one gall-bladder in which pumping had been partially inhibited by cyanide alone, and in another in which the pumping rate had been lowered by reducing luminal [NaCl] from 144 to 50 mM. Grim & Smith (1957) have observed water movement against osmotic gradients across the gall-bladder *in vivo*.

The osmotic permeability to water (P_{osm}) may be defined as the change in water flux produced by a change in osmotic gradient. P_{osm} was measured by adding sucrose to one side of a gall-bladder initially separating two isotonic solutions under four different conditions: (1) initially both solutions were isotonic NaCl Ringer's solution, and there was a net water efflux due to pumping; (2) initially both solutions were isotonic NaCl Ringer's solution, and there was no water flux because the preparation had

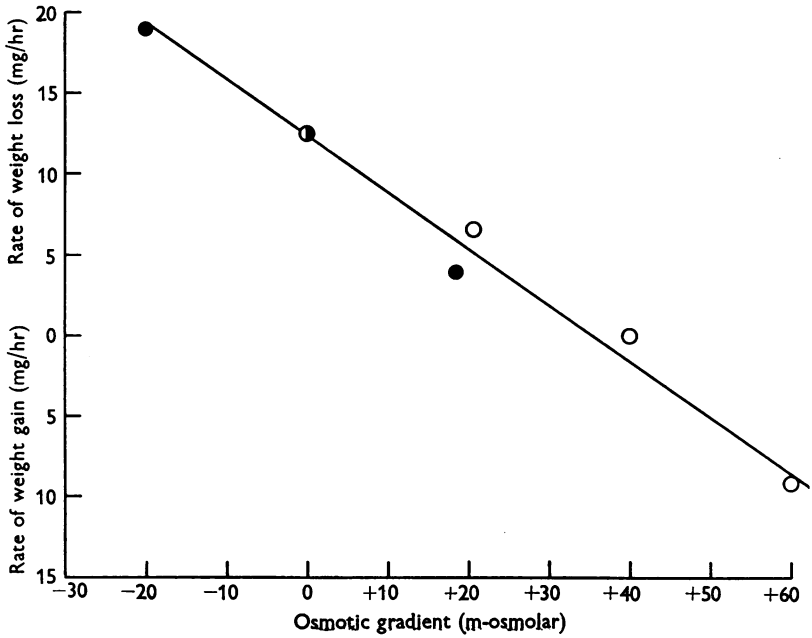

Fig. 4. Effect of osmotic gradients on water absorption by a normal gall-bladder. Abscissa, osmolarity of luminal solution minus osmolarity of outer solution. Ordinate, rate of change of weight of a gall-bladder preparation (weight loss means that fluid is being absorbed from the lumen). ○, luminal solution anisotonic, outer solution isotonic; ●, outer solution anisotonic, luminal solution isotonic; ◑, both bathing solutions isotonic. In the experimental point at 18·6 m-osmolar, 3·9 mg/hr, the outer solution was made hypotonic by removal of some NaCl; all other osmotic gradients involved making one bathing solution hypertonic by addition of sucrose.

been poisoned with cyanide-iodoacetate; (3) initially the lumen contained an isotonic NaCl–sucrose mixture, the outside was isotonic NaCl Ringer's solution, and there was a small water efflux; (4) initially the lumen contained isotonic sucrose solution, the outside was isotonic NaCl Ringer's solution, and there was a net water influx due to inward diffusion of salt. For any one gall-bladder P_{osm} was independent of the osmotic gradient up to at least 70 m-osmolar, and approximately the same under all four

conditions. The average value of P_{osm} for ten gall-bladders was $0{\cdot}38 \pm 0{\cdot}16\,\mu$l. water/hr. cm^2. m-osmolar. The antidiuretic hormone, which has been shown to increase P_{osm} in urinary bladder (Bentley, 1961) and other tissues, had no effect on P_{osm} in either roach or frog gall-bladders.

Thus, if physiological water transport depended upon NaCl transport setting up a difference in osmotic pressure between the bulk solutions on opposite sides of the gall-bladder, the osmotic gradient thus established would have to be 40 m-osmolar (15·3/0·38, since the normal rate of water transport is 15·3 μl./cm^2.hr). Since the gall-bladder can transport water not only between isotonic solutions but also into hypotonic solutions, classical osmosis cannot be the physiological mechanism of water absorption. Similarly, from published figures for water transfer rates and P_{osm} in kidney proximal tubule (Whittembury, Oken, Windhager & Solomon, 1959), frog skin (Andersen & Ussing, 1957; Ussing, Kruhøffer, Hess Thaysen & Thorn, 1960), small intestine (Curran & Solomon, 1957) and the ciliary body of the eye (Auricchio & Bárány, 1957) one can calculate that these tissues would require osmotic gradients of 20, 15–70, 61 and 30 m-osmolar, respectively, to reabsorb urine, absorb saline, and secrete the aqueous humour by classical osmosis; but all four tissues can operate isotonically. Furthermore, the small intestine (Parsons & Wingate, 1961) and Malphigian tubule (Ramsay, 1954) can transport water against osmotic gradients. Some explanation other than classical osmosis must also be sought in these five preparations.

Since 1000 m-osmolar is equivalent to 22·4 atmospheres, P_{osm}, which is effectively a filtration coefficient or the hydraulic conductivity, may be expressed as 17 μl./cm^2. hr atmosphere. If the normal water flux of 15·3 μl./cm^2.hr had been due to hydrostatic filtration, the pressure difference between the lumen and the outside would have had to be 15·3/17 = 0·9 atmosphere, more than sufficient to burst the gall-bladder. It is not surprising that pressures of 6–20 cm Ringer's solution (0·006–0·020 atmosphere) produced no filtration detectable by the horizontal-capillary method.

Co-diffusion

Although movement of water against osmotic gradients is sometimes taken as evidence of active water transport, it should be noted that even in inanimate systems water can move up osmotic gradients if a membrane separates solutions of two solutes to which it is not equally permeable. For instance, in the experiments of Meschia & Setnikar (1959), when a collodion membrane separated a dextran solution from a urea solution 87 times as concentrated as the dextran, water moved from the urea to the dextran—i.e. into the solution with the higher water activity. This occurred because the membrane was permeable to urea but not to dextran, so the

diffusion of urea down its concentration gradient supplied the energy for the water flux. The term 'co-diffusion' has been used for such a phenomenon, in which the flux of one component down its activity gradient produces a flux of another component. Similar 'co-diffusional' effects can carry water against osmotic gradients in the gall-bladder. For instance, fluid enters the gall-bladder when isotonic sucrose solution is in the lumen and isotonic NaCl Ringer's solution outside, and this water influx is unrelated to pumping (Diamond, 1962*a*). Analysis of luminal fluid at the end of these experiments showed that salt had also diffused into the lumen. Since the gall-bladder is virtually impermeable to sucrose, the driving force for the water movement must have come from the diffusion of NaCl down its concentration gradient. When the lumen was made hypotonic by removing some sucrose, fluid movement varied linearly as the osmotic gradient, and ceased with the lumen hypotonic by 18 m-osmolar. Thus, co-diffusion of water during net salt movements can proceed against an osmotic gradient in the gall-bladder.

Kirschner, Maxwell & Fleming (1960) noted that when frog skin had isotonic sucrose outside and isotonic saline inside, water moved into the sucrose. This must also be unrelated to active salt transport, since frog skin transports salt from the outside to the inside, and the explanation might be the same co-diffusional effect.

Electro-osmosis

Electro-osmosis is the flow of water often observed when an electric current is passed through a charged artificial membrane. Since many biological membranes carry fixed charges, electro-osmosis has been frequently postulated as a mechanism of biological water flow—e.g. in frog skin (Capraro & Garampi, 1956), intestine (Parsons & Wingate, 1958), plant roots (Spanner, 1958), and secretory glands (Mudd, 1926). Despite its popularity in this regard, electro-osmosis has never been observed in any living animal membrane. Apparently the only attempt to detect it was due to Mudd (1925), who applied 220 V to mammalian serous membranes in solutions with a total electrolyte concentration 1/25 that of blood at various pH down to 1·5. Since the iso-electric reversal point of the 'electro-osmosis' he observed remained unchanged for 8 days post mortem his results must bear little relevance to living membranes. Blinks & Airth (1957) could not observe electro-osmosis in a plant cell (the alga *Nitella*).

An experimental test of the possible role of electro-osmosis in the gall-bladder arose from observation of the converse electro-kinetic phenomenon, streaming potentials. When initially both sides of the gall-bladder were bathed by identical solutions and there was no potential difference

(p.d.) across the preparation, it was found that making one of the bathing solutions anisotonic by addition or subtraction of sucrose set up a p.d. As is seen in Fig. 5 (an experiment in 'sulphate solution'), outside-hypertonic or lumen-hypotonic solutions make the lumen increasingly negative, while outside-hypotonic or lumen-hypertonic solutions make the lumen positive. Clearly the sign of the p.d. is determined by the direction of the

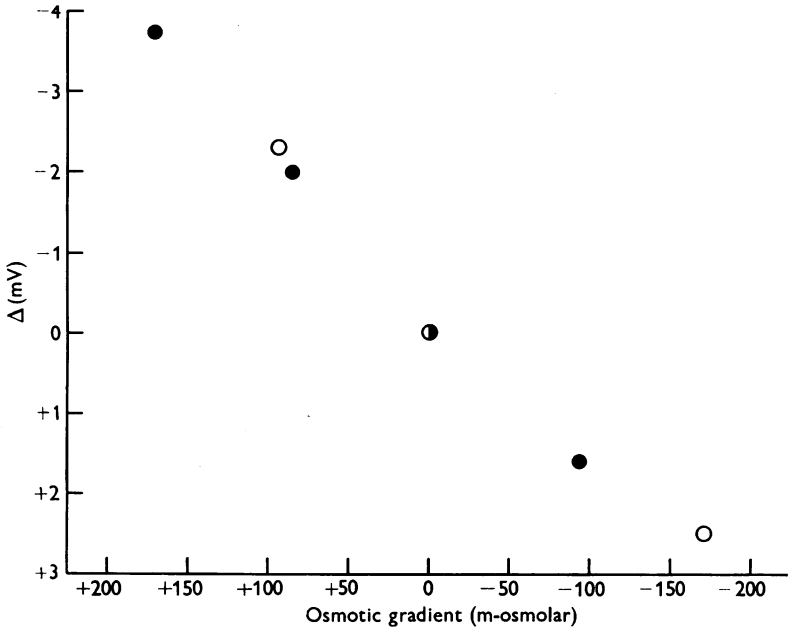

Fig. 5. Streaming potentials as a function of the osmotic gradient. Abscissa, osmolarity of outer solution minus osmolarity of luminal solution; ordinate, change in the potential difference across the gall-bladder from the value observed with both bathing solutions isotonic. ◑, both bathing solutions isotonic Na_2SO_4 solution (*G*, Table 2; Diamond, 1962*a*); ●, outer solution anisotonic, luminal solution isotonic; ○, luminal solution anisotonic, outer solution isotonic. In every case anisotonicity resulted from adding or subtracting sucrose in one of the bathing solutions, without changing concentrations of other constituents. A negative value of Δ (mV) means that the potential of the lumen with respect to the outer solution has gone more negative.

osmotic gradient rather than by hyper- or hypotonicity *per se*. The magnitude of the p.d. seems directly proportional to the magnitude of the osmotic gradient, with a proportionality coefficient of 0·019 mV/m-osmolar. Similar p.d.s were observed in two gall-bladders in NaCl Ringer's solution, with coefficients of 0·017 and 0·027 mV/m-osmolar (the concentration of sodium in chloride and sulphate solutions is the same). These osmotic p.d.s were independent of diffusion potentials, and could be set up between

two solutions of identical electrolyte composition, when the gall-bladder showed no diffusion potentials. Furthermore, between two solutions of different electrolyte composition the osmotic potentials simply added to or subtracted from the diffusion potentials. For instance, a diffusion potential of + 8·5 mV was set up across one gall-bladder by partial replacement of luminal NaCl with sucrose. The p.d. dropped by 4·5 mV to 4·0 if the outside was made hypertonic, rose by 5·0 mV to 13·5 if the lumen was hypertonic, and returned to 8·5 mV with both sides of the preparation isotonic. Evidently the 'osmotic' p.d. of ± 4·5 mV and the diffusion potential of + 8·5 mV combine linearly. Thus, as expected, dilution or concentration of the intracellular fluid by anisotonic solutions does not produce or change a diffusion potential, since the inner and outer membranes of the epithelial cells have the same relative permeability coefficients. In asymmetrical membranes, where changes in osmolarity may change diffusion potentials and active transport potentials, detection of true streaming potentials will be more difficult.

Similar 'osmotic' potentials have frequently been observed in charged artificial membranes through which water is being forced under pressure gradients. This phenomenon is the opposite of electro-osmosis, and is termed a streaming potential. The side of the membrane towards which water is flowing acquires a charge opposite to that of the membrane. Hence the gall-bladder must be negatively charged, a conclusion also reached from its anion permeability. As in artificial membranes, streaming potentials in the gall-bladder are linearly related to the pressure difference, and depend upon the concentration of the permeant ion (sodium) but are independent of the valency of the impermeant counter-ion (chloride or sulphate). In gall-bladders subjected to too many changes of solutions cation diffusion potentials and streaming potentials decline in parallel, indicating that the same fixed charge is responsible for both.

Streaming potentials and electro-osmosis in the same membrane are related by the formula

$$H/p = v/i,$$

where H is the electrical potential (stat V), and

p the pressure difference (dynes/cm^2) in a streaming potential experiment;

i is the electric current (stat V), and

v the volume flow (ml./sec) in an electro-osmosis experiment.

The average value of H/p for the gall-bladder is 0·021 mV/milliosmolar = $3{\cdot}08 \times 10^{-12}$ statV-cm^2/dyne. If water absorption by the normal gall-bladder (15·3 μl./cm^2. hr = $4{\cdot}25 \times 10^{-6}$ ml./cm^2. sec) is due to electro-osmosis, the

necessary current is $4{\cdot}25 \times 10^{-6}/3{\cdot}08 \times 10^{-12} = 1{\cdot}38 \times 10^{6}$ statA/cm^2. The resistance of the gall-bladder is 113 Ω cm^2, and 1 statA = $3{\cdot}34 \times 10^{-10}$ A, hence the required voltage is $(1{\cdot}38 \times 10^{6})$ (113) $(3{\cdot}34 \times 10^{-10}) = 52$ mV, lumen positive. Since the observed potential is $-0{\cdot}8 \pm 0{\cdot}6$ mV, the actual electro-osmosis is two orders of magnitude too low and in the wrong direction.

Channels for water flow

Passive water flow in many biological and artificial membranes behaves as if it occurred in narrow molecular-sized pores, which produce 'single-file' effects in tracer experiments. In such cases the water flux ratio is proportional, not to the first power of the activity ratio (as would be true if water molecules traversed the membrane without mutual interference), but to some higher power.

To determine whether the gall-bladder shows interference effects when the water flux is known to be passive, a preparation was filled with NaCl Ringer's solution, poisoned by ouabain to eliminate the pump, and placed in a solution made hypertonic by 40 mM sucrose. The net osmotic efflux of water, measured by weighing, was 66·3 μl./hr; the total efflux, measured by tritiated water, was 1680 μl./hr; hence the influx was $1680 - 66 = 1614$ μl./hr, and the flux ratio, 1680/1614 = 1·041. Since the water activity ratio was 1·00073, the flux ratio equalled the 56th rather than the first power of the activity ratio. Thus, a net flux of water through the passive channels exaggerates the one-way flux ratio, and water behaves as if it moved through narrow 'single-file' pores 56 water-molecules long.

For three gall-bladders in NaCl Ringer's solution the average efflux of tritiated water was 298 ± 51 μl./cm^2.hr. This is in the same range as for other epithelial membranes: dog gall-bladder 396 (Grim & Smith, 1957), frog skin 356 (Koefoed-Johnsen & Ussing, 1953), frog stomach 170 (Durbin, Frank & Solomon, 1956) (all in μl./cm^2.hr). Since the net water flux due to pumping is 15·3 μl./cm^2.hr, the total efflux is 298/15·3 = 19·5 times the net efflux.

Thermodynamics of water transport

From classical thermodynamics, the flux of water (M_{H_2O}) across a membrane under reversible conditions of equilibrium is determined by the hydrostatic pressure (Δp) and by the osmotic pressure resulting from solute concentration gradients ΔC_s: i.e. $M_{H_2O} = k(\Delta p - RT\Sigma\Delta C_s)$, where k is the filtration coefficient (Starling's law). The validity of Starling's law has been amply confirmed for membranes strictly impermeable to the solutes responsible for the concentration gradient, but the law breaks down experimentally if the membrane has a finite permeability to solutes. This is

clearly illustrated in the experiments of Durbin (1960) on a cellulose membrane with solutes of increasing permeability (decreasing molecular size). Albumin exerted the full van't Hoff osmotic pressure ($\Delta p = RT\Delta C_s$); the polysaccharide inulin, trisaccharide raffinose, disaccharide sucrose, and monosaccharide glucose gave 1/1·3, 1/2·3, 1/2·7 and 1/5 of the van't Hoff values; and urea and D_2O, to which the membrane was as permeable as it was to water, exerted virtually no osmotic pressure. Evidently the flow of water in 'leaky' membranes is determined by an equation

$$M_{H_2O} = k(\Delta p - RT\Sigma\sigma\Delta C_s),$$

where σ (the so-called reflexion coefficient) for a given solute and membrane is the ratio of the observed osmotic pressure to the theoretical van't Hoff value. σ is 1 for a completely impermeant solute, 0 for a solute to which the membrane is as permeable as to water, and between 0 and 1 if the membrane discriminates somewhat between solute and water. However, σ has more significance than just an empirical correction factor, for it arises as a constant from irreversible thermodynamics and can be calculated from the molecular radius and membrane porosity.

Thus, Starling's law may not be applied to membranes with finite solute permeability or active transport, which violate its assumptions of equilibrium conditions and reversible changes. The correct laws for such membranes must be derived from irreversible thermodynamics, which have been applied to the case of finite solute permeability by Kedem & Katchalsky (1958) and are extended to cover active transport of water or solute in the appendices to this paper.

Passive properties of the gall-bladder

Before the case of active transport can be treated, three constants must be calculated from the passive behaviour of the gall-bladder and applied to co-diffusion. Consider the situation where sucrose solution is in the lumen of the gall-bladder and NaCl Ringer's solution outside. There is then no active transport, and NaCl may be regarded as the only permeant solute, since sucrose is practically impermeant and other solutes are present only at negligible concentrations.

In the following equations let superscripts o and l refer to outer and luminal solutions,

M = flux (moles/unit area × time) from the luminal to the outer solution,

C = mean concentration,

$\Delta C = C^l - C^o$,

v = partial molar volume,

J_v = volume of fluid crossing unit area of the gall-bladder in unit time ($J_v = M_{H_2O} v_{H_2O} + M_{NaCl} v_{NaCl}$),

L_p = filtration coefficient or hydraulic conductivity (for instance, from eqn. 1 the volume flow in the absence of concentration gradients ($\Delta C_{sucrose} = 0 = \Delta C_{NaCl}$) is simply $J_v = L_p \Delta p$),

σ_{NaCl} = reflexion coefficient for NaCl in the gall-bladder, as defined above, and

$\omega_{NaCl} RT$ is the NaCl permeability coefficient at constant volume (from eqn. 2*b* the NaCl flux at constant volume ($J_v = 0$) is simply $M_{NaCl} = \omega_{NaCl} RT \Delta C_{NaCl}$).

Then the following equations hold (after eqns. (39) and (40) of Kedem & Katchalsky, 1958):

$$J_v = L_p(\Delta p - RT\Delta C_{sucrose}) - \sigma_{NaCl} L_p RT \Delta C_{NaCl}, \tag{1}$$

and

$$\begin{aligned} M_{NaCl} &= C_{NaCl} L_p (1 - \sigma_{NaCl})(\Delta p - RT\Delta C_{sucrose}) \\ &\quad + RT\Delta C_{NaCl}[\omega_{NaCl} - \sigma_{NaCl} L_p C_{NaCl}(1 - \sigma_{NaCl})] \qquad (2a) \\ &= C_{NaCl} J_v (1 - \sigma_{NaCl}) + RT\Delta C_{NaCl}\omega_{NaCl}. \qquad (2b) \end{aligned}$$

These equations give the total volume flow J_v and the NaCl flux M_{NaCl} in terms of three constants of the membrane, L_p, ω_{NaCl}, and σ_{NaCl}. If there is no sucrose concentration gradient ($\Delta C_{sucrose} = 0$), from eqn. (1) the hydrostatic pressure required to reduce the volume flow to zero is $\Delta p = \sigma_{NaCl} RT \Delta C_{NaCl}$. If, however, the gall-bladder were impermeable to NaCl ($\omega_{NaCl} = 0$, $\sigma_{NaCl} = 1$), eqn. (1) would give the result of classical thermodynamics: the hydrostatic pressure required to reduce the volume flow to zero would be $\Delta p = RT\Delta C_{NaCl}$, the van't Hoff value. Equations (1) and (2) make no assumption about the structure of the membrane, but do assume that the solutions are dilute and well stirred.

Since the hydrostatic pressure gradients present across the gall-bladder are too small to be of any significance, eqns. (1) and (2) may be simplified to:

$$J_v = -L_p RT(\Delta C_{sucrose} + \sigma_{NaCl}\Delta C_{NaCl}), \tag{3}$$

$$\begin{aligned} M_{NaCl} &= -L_p C_{NaCl}(1 - \sigma_{NaCl})(RT\Delta C_{sucrose}) \\ &\quad + RT\Delta C_{NaCl}[\omega_{NaCl} - \sigma_{NaCl} L_p C_{NaCl}(1 - \sigma_{NaCl})]. \qquad (4) \end{aligned}$$

Equations 3 and 4 lead to the following three calculations, which show that the experimental observations concerning co-diffusion of water with salt in the gall-bladder are thermodynamically consistent and predictable.

(1) σ_{NaCl}. Equation (3) states that unless σ_{NaCl} is 1, the osmolarity of the NaCl Ringer's solution outside the gall-bladder must be higher than that

of the sucrose solution inside to keep the gall-bladder at constant volume (which would be virtually the same as constant weight). Experimentally, as described above in the section entitled 'Co-diffusion', change of weight ceased with an osmotic gradient of 18 m-osmolar; i.e. $\Delta C_{\text{sucrose}} = 249$ m-osmolar, $\Delta C_{\text{NaCl}} = -267$ m-osmolar. Thus, $\Delta C_{\text{sucrose}} = \sigma_{\text{NaCl}} \Delta C_{\text{NaCl}}$, and $\sigma_{\text{NaCl}} = 249/267 = 0{\cdot}93$. A similar principle has been used to find σs for red blood cells by Goldstein & Solomon (1960).

(2) *Co-diffusion of water with NaCl.* From eqn. (3), when the sucrose solution in the lumen and the NaCl Ringer's solution outside have the same osmolarity, there should be a volume flow, since $\sigma_{\text{NaCl}} \neq 1$. Since the filtration coefficient L_p is 17×10^{-3} ml./cm^2.hr.atmosphere and RT is 25 atmosphere.ml./m-mole at 25° C, the expected volume flow is:

$$J_v = (-17 \times 10^{-3}) \times (25) \times (267 - 0{\cdot}93 \times 267) \times (10^{-3})$$
$$= -7{\cdot}9 \times 10^{-3} \text{ ml./cm}^2\text{.hr},$$

the sign indicating a net influx into the lumen. The experimental influx was $7{\cdot}4 \pm 2{\cdot}2 \times 10^{-3}$ ml./cm^2.hr (3). The close agreement between the expected and observed volume change serves as a check on the estimates of σ_{NaCl} and L_p, the only parameters of the membrane appearing in this calculation.

Thermodynamics make no stipulations about kinetic mechanisms. Although this flux of water between two solutions of the same osmolarity and vapour pressure is clearly coupled in some way to the diffusion of NaCl, the detailed mechanism of the coupling is not clear. However, a possible mechanism is worth mentioning to provide a more concrete, though perhaps incorrect, picture: diffusion of NaCl across the membrane may set up a bulk flow of solution through the membrane in the direction of NaCl diffusion.

(3) *Osmolarity of the 'co-diffusate'.* With isotonic sucrose solution in the lumen and isotonic NaCl Ringer's solution outside, the concentration of NaCl in the solution diffusing into the gall-bladder may be calculated by dividing eqn. (4) by eqn. (3). Since $\Delta C_{\text{sucrose}} = -\Delta C_{\text{NaCl}}$,

$$\frac{M_{\text{NaCl}}}{J_v} = \frac{\omega_{\text{NaCl}} + L_p C_{\text{NaCl}}(1 - \sigma_{\text{NaCl}})^2}{L_p(1 - \sigma_{\text{NaCl}})}. \tag{5}$$

To evaluate this expression one must know the remaining thermodynamic constant for the membrane, ω_{NaCl}. Since $\omega_{\text{NaCl}} RT$ is the absolute permeability coefficient $\bar{P}_{\text{NaCl}} (= M_{\text{NaCl}}/\Delta C_{\text{NaCl}})$ at constant volume, it may be calculated in two ways.

First, the absolute permeability coefficients $\bar{P}$ for Na and Cl may be calculated from measurements of resistance and relative permeability coefficients in a previous paper (Diamond, 1962*b*) as: $\bar{P}_{\text{Na}} = 14{\cdot}5 \times 10^{-6}$ cm/sec, $\bar{P}_{\text{Cl}} = 0{\cdot}58 \times 10^{-6}$ cm/sec. $\bar{P}_{\text{NaCl}}$ is not

independent of the NaCl concentration gradient, since this gradient sets up a diffusion potential, but the following expression may be derived from the constant-field equation

$$\bar{P}_{NaCl} = \frac{\bar{P}_{Na}\bar{P}_{Cl}(C^o + C^l)}{(\bar{P}_{Na} - \bar{P}_{Cl})\,(C^o - C^l)} \ln \frac{\bar{P}_{Na}C^o + \bar{P}_{Cl}C^l}{\bar{P}_{Na}C^l + \bar{P}_{Cl}C^o}. \quad (6)$$

(If $C^o - C^l \ll C^o$, C^l, eqn. (6) reduces to an expression analogous with the Nernst limiting formula for diffusion coefficients: $D_{NaCl} = 2D_{Na}D_{Cl}/(D_{Na}+D_{Cl})$. Substituting $\bar{P}_{Na}$ and $\bar{P}_{Cl}$ as given above ($C^o = 144$ mM, $C^l = 0$) in eqn. (6) yields: $\bar{P}_{NaCl} = 1{\cdot}9 \times 10^{-6}$ cm/sec. Since $\bar{P}_{NaCl} = \omega_{NaCl}RT$, $\omega_{NaCl} = 2{\cdot}7 \times 10^{-7}$ mole/cm^2.hr.atmosphere.

Secondly, ω_{NaCl} may be obtained from the experimental value of M_{NaCl} and eqn. (4), since ω_{NaCl} is now the only unknown in this equation. By this method the experimental value of ω_{NaCl} is $3{\cdot}2 \times 10^{-7}$ mole/cm^2.hr.atmosphere, in good agreement with the calculated value of the first method.

Inserting experimental values into eqn. (5), one obtains $M_{NaCl}/J_v = 269$ m-osmolar. Since the osmotic coefficient of NaCl is 2 (0·928), [NaCl] in the co-diffusate should be 269/(2) (0·928) = 145 mM, practically the same as [NaCl] in Ringer's solution (144 mM). The actual experimental value was 191 mM, from the amounts of NaCl and water entering the lumen. As these amounts were necessarily small and their accurate measurement consequently difficult, the difference between 191 and 144 is probably not significant.

Active water transport

Consider an isothermal system consisting of a membrane separating two identical, well stirred solutions of a permeant solute dissolved in water, with no hydrostatic pressure differences. One assumes that water is actively transported, the solute moves only passively, solute–water interactions (i.e. σ) are the same for passive and active water movements, and the solutions are dilute. Then it is shown in Appendix 1 (eqn. (12)) that active transport of water may give rise to a flux of solute (e.g. by solvent drag), depending upon the parameters of the membrane; and that the expression for the osmolar concentration of solute in the absorbate is

$$\frac{M_s}{J_v} = \frac{C_s L_p (1 - \sigma_s) - C_s v_s \omega_s}{L_p}. \quad (7)$$

The only new symbol in this expression is the subscript s, referring to solute. The derivation makes no further assumptions about membrane structure or the mechanism of the water pump, but the structure of any particular membrane will obviously be expressed in experimental values of the filtration coefficient, reflexion coefficient, and solute permeability coefficient (L_p, σ_s and ω_s). For instance, if the membrane is impermeable to the solute, $\omega = 0$ and $\sigma = 1$, and by eqn. (7) no solute would appear in the absorbate.

Since eqn. (7) contains only quantities that may be determined by experiment, this equation provides a test for the presence of active water trans-

port without active solute transport. The experimental values for the gall-bladder (s corresponds to NaCl) are:

$$C_s = 267 \text{ m-osmolar}, \quad L_p = 17 \times 10^{-3} \text{ cm/hr.atmosphere},$$

$$\sigma_s = 0{\cdot}93, \quad \omega_s = 3{\cdot}2 \times 10^{-4} \text{ m-mole/cm}^2\text{.hr.atmosphere},$$

$v_s = 22$ ml./mole (from tabulated values for the density of NaCl solutions). Substituting these values in eqn. (7) gives only 10 mM for [NaCl] in the absorbate. Since [NaCl] in the absorbate was experimentally 149 mM, a water pump may be rejected as the explanation of salt and water absorption by the gall-bladder.

Active transport of solute

Osmolarity of the absorbate. Again, consider an isothermal system consisting of a membrane separating two identical, well stirred solutions of a permeant solute dissolved in water, with no hydrostatic pressure differences. One now assumes that solute is actively transported, water moves only passively, the reflexion coefficient σ is the same for passively and actively transported solute, and the solutions are dilute. Then it is shown in Appendix 2 (eqn. (18)) that active transport of solute may give rise to a flux of water, depending upon the parameters of the membrane; and that the volume of fluid absorbed per mole solute transported is

$$\frac{J_v}{M_s} = \frac{L_p(1-\sigma_s)}{\omega_s + C_s L_p (1-\sigma_s)^2}. \tag{8}$$

The reciprocal of this expression will be the absorbate osmolarity.

Again the derivation makes no further assumptions about membrane structure or the mechanism of active solute transport, and since all quantities in eqn. (8) may be determined by experiment, this equation provides a test for the nature of water transport in the presence of active solute transport. Inserting the experimental values given previously for the gall-bladder, one finds 6·9 ml. fluid/m-mole NaCl predicted for the absorbate. This is in good agreement with the experimental value of 6·7, and since the value for Ringer's solution is 6·5, the absorbate should be and is virtually isotonic.

A point requiring comment is that σ_{NaCl} and ω_{NaCl} were evaluated for the case of isotonic NaCl Ringer's solution outside, isotonic sucrose solution in the lumen; whereas the calculation refers to Ringer's solution on both sides of the preparation. As derived from the constant-field equation, ω_{NaCl} varies slightly with the concentration gradient, and with NaCl Ringer's solution on both sides of the gall-bladder, ω_{NaCl} should be 58% of its value in the NaCl versus sucrose case. However, since the term $C_s L_p (1-\sigma_s)^2$ in eqn. (8) is negligible in the gall-bladder, the absorbate

volume is proportional to $(1-\sigma_s)/\omega_s$, and as the percentage change in ω_s and $(1-\sigma_s)$ can be shown to be the same, the absorbate volume will be unchanged.

While this thermodynamic calculation shows that the observed water absorption in the gall-bladder can be explained passively, one is left no better informed as to the kinetic coupling mechanism by which active solute transport can carry water between identical solutions. However, a useful way to visualize what might be going on is provided by the suggestion that active solute transport raises the concentration of solute in a small region within the membrane, and water then comes across by a kind of local osmosis (Curran, 1960).

Effect of osmotic gradients on the water flux. In the same membrane, if there is no hydrostatic pressure difference or concentration gradient of the actively transported solute, but if an impermeant solute (subscript i) is present on one side of the membrane, then the rate of fluid transport is given by eqn. (19) in Appendix (2): $J_v = J_v^o - L_p RT\Delta C_i$. J_v^o is the rate of fluid transport between identical solutions in the absence of an osmotic gradient.

Thus, if solute is actively transported and if one further assumes that the active transport mechanism is not directly affected by an osmotic gradient, water will move against a finite osmotic gradient ($RT\Delta C_i$) even though water itself is not actively transported. The water flux should vary linearly as the osmotic gradient; the proportionality coefficient (L_p) between change of volume flow and change of osmotic gradient should be the same in the presence or absence of active transport; and there should be no movement of fluid ($J_v = 0$) at an osmotic adverse gradient of J_v^o/L_p. All these statements were found to hold true in the gall-bladder, as already described. For instance, since the water flux between identical solutions (J_v^o) is 15·3 μl./cm^2.hr and the filtration coefficient is $0{\cdot}38 \pm 0{\cdot}16$ μl./cm^2.hr m-osmolar, there should have been no volume change at an osmotic gradient of $15{\cdot}3/0{\cdot}38 = 40 \pm 17$ m-osmolar. The experimental value was 40 ± 9 m-osmolar, suggesting that the active transport mechanism is in fact independent of the osmotic gradient. Similarly, Parsons & Wingate (1961) found water moving against osmotic gradients in rat jejunum and rat ileum, which also transport NaCl actively.

Effect of osmotic gradients on the solute flux. Again, if the concentrations of the actively transported solute are left unchanged but if the rate of fluid transport is varied by concentration gradients of impermeant solutes, then the solute flux is given by eqn. (20), Appendix 2: $M_s = M_s^o + C_s(1-\sigma_s)J_v$. M_s^o is the solute flux in the absence of net fluid transport. If one substitutes experimental values for the gall-bladder, the term $C_s(1-\sigma_s)J_v$ represents only 7 % of the total NaCl flux between identical solutions. Thus,

if fluid transport is reduced to zero by an adverse osmotic gradient, the flux of NaCl should remain practically unchanged. This prediction has not yet been tested for active transport of NaCl in the gall-bladder but has been confirmed for the analogous case of NaCl diffusion down concentration gradients. In rat small intestine, which also absorbs isotonic NaCl and also has σ_{NaCl} near 1, Parsons & Wingate (1961) observed no change in active solute transport when fluid movement was increased, reversed, or reduced to zero by varying osmotic gradients. Similarly, Whittembury *et al.* (1959) found no effect of moderate osmotic gradients on the NaCl flux in the proximal tubule of the kidney. In general, the effect of a water flux (e.g. by solvent drag) on the flux of a solute for which $\sigma_s \sim 1$ will be slight.

Effect of solute concentration on absorbate osmolarity. If one substitutes experimental values for the gall-bladder in the expression for absorbate osmolarity ($\{\omega_s + C_s L_p (1-\sigma_s)^2\}/\{L_p(1-\sigma_s)\}$, the reciprocal of eqn. (8)), the term $C_s L_p (1-\sigma_s)^2$ is only 1/27 times ω_s. Hence the absorbate tonicity should be practically independent of [NaCl] as long as the bathing solutions are kept isotonic. This was confirmed experimentally, in that the absorbate remained isotonic when half the luminal NaCl was replaced by KCl. The same observation has been made for NaCl–mannitol substitutions in small intestine, large intestine, and proximal tubule of the kidney.

DISCUSSION

From irreversible thermodynamics three new criteria can be suggested for deciding whether water transport is active or passive: (1) Comparison of the equations for the absorbate osmolarity during co-diffusion (eqn. (5)) and during active solute transport (reciprocal of eqn. (8)) shows that they are formally identical. Hence, if water transport is passive but solute transport active, the absorbate should have the same osmolarity as the solution transferred during co-diffusion. (2) Equation (8) predicts the absorbate osmolarity from the experimental values of the solute permeability coefficient, reflexion coefficient, and the hydraulic conductivity if solute transport is active and water passive. (3) Equation (7) predicts the absorbate osmolarity from the same parameters if water transport is active and solute passive. By the first two of these criteria, water transport in the gall-bladder behaves as if it were passive, while by the third, it does not behave as if it were active.

It should be stressed that whenever active solute transport is going on, movement of water against osmotic gradients is not a sufficient qualitative condition for assuming active water transport. In fact, the following theorems were derived from thermodynamics: (1) Water should be carried passively against osmotic gradients up to J_v^o/L_p (where J_v^o is the rate of fluid transport in the absence of osmotic gradients, and L_p the hydraulic

conductivity). (2) A given osmotic gradient should produce the same change in fluid transport rate whether or not active solute transport is operating. (3) In membranes which are not exceptionally permeant to solute (i.e. $\sigma_s \sim 1$) the solute flux should be practically independent of osmotic gradients, and (4) the absorbate osmolarity should be practically independent of the concentration of actively transported solute. All these predictions, based on the assumption of active solute transport and passive water transport, have been verified in the gall-bladder. The fact that these predictions also hold in several other epithelial preparations suggests that in general there may be no need to assume active water transport when considering absorption and secretion, but application of the three above-mentioned criteria to more preparations will provide a clearer test. In particular, it will be interesting to see if eqn. 8 makes correct predictions for those glands which are known to transport hypertonic or hypotonic fluids.

An outstanding unsolved question is the exact kinetic mechanism of the passive water transport associated with active solute transport. All that can be asserted for the gall-bladder is that this mechanism is neither filtration, classical osmosis, nor electro-osmosis. Since no artificial membrane transports solute actively, the problem can unfortunately not be approached by means of model experiments. However, it is suggestive that the ratio of fluid transported to solute transported is the same during co-diffusion or active solute transport. The force responsible for water absorption is thus formally analogous to co-diffusion, and this analogy between pump-linked and co-diffusional water movements might exist also on the kinetic level.

Whatever this kinetic mechanism turns out to be, it is clear that most observed properties of water transport by the gall-bladder are secondary consequences of active NaCl transport. Previous papers (Diamond, 1962*a*, *b*) demonstrated such an active transport mechanism in the form of a neutral NaCl pump, which therefore provides at present a satisfactory driving force for both salt and water transport in the gall-bladder. Since the possibility of electro-osmotic effects has been ruled out, the coupling of water transport to such a neutral pump need not be any different from the coupling to the voltage-producing pumps of most epithelia, and, in fact, most experimental features of water transport in the gall-bladder have parallels in other epithelia. The principal idiosyncrasy of isotonic NaCl absorption by the gall-bladder is therefore the direct coupling of Na and Cl transport.

SUMMARY

1. The mechanism of isotonic water transport has been studied *in vitro* in the gall-bladder of fresh-water fish.

2. Filtration, classical osmosis, and electro-osmosis can be ruled out quantitatively as possible mechanisms. Filtration would be two orders of magnitude too small; electro-osmosis two orders of magnitude too small and in the wrong direction; and the gall-bladder can transfer water against osmotic gradients of up to 40 m-osmolar.

3. During osmotic water flow, streaming potentials have been observed (apparently for the first time in a biological membrane). Their sign indicates the presence of negative fixed charges.

4. During diffusion of NaCl across the gall-bladder co-diffusion can carry water passively against osmotic gradients.

5. The passive one-way water fluxes show single-file interference effects.

6. Three general criteria have been derived from irreversible thermodynamics to distinguish active water transport from passive water transport in the presence of active solute transport. By these criteria the gall-bladder could not function by an active water pump, but active NaCl transport could account quantitatively for passive isotonic water transport.

7. Active solute transport can carry water against an osmotic gradient, whose magnitude is predictable from thermodynamics. The expected value of the absorbate osmolarity and three other theorems concerning active transport have also been derived.

8. The kinetic mechanism by which active solute transport produces water flow is formally analogous to co-diffusion.

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APPENDIX 1. *Active transport of water*

Kedem & Katchalsky (1958) should be consulted for a fuller account of procedure in applying irreversible thermodynamics to membranes.

Consider an isothermal system consisting of water, one permeant solute (subscript s), and a membrane separating two well stirred compartments (arbitrarily indicated by superscripts l and o). Symbols: μ, chemical potential; M, a flux in moles/area × time from compartment l to compartment o; v, a partial molar volume (moles/ml.); C, a concentration; $\Delta C \equiv C^l - C^o$; Δp, the hydrostatic pressure of compartment l relative to compartment o; R, the gas constant; T, the absolute temperature; ϕ, the volume fraction of solute ($\phi \equiv C_s v_s$). The starting point in irreversible thermodynamics is the dissipation function per unit membrane area,

Φ (temperature times rate of entropy production per unit area), which in such a system is given by

$$\Phi = (\mu^l_{H_2O} - \mu^o_{H_2O})\, M_{H_2O} + (\mu^l_s - \mu^o_s)\, M_s. \tag{1}$$

For ideal dilute solutions

$$\left.\begin{aligned} \mu^l_{H_2O} - \mu^o_{H_2O} &= v_{H_2O}\Delta p \frac{RT\,\Delta C_s}{C_{H_2O}} + \mu^*_{H_2O}; \\ \mu^l_s - \mu^o_s &= v_s \Delta p + RT\,\Delta C_s/C_s. \end{aligned}\right\} \tag{2}$$

The concept of active transport of water is expressed mathematically by introducing an active transport potential $\mu^*_{H_2O}$ into the chemical potential of water. This mode of expression can be shown to involve the assumption that the reflexion coefficient (σ) is the same for interaction of solute with active and with passive water movements. $\mu^*_{H_2O}$ cancels in the final formula for absorbate osmolarity and thus need not be assumed to be constant.

From (1) and (2),

$$\Phi = \Delta p(M_{H_2O} v_{H_2O} + M_s v_s) + RT\,\Delta C_s \left(\frac{M_s}{C_s} - \frac{M_{H_2O}}{C_{H_2O}}\right) + M_{H_2O}\mu^*_{H_2O}. \tag{3}$$

The general theory of irreversible thermodynamics states that $\Phi = \sum_i J_i X_i$, where J_i is a generalized flow and X_i its conjugated force. A convenient choice of flows is J_v, the total volume flow crossing unit area of the membrane in unit time

$$J_v \equiv M_{H_2O} v_{H_2O} + M_s v_s, \tag{4a}$$

and J_D, the relative velocity of solute versus solvent

$$J_D \equiv \frac{M_s}{C_s} - \frac{M_{H_2O}}{C_{H_2O}}. \tag{4b}$$

Then
$$\Phi = (M_{H_2O} v_{H_2O} + M_s v_s)\, X_v + \left(\frac{M_s}{C_s} - \frac{M_{H_2O}}{C_{H_2O}}\right) X_D. \tag{5}$$

Equating coefficients in (3) and (5)

$$X_v = \Delta p + C_{H_2O}\mu^*_{H_2O};$$
$$X_D = RT\,\Delta C_s - \phi C_{H_2O}\mu^*_{H_2O}.$$

According to Onsager's Law, $J_i = \sum_j L_{ij} X_j$, where $L_{ij} = L_{ji}$. In this case the Ls are parameters of the membrane.

Thus,
$$J_v = L_p(\mu^*_{H_2O} C_{H_2O} + \Delta p) + L_{pD}(RT\,\Delta C_s - \phi C_{H_2O}\mu^*_{H_2O});$$
$$J_D = L_{Dp}(\mu^*_{H_2O} C_{H_2O} + \Delta p) + L_D(RT\,\Delta C_s - \phi C_{H_2O}\mu^*_{H_2O}); \quad L_{pD} = L_{Dp}. \tag{6}$$

By definition:

$$\sigma \equiv \frac{-L_{Dp}}{L_p} = \frac{-L_{pD}}{L_p}; \quad \omega \equiv \frac{L_p L_D - L^2_{pD}}{L_p} C_s = (L_D - L_p\sigma^2)\, C_s. \tag{7}$$

Substituting (7) in (6):

$$J_v = L_p(\mu^*_{H_2O} C_{H_2O} + \Delta p) - \sigma L_p(RT\,\Delta C_s - \phi C_{H_2O}\mu^*_{H_2O}); \tag{8a}$$

$$J_D = -\sigma L_p(\mu^*_{H_2O} C_{H_2O} + \Delta p) + \left(\frac{\omega}{C_s} + L_p\sigma^2\right)(RT\,\Delta C_s - \phi C_{H_2O}\mu^*_{H_2O}). \tag{8b}$$

From (4a) and (4b), since $C_s v_s + C_{H_2O} v_{H_2O} = 1 \doteq C_{H_2O} v_{H_2O}$:

$$M_s = C_s(J_v + J_D). \tag{9}$$

From (8) and (9)

$$M_s = C_s L_p(1-\sigma)\,(\mu^*_{H_2O} C_{H_2O} + \Delta p) + (RT\,\Delta C_s - \phi C_{H_2O}\mu^*_{H_2O})\,[\omega - \sigma L_p C_s(1-\sigma)]. \tag{10}$$

If the water pump is operating between two identical solutions in the absence of hydrostatic pressure gradients (i.e. $\Delta p = 0 = \Delta C_s$), then (8a) and (10) reduce to

$$J_v = L_p\mu^*_{H_2O} C_{H_2O}(1+\sigma\phi) \doteq L_p\mu^*_{H_2O} C_{H_2O}; \tag{11a}$$

$$M_s = \mu^*_{HO_2} C_{H_2O} C_s L_p(1-\sigma)\,(1+\sigma\phi) - \phi\omega C_{H_2O}\mu^*_{H_2O} \doteq \mu^*_{H_2O} C_{H_2O}[C_s L_p(1-\sigma) - \phi\omega]; \tag{11b}$$

since $\sigma \leqslant 1$, $\phi \ll 1$. Dividing (11b) by (11a) gives the concentration of solute in the absorbate

$$\frac{M_s}{J_v} = \frac{C_s L_p(1-\sigma) - \phi\omega}{L_p}. \tag{12}$$

Equation (12) describes the solute transfer caused by a water pump when no other driving forces act on the solute.

APPENDIX 2. *Active transport of solute*

In order to be able to consider the effect of an osmotic gradient on pumping, the same isothermal system is now supposed to contain one impermeant solute (subscript i), in addition to water, the permeant solute, and the membrane separating two well stirred compartments.

If only the permeant solute is actively transported and if the solutions are ideal and dilute

$$\mu^l_{H_2O} - \mu^o_{H_2O} = \frac{-RT\Delta C_s}{C_{H_2O}} - \frac{RT\Delta C_i}{C_{H_2O}} + v_{H_2O}\Delta p; \tag{13}$$

$$\mu^l_s - \mu^o_s = \frac{RT\Delta C_s}{C_s} + v_s\Delta p + \mu^*_s.$$

Active transport of solute is expressed as an active transport potential μ^*_s appearing in the chemical potential of the solute only. This can be shown to involve the assumption that the reflexion coefficient σ is the same for actively and passively transported solute. μ^*_s cancels in the expression for the absorbate osmolarity and need not be assumed to be constant. From (1) and (13)

$$\Phi = \Delta p(M_{H_2O}v_{H_2O} + M_s v_s) + RT\Delta C_s\left(\frac{M_s}{C_s} - \frac{M_{H_2O}}{C_{H_2O}}\right) - RT\Delta C_i\frac{M_{H_2O}}{C_{H_2O}} + \mu^*_s C_s. \tag{14}$$

Choose the same flows J_v and J_D as before ((4a) and (4b)). Equating coefficients in (5) and (14)

$$X_v = \Delta p + C_s\mu^*_s - RT\Delta C_i;$$

$$X_D = C_s\mu^*_s(1-\phi) + \phi RT\Delta C_i + RT\Delta C_s.$$

By Onsager's Law and substitutions (7)

$$J_v = L_p(\mu^*_s C_s + \Delta p - RT\Delta C_i) - \sigma L_p[RT\Delta C_s + \mu^*_s C_s(1-\phi) + \phi RT\Delta C_i]; \tag{15a}$$

$$J_D = -\sigma L_p(\mu^*_s C_s + \Delta p - RT\Delta C_i)\left(\frac{\omega}{C_s} + L_p\sigma^2\right)[RT\Delta C_s + \mu^*_s C_s(1-\phi) + \phi RT\Delta C_i]. \tag{15b}$$

From (9) and (15)

$$M_s = C_s L_p(1-\sigma)(\mu^*_s C_s + \Delta p - RT\Delta C_i) + [RT\Delta C_s + \mu^*_s C_s(1-\phi) + \phi RT\Delta C_i] \times [\omega - L_p\sigma C_s(1-\sigma)]. \tag{16}$$

If the solute pump is operating between two identical solutions in the absence of a hydrostatic pressure gradient (i.e. $\Delta p = 0 = \Delta C_s = \Delta C_i$), then (15$a$) and (16) reduce to

$$J_v = L_p\mu^*_s C_s[1-\sigma(1-\phi)] \doteq L_p\mu^*_s C_s(1-\sigma); \tag{17a}$$

$$M_s = \mu^*_s C_s[C_s L_p(1-\sigma) + (1-\phi)(\omega - \sigma L_p C_s + \sigma^2 L_p C_s)] \doteq \mu^*_s C_s[\omega + C_s L_p(1-\sigma)^2]. \tag{17b}$$

Dividing (17a) by (17b) gives the volume of fluid transported per mole of solute transported

$$\frac{J_v}{M_s} = \frac{L_p(1-\sigma)}{\omega + C_s L_p(1-\sigma)^2}. \tag{18}$$

The reciprocal of this expression is the absorbate osmolarity.

The effect of an osmotic gradient is found by taking $\Delta p = 0 = \Delta C_s$, $\Delta C_i \neq 0$. If one defines the volume flux in the absence of osmotic gradients as J_v^o ($J_v^o \equiv L_p \mu_s^* C_s(1-\sigma)$), then from (15*a*)

$$J_v = J_v^o - L_p RT \Delta C_i. \tag{19}$$

(15*a*) and (16) may be combined to correct M_s for the effect of J_v

$$\begin{aligned} M_s &= C_s(1-\sigma) J_v + \omega[RT \Delta C_s + (1-\phi) C_s \mu_s^* + \phi RT \Delta C_i] \\ &\doteq C_s(1-\sigma) J_v + \omega[RT \Delta C_s + C_s \mu_s^*]. \end{aligned}$$

When $\Delta C_s = 0$, $M_s = C_s(1-\sigma) J_v + \omega C_s \mu_s^*$. If one defines the solute flux in the absence of volume flow as M_s^o ($M_s^o \equiv \omega C_s \mu_s^*$), this becomes

$$M_s = C_s(1-\sigma) J_v + M_s^o. \tag{20}$$