Transport of Salt and Water in Rabbit and Guinea Pig Gall Bladder

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ABSTRACT A simple and reproducible method has been developed for following fluid transport by an *in vitro* preparation of mammalian gall bladder, based upon weighing the organ at 5 minute intervals. Both guinea pig and rabbit gall bladders transport NaCl and water in isotonic proportions from lumen to serosa. In the rabbit bicarbonate stimulates transport, but there is no need for exogenous glucose. The transport rate is not affected by removal of potassium from the bathing solutions. Albumin causes a transient weight loss from the gall bladder wall, apparently by making the serosal smooth muscle fibers contract. Active NaCl transport can carry water against osmotic gradients of up to two atmospheres. Under passive conditions water may also move against its activity gradient in the presence of a permeating solute. The significance of water movement against osmotic gradients during active solute transport is discussed.

The gall bladder concentrates bile by reabsorbing sodium chloride and water in isotonic proportions. From *in vitro* studies on fish gall bladder the mechanism of reabsorption was shown to be the active transport of both Na and Cl, coupled so that the transfer of one cation is linked directly to the transfer of one anion (Diamond, 1962 *a*, *b*). Because of this electrically neutral NaCl pump the gall bladder absorbed salt without giving rise to any electrical potential difference. Water movement followed salt movement passively by means of an unidentified coupling mechanism (Diamond, 1961 and 1962 *c*). Similar findings apply to rabbit gall bladder (Wheeler, 1963; Dietschy, 1964), which has the advantage of maintaining higher transport rates. The present paper is concerned with establishing optimal conditions for transport by the mammalian gall bladder *in vitro* and with studying the effects of osmotic gradients in the presence and absence of active salt transport. While exact comparison is difficult because the degree of microscopic folding cannot be easily estimated, on the basis of apparent surface area rabbit gall bladder maintains the highest rate of water transport reported for an epithelial membrane. Guinea pig gall bladder also offers a stable preparation, maintaining constant rates of transport for many hours.

METHODS

Experimental techniques were in general similar to those described for fish gall bladder in previous papers (Diamond, 1962 *a*, *b*, *c*), which may be consulted for further details. White rabbits of either sex weighing 3 to 5 kg were anesthetized intraperitoneally with nembutal, and the lobe of liver with the gall bladder was removed through a ventral incision. By reflecting the gall bladder from side to side, it was usually possible to visualize and carefully cut the membranes holding the gall bladder to the liver, thus obtaining a preparation free of adhering liver tissue. The remainder of the dissection was carried out in a dish of Ringer's solution, which was changed frequently to prevent leakage of bile from damaging the preparation. Since the cystic duct in the rabbit clamps down and is difficult to cannulate, it was cut off where it joined the gall bladder. A needle attached to a syringe was inserted into the resulting hole in the neck of the gall bladder to withdraw bile and wash out the lumen several times with Ringer's solution. Finally a polythene cannula (internal diameter, 1.57 mm) was secured in the hole by two ligatures, the gall bladder was refilled with Ringer's solution, and the upper end of the cannula was closed by a glass plug with a wire hook attached. Care was taken not to distend the gall bladder or apply too much pressure in filling it. The lower end of the cannula projecting below the ligatures into the lumen of the gall bladder had a few lateral flute-holes, to facilitate complete withdrawal of fluid from the gall bladder while changing solutions. 250 to 500 gm guinea pigs were killed by a blow on the head, and the gall bladder was prepared as just described for rabbits, except that it proved feasible to cannulate the cystic duct instead of having to make a hole in the neck of the gall bladder.

The cannulated gall bladder was suspended in a beaker of Ringer's solution, periodically renewed, in a water bath whose temperature was maintained at 37.0 ± 0.3°C. The bathing solution was stirred throughout the experiment by a stream of oxygen bubbles saturated with water vapor (or by 5 per cent CO_2–95 per cent O_2 when the bathing solution contained 25 mM bicarbonate). As the levels of fluid in the beaker and within the gall bladder were approximately the same, the hydrostatic pressure difference across the organ was negligible. Every 5 minutes the preparation was removed from the beaker, suspended by the wire hook from the hook of a Mettler balance, and weighed to the nearest milligram. Since the gall bladder wall and the cannula were very light, most of the weight of the preparation represented the fluid in the lumen, hence the decline of weight with time provided a simple and accurate method of following fluid absorption (transport of fluid out of the lumen across the gall bladder wall). To minimize the weighing error resulting from variable amounts of superficial fluid, a consistent procedure was adopted for rapidly draining the gall bladder against the inner lip of the beaker before a weighing. Each weighing involved the gall bladder being in air for 20 to 25 seconds.

At the end of an experiment the luminal fluid could be recovered for analysis by cutting the drained gall bladder over a tared pyrex centrifuge tube, in which the luminal fluid was collected and which was then promptly stoppered. The volume of solution was obtained from its known specific gravity and the gain in weight of the tube. During an experiment the luminal solution could be changed by unplugging

the cannula, and alternately withdrawing the luminal contents and refilling with fresh solution several times, using polythene capillaries mounted on hypodermic needles and syringes (Diamond, 1962 *a*). The volume of a gall bladder was obtained from the difference between the initial weight of the preparation with full lumen and the final weight with the gall bladder cut and the luminal fluid drained out. The formulae for a sphere may then be used to calculate the area from the volume, and although the organ is actually ellipsoidal in shape, this approximation suffices for purposes of comparison of results between different preparations.

Sodium and potassium analyses were carried out in duplicate with a modified Perkin-Elmer model 52A flame photometer, using lithium sulfate as an internal standard. The standard deviation of sodium determinations from twelve separate dilutions of the same sample was ±0.6 per cent. Chloride was titrated potentiometrically with 10 mM $AgNO_3$ (Sanderson, 1952). Evans Blue was measured colorimetrically at 610 mμ with the Beckman model B spectrophotometer.

TABLE I

COMPOSITION OF EXPERIMENTAL SOLUTIONS

	NaCl	$NaHCO_3$	KCl	$CaCl_2$	$MgSO_4$	Glucose	NaH_2PO_4	Na_2HPO_4	KH_2PO_4	K_2HPO_4	Sucrose
	mM	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*	*mM*
A	110.0	25.0	7.0	2.0	1.2	11.1	1.2	—	—	—	—
B	132.5	—	7.0	2.0	1.2	11.1	0.375	2.125	—	—	—
C	138.5	—	7.0	2.0	1.2	—	0.375	2.125	—	—	—
D	140.0	—	—	2.0	1.2	11.1	—	—	0.375	2.125	—
E	—	—	—	2.0	1.2	11.1	—	—	0.375	2.125	254

Table I gives the composition of experimental solutions, all of which were calculated to have the same osmolarity. In the experiments to be reported it may be assumed that solution A was used if specific mention of a solution is omitted. Pyrex glassware was used throughout.

RESULTS

Guinea Pig When a guinea pig gall bladder had identical bathing solutions containing bicarbonate and glucose (solution A) in the lumen and on the serosa, the preparation lost weight steadily for as long as observed (up to 4 hours). The upper curve in Fig. 1 illustrates changes in weight of a gall bladder preparation initially weighing 2687 mg, of which 2326 mg was fluid in the lumen, 181 mg was the gall bladder wall, and 180 mg was the hook, plug, and cannula. In 3 hours 10 minutes the preparation lost a total of 729 mg, so that each hour 230 mg, or 9.9 per cent (230 ÷ 2326) of the initial luminal volume, was transported across the gall bladder wall into the external bathing solution. The lower curve refers to a gall bladder initially weighing 2072 mg, of which 1748 mg was luminal fluid, 147 mg the gall bladder wall, and 177 mg the hook, plug, and cannula. After the gall bladder had lost

weight steadily for 1 hour 55 minutes at 139 mg/hour (8.0 per cent of the initial luminal contents reabsorbed each hour), the preparation was poisoned with cyanide and iodoacetate and after 20 minutes ceased to lose weight. As an example of the reproducibility of the weighing method for following fluid transport, the average of fourteen weighings after the gall bladder had ceased to lose weight was 1782 mg, and the standard deviation 3 mg, so that the weighing error was 3/1782, or 0.17 per cent. Since the luminal and serosal

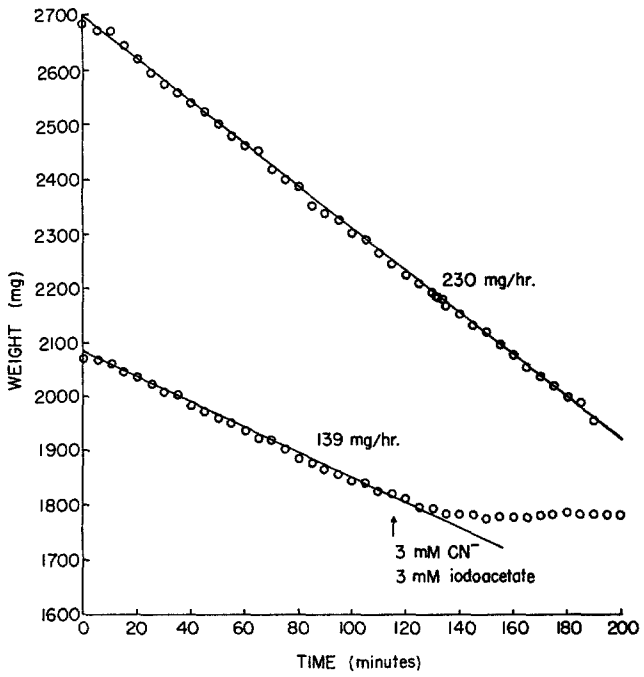

FIGURE 1. Fluid absorption by two gall bladders from guinea pigs. Ordinate, weight of the gall bladder preparation (most of the weight is luminal fluid). At $t = 0$ the lumen of each preparation was filled with Ringer's solution (solution A) identical in composition to the outer (serosal) bathing solution. The gall bladder illustrated in the lower curve was poisoned at $t = 115$ minutes by addition of 1 ml of a neutral isotonic solution of 103 mM NaCN, 103 mM iodoacetic acid to 35 ml of the outer solution.

solutions were identical in these experiments, the bathing solutions provided no external driving force for reabsorption of the luminal fluid, and fluid transport must have been powered by metabolism in the gall bladder wall, as is also implied by cessation of fluid transport after metabolic poisoning.

In a total of twelve similar experiments, guinea pig gall bladder reabsorbed its luminal contents at 9.6 ± 4.4 per cent/hour (this and all other errors to be reported are standard deviations), or 23.4 μl/hour, cm^2 of gall bladder surface. No preparation failed to transport fluid. In no preparation did the

rate of transport show signs of falling off with time, although weight loss was always followed for at least 2 to 3 hours. At the end of one experiment the residual fluid was analyzed and found to contain Na and Cl at within 3 per cent of their original concentrations. Thus, NaCl as well as water was reabsorbed, and the transported solution must have approximated an isotonic salt solution.

Rabbit. Rabbit gall bladders with identical bathing solutions on both sides also lost weight at a steady rate for many hours. As in guinea pig gall bladder, simultaneous addition of cyanide and iodoacetate to the serosal bathing solution at 3 mM stopped loss of weight within 20 to 30 minutes, while a 2 minute exposure to temperatures of 55–70°C stopped weight loss within 5 minutes. The results were qualitatively identical to those depicted for guinea pig in Fig. 1, but quantitatively the rate of transport in the rabbit was higher. On the average (28 gall bladders), rabbit gall bladder reabsorbed 24.6 per cent of its luminal volume per hour (52.8 μl/cm^2, hour), and the most active preparations reabsorbed half their volume in an hour. Wheeler (1963) and Dietschy (1964) obtained similar rates of transport (*ca.* 90 and 43 μl/cm^2, hour, respectively). Hence rabbit gall bladder was chosen as the material for all the remaining experiments to be described.

To test whether weight loss could be quantitatively attributed to reabsorption of luminal fluid, the dye Evans Blue was added to the luminal fluid of three gall bladders, and its increase in concentration was measured after the preparations had lost weight for several hours. Even when the gall bladder held 2 ml of solution with an optical density of 16 (as calculated from measurements on a diluted aliquot) at the absorption maximum of Evans Blue, the external bathing solution (volume 50 ml) still had an optical density of 0.000 when checked at half-hour intervals. At the end of an experiment the fraction of the luminal fluid absorbed was calculated (*a*) from the rise in concentration of Evans Blue, as $(r - 1)/r$, where r is the ratio of the final to the initial concentration of Evans Blue; (*b*) from the loss of weight, on the assumption that all weight lost was at the expense of luminal fluid. On the average the two methods of calculation gave figures for reabsorption differing by only 2.3 per cent. Hence there can be no bulk leakage of luminal fluid or weight loss by the gall bladder wall itself, and weight loss must be attributed quantitatively to selective reabsorption of luminal fluid. Later experiments using phenol red and C^{14}-inulin as volume indicators confirm this conclusion (Diamond, 1964).

Analyses performed in nine cases on the residual luminal fluid at the end of an experiment showed that [K] in the lumen always rose (on the average, by 21 per cent), while [Na] decreased (on the average, by 10 per cent) and [Cl] decreased more (on the average, by 27 per cent). The initial and final volumes and Na and K concentrations in the lumen were used to calculate the com-

position of the absorbed fluid, and then its osmolarity on the assumption that the absorbate consists of Na and K plus monovalent anions (*i.e.*, that absorption of the minor components of Ringer's is quantitatively negligible). The absorbate osmolarity thus computed was 98.5 ± 3.2 per cent of the bathing solution osmolarity. Hence the absorbate is isotonic, and the rise in luminal [K] implies that K diffuses out passively as reduction in luminal volume raises its luminal concentration. The decline in luminal [Cl] means that rabbit gall bladder transports chloride in preference to the principal other available

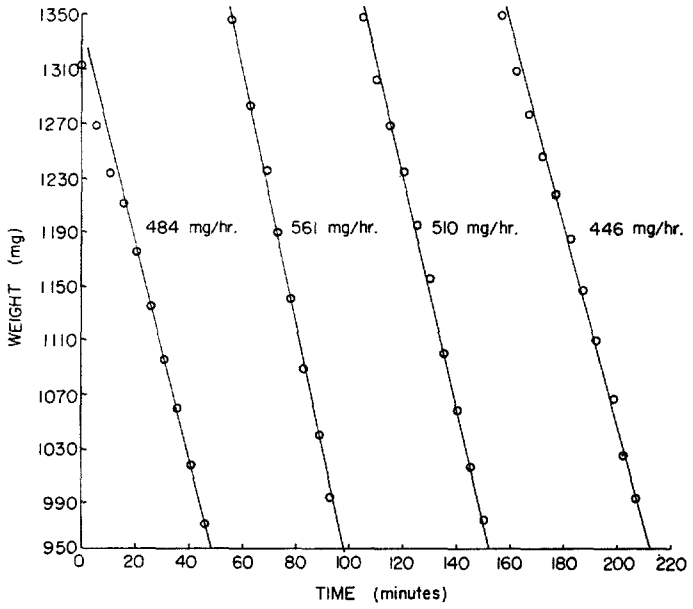

FIGURE 2. Reproducibility of fluid transport determinations. Ordinate, weight of a preparation of rabbit gall bladder. The luminal and outer bathing solutions were solution A. Before each of the four series of weighings the cannula was unplugged, the gall bladder refilled with fresh solution A, and the cannula replugged.

anion, bicarbonate. These findings are confirmed by direct analyses of the transported fluid obtained by another method (Diamond, 1964).

Reproducibility of Fluid Transport Determinations As a control for experiments in which the rates at which the gall bladder absorbed solutions of differing composition were to be compared, Fig. 2 illustrates measurements of the rate of weight loss when a gall bladder was filled up four successive times with the same solution (solution A) to approximately the same volume. The resulting four curves for weight loss under identical conditions are qualitatively and quantitatively similar. The average of the four determinations of fluid absorption rate was 500 mg/hour, with a standard deviation of 9.6 per cent;

and the average initial weight of luminal fluid was 1340 mg, with a standard deviation of 1.3 per cent. Hence the gall bladder can be repeatedly filled up to the same volume, and the rate of fluid transport can then be measured reproducibly.

The Effect of Glucose and Bicarbonate In Ringer's solutions without bicarbonate the rate of weight loss was approximately the same with glucose present in both bathing solutions at 11.1 mM (solution B) as in the absence of glucose (solution C). In solutions containing bicarbonate at 25 mM, neither the addition of glucose at 11.1 or 24 mM to initially glucose-free solutions nor the removal of glucose when it was initially present at 11.1 mM affected the rate of fluid transport. Thus, transport in rabbit gall bladder is powered by metabolism of endogenous substrates and not by exogenous glucose. Similarly, glucose has no effect in frog skin on the transport rate, and urinary bladder, rat ileum, and frog sartorius muscle can also transport ions in the absence of glucose.

The removal of bicarbonate, initially present in both bathing solutions at 25 mM, produced on the average a 47 per cent decrease in the rate of fluid transport, which then returned reversibly to its former level when bicarbonate was restored. Since bicarbonate was replaced mole for mole with chloride (solution A changed to solution B) and the gas was changed from 5 per cent CO_2–95 per cent O_2 to pure oxygen, the cation and glucose concentrations and pH remained unchanged, and the effect must be caused by the bicarbonate system itself. Replacement of bicarbonate with chloride reduced fluid transport by about the same percentage in the absence of glucose. As chloride is transported preferentially over bicarbonate, the effect cannot be due to bicarbonate transport *per se*, and bicarbonate must act by stimulating NaCl transport.

The Effect of Potassium In some epithelial membranes, such as frog skin (Koefoed-Johnsen and Ussing, 1958) and urinary bladder (Bentley, 1959), the presence of potassium on the serosal side of the preparation is essential for sodium transport from the mucosa to the serosa. Removal of potassium from the serosal side of fish gall bladder inhibits fluid transport by 52 per cent (Diamond, 1962 *a*). However, it has not been possible to establish any effect of potassium upon fluid transport by rabbit gall bladder, which was found to absorb fluid at normal rates even after a total of 86 minutes in potassium-free solution, with frequent changes of both luminal and serosal bathing solution to ensure that they remained as potassium-free as possible. Addition of K to both bathing solutions at 8 mM then produced no change in the absorption rate. Similarly, when both bathing solutions were initially potassium-free and potassium was then added to the outer bathing solution alone at 4.0 or 8.3 mM, there was still no detectable increase in the fluid transport rate. The K-free

effect in fish gall bladder could not have been a direct one on transport at the serosal membrane, as postulated for frog skin (Koefoed-Johnsen and Ussing, 1958), but may just have meant that intracellular K stimulated transport and was maintained by K uptake at the serosal face of the epithelial cells. The absence of the effect in rabbit gall bladder, with a thicker serosa, might be due to greater difficulty in depleting the cells of K.

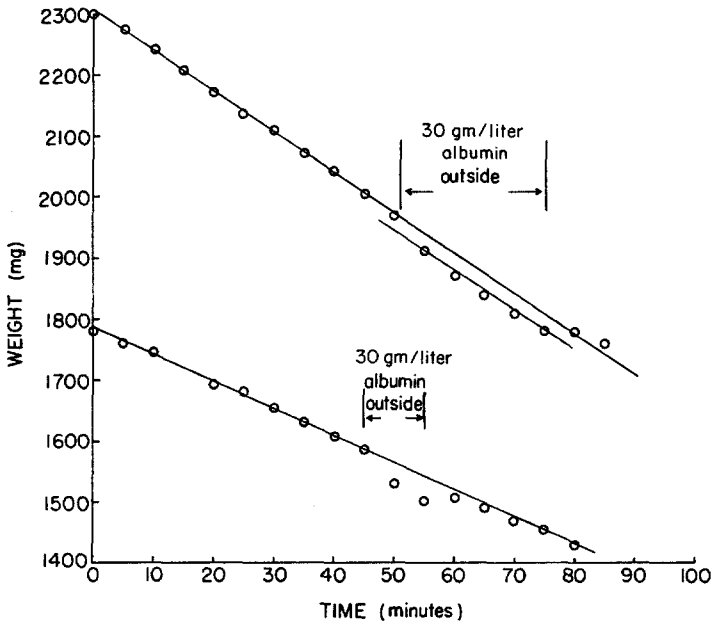

FIGURE 3. Effect of albumin upon fluid absorption by rabbit gall bladder. Ordinate, weight of the gall bladder preparation. Both sides of the preparation were bathed by solution A. Between the arrows bovine albumin was present in the outer bathing solution at 30 gm/liter.

The Effect of Albumin Fig. 3 illustrates two experiments in which albumin was added to the outer bathing solution. In the upper curve the preparation was losing weight at 395 mg/hour (33 mg/5 minutes), but in the 5 minutes after addition of albumin the gall bladder lost 61 mg, 28 mg more than expected. While the albumin remained outside, the gall bladder then continued to lose weight at the initial rate, but in the 5 minutes after removal of albumin it lost only 2 mg, 31 mg less than expected. The fact that the weighings before and after albumin fall on the same straight line in both the upper and lower curves of Fig. 3 means that albumin does not change the steady-state rate of water transport. It simply causes the gall bladder to lose all at once a certain weight, which is regained on removal of albumin. On the average (eight determinations) this reversible weight change represented 10.0 ± 1.9 per cent of the wet weight of the gall bladder wall.

Several possible explanations of the effect can be eliminated immediately. The effect cannot be an osmotic one, since the concentration of albumin was only 0.4 mOsM. The effect of colloid osmotic pressure is unimportant in the gall bladder, across which salt exerts fully 90 per cent the osmotic pressure of protein. There is no effect on the transport rate across the gall bladder. Since the gall bladder epithelial cells are separated from the outer bathing solution by the serosa, the speed with which the effect comes on and is reversed makes it likely that albumin simply causes a contraction in size of the serosa, decreasing the weight of the gall bladder wall itself. Upon removal of albumin the serosa would regain its former size. In fact, albumin is known to increase the resting potential and augment the contractile tension of muscle (Green, Giarman, and Salter, 1952; Kernan, 1960; Page, 1962). The simplest explanation of the albumin effect, then, would be that it increases the tone of the smooth muscle fibers in the serosa, squeezing some water out of the serosal extracellular space; and that on removal of albumin the muscle relaxes, allowing the serosa to reexpand to its original volume. If this explanation is correct, it would be additional evidence that the smooth muscle fibers in the serosa of the gall bladder have nothing to do with transport by the epithelial cells, since the fibers can go through a complete contraction-relaxation cycle without affecting the transport rate.

Transport of Water against Osmotic Gradients In the experiments described so far, the solutions on opposite sides of the gall bladder have been of identical osmolarity and usually of identical composition, so that no osmotic gradient was present either to oppose or to explain water movement. However, when the luminal solution was made hypertonic by addition of sucrose or raffinose, which should make water enter the lumen by osmosis, the gall bladder was still able to transport water outwards; *i.e.*, against the osmotic gradient. The experiment of Fig. 4 demonstrates that a gall bladder which transported fluid between identical solutions at 432 mg/hour could still absorb water against an osmotic gradient of 40 mM sucrose (0.9 atmosphere) at a rate of 159 mg/hour. Rabbit gall bladder is impermeable to sucrose and raffinose, which do produce osmotic water flow across it (Pidot and Diamond, 1964), and analysis of the residual luminal fluid showed that the lumen was still hypertonic at the end of these experiments. Hence there is no doubt that the preparation was transporting water against effective osmotic pressure gradients. When the concentration of sucrose in the lumen was increased to 80 mM, there was no net water movement. With luminal sucrose at 150 mM, the direction of net water movement was reversed, so that water flowed from the outer solution into the lumen at 32.6 mg/hour. Thus, increasing osmotic gradients cause increasing osmotic water flow into the lumen, but superimposed on osmosis is water transport out of the lumen in association with salt transport.

The maximum osmotic gradient against which rabbit gall bladder can carry fluids may also be estimated from experiments in which water movement was measured in different gall bladders with the lumen made hypertonic by different concentrations of sucrose or raffinose. Ten preparations continued to absorb water when 15, 25, 30, 50, or 70 mM raffinose or 25, 40 (two experiments), 50, or 105 mM sucrose, respectively, were added to the lumen. In four other gall bladders there was no net movement of water with the lumen made hypertonic by 60, 75, 90, and 100 mM sucrose, respectively. Finally, a reversed

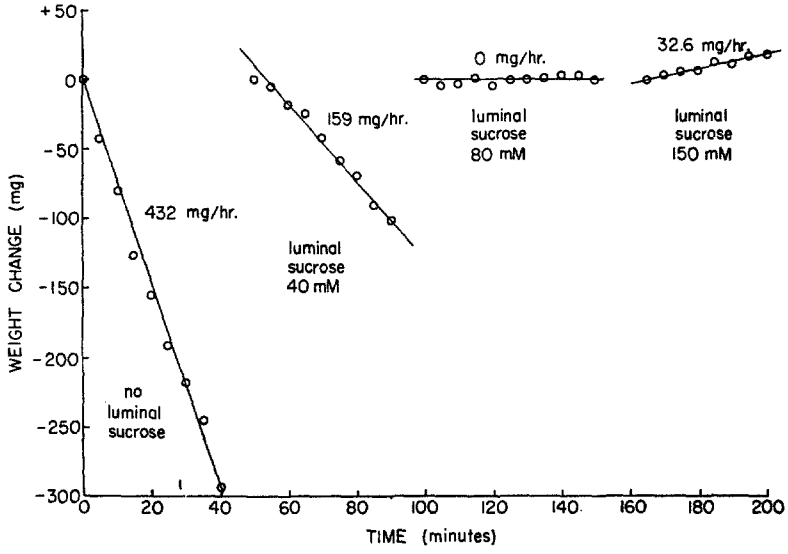

FIGURE 4. Effect of osmotic gradients upon fluid transport by rabbit gall bladder. Ordinate, change in weight of the gall bladder preparation after the first weighing in each series (*i.e.*, a negative value means weight loss, or movement of fluid out of the lumen). The outer bathing solution was solution A throughout. The luminal bathing solution was identical to solution A except for containing in addition sucrose at the indicated concentration. Thus, the luminal solution was isotonic in the first series of weighings and hypertonic during the other three series.

movement of water (into the lumen) was observed in two gall bladders when 150 or 200 mM raffinose was added to the lumen. Thus, osmotic gradients of about two atmospheres (39 mOsM = 1 atmosphere at 37°C) are required to stop water transport in this preparation. Smaller gradients diminish but do not stop net fluid absorption, while larger gradients reverse the direction of water movement, making it enter the lumen. Water transport against osmotic gradients during active solute transport has also been demonstrated in fish gall bladder (Diamond, 1962 *c*), rat small intestine (Parsons and Wingate, 1961), and the cerebrospinal fluid system (Heisey, Held, and Pappenheimer, 1962). The observation does not imply active water transport but means that

active solute transport exerts a force on water sufficient to carry water against a finite osmotic gradient (Diamond, 1961, 1962 *c*).

Passive Water Movement against Its Activity Gradient It is known from studies of artificial membranes that water can also move between solutions with identical water activities or against water activity gradients when a concentration gradient of a permeant solute is present. This phenomenon was

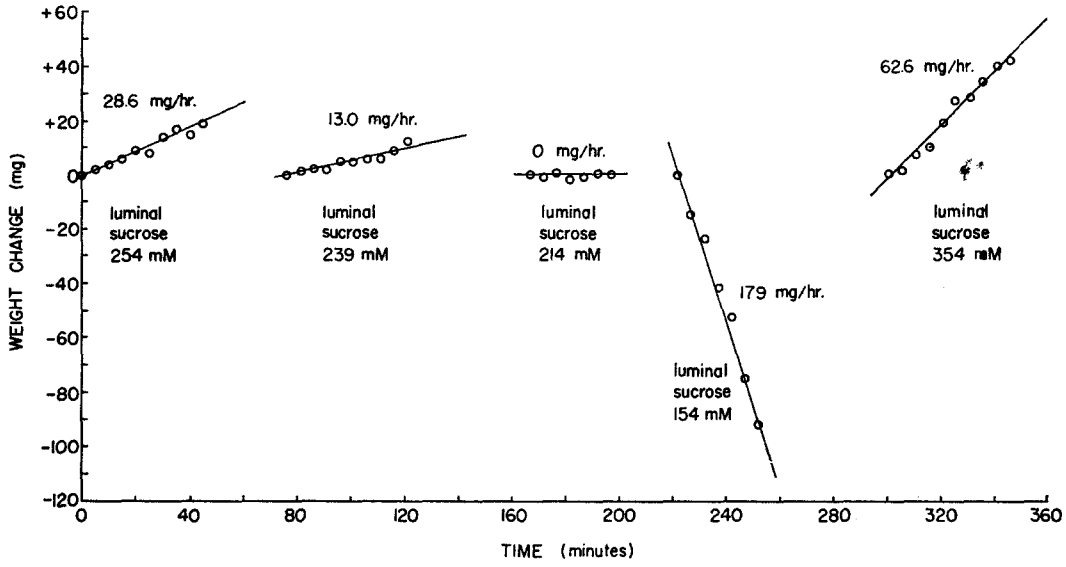

FIGURE 5. Passive water movement in rabbit gall bladder in the presence of a permeating solute. Ordinate, change in weight of the gall bladder preparation after the first weighing in each series (*i.e.*, a positive value means movement of fluid into the lumen, a negative value means movement of fluid out of the lumen). The outer bathing solution was solution D (mostly NaCl) throughout. The luminal solution was solution E (as solution D, but contains sucrose and no NaCl) with sucrose at the indicated concentration. Since solution E with 254 mM sucrose is isotonic to solution D, the luminal solution was isotonic during the first series of weighings; hypotonic by 15, 40, and 100 mOsM, respectively, during the next three; and hypertonic by 100 mOsM during the last series.

observed in fish gall bladder (Diamond, 1962 *c*), and Fig. 5 shows that it also occurs in rabbit gall bladder. The outer bathing solution contained as the main solute NaCl, to which the gall bladder is somewhat permeable. The lumen first contained an otherwise identical solution with the same osmolarity except that NaCl was replaced by 254 mM sucrose, to which the gall bladder is impermeable. Although the water activity on both sides was the same, the gall bladder gained weight; *i.e.*, water flowed from the outer bathing solution into the lumen. This is a purely passive phenomenon that persists after active salt transport has been stopped with metabolic poison, and in any case, with-

out NaCl in the lumen there is no active transport. In the second curve of Fig. 5 the luminal sucrose concentration was reduced to 239 mM, so that the lumen was now hypotonic by 15 mOsM and there was an activity gradient driving water out of the lumen. Nevertheless, the direction of net movement of water was opposite to its activity gradient and still into the lumen, at 13.0 mg/hour, although analysis of the residual luminal fluid at the end of such experiments showed that the lumen was still hypotonic. A gradient of 40 mOsM (luminal sucrose 214 mM) brought water movement to zero, while with larger gradients (luminal sucrose 154 or 354 mM) water moved down its activity gradient. The interpretation of these results is related to the fact that the gall bladder is impermeable to sucrose but somewhat permeable to NaCl. They may be formally described in terms of irreversible thermodynamics by use of a reflection coefficient σ for each solute (Kedem and Katchalsky, 1958), and one may then calculate that σ for NaCl in the rabbit gall bladder of Fig. 5 is 0.85 but that σ for the impermeant sucrose is 1.

DISCUSSION

The present study has shown that rabbit gall bladder utilizes metabolic energy to transport NaCl and water in isotonic proportions between identical bathing solutions and can also transport water against osmotic gradients. These findings are quite similar to those obtained in the earlier analysis of gall bladders from fish (Diamond, 1961, 1962, *a*, *b*, *c*). As in the fish, there is coupled active transport of both Na and Cl by means of an electrically neutral NaCl pump (Wheeler, 1963; Dietschy, 1964; Pidot and Diamond, 1964). The main problem requiring further discussion is the significance of water movement against osmotic gradients during active solute transport.

Just as in fish and rabbit gall bladders, water transport against osmotic gradients as well as absorption of isotonic NaCl without an electrical potential difference has been observed in the dog gall bladder by Grim (1963). However, he interpreted this to mean active transport of water carrying along NaCl passively and proposed that "an active mechanism transports luminal solution [by pinocytosis] from lumen to blood at a constant rate independent of concentration." In fish and rabbit gall bladder this interpretation would be rendered improbable by one fact and impossible by three others. Summarizing the evidence for fish (Diamond, 1962 *a*, *b*, *c*) and rabbit (Wheeler, 1963; Dietschy, 1964; Pidot and Diamond, 1964; Diamond, 1964, and present study) gall bladders, the suggestive fact favoring specific active transport of NaCl rather than of water is that: (*a*) Water transport is observed when the luminal solute is NaCl but not when it is another salt (with few exceptions, as NaBr) or any organic molecule so far tested. The three conclusive facts are that: (*b*) NaCl is virtually the only solute transported, while other salts (with few exceptions) and all organic molecules so far tested are not. Specif-

ically, there is no transport of choline chloride, tetraethyl ammonium chloride, KCl, Na_2SO_4, $NaCH_3SO_4$, malonamide, erythritol, arabinose, xylose, mannitol, galactose, sucrose, lactose, raffinose, inulin, Evans Blue, phenol red, bromsulfalein, bile pigment, cholate, hemoglobin, or albumin. If the lumen is filled with NaCl plus one or several of these twenty-two other compounds, only NaCl is absorbed, and the other compounds remain behind. Therefore, there can be no transport of luminal solution as such. (*c*) Net transport of both Na and Cl can go on against their electrochemical gradients when there is no water movement or even when the water movement is in the opposite direction. Therefore, there must be active transport of both Na and Cl. (*d*) There is no net water transport in the absence of net NaCl transport, and the amount of water transported is directly proportional to the amount of NaCl transported. Therefore, there is no separate mechanism for water transport, and all the water transport is associated with NaCl transport. Hence in fish and rabbit gall bladder NaCl transport is active and water secondary, not *vice versa*. The basic experimental findings in dog gall bladder (isotonic NaCl transport without a PD, ability to move water against osmotic gradients) are the same, and it will be surprising if the interpretation proves radically different. In fact, the classical experiments of Ravdin, Johnston, Riegel, and Wright (1932), demonstrating that dog gall bladder concentrates hepatic bile by absorbing NaCl but not bile pigment, bile salt, cholesterol, or calcium, make pinocytosis unlikely in the dog as well. It is now known that movement of water against osmotic gradients, which formerly was sometimes taken as a sign of active water transport, can result secondarily from active solute transport (Diamond, 1961, 1962 *c*). The mechanism by which active NaCl transport in the gall bladder gives rise to isotonic water movement is considered in the following paper (Diamond, 1964).

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