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TRANSCELLULAR TRANSPORT OF ISOSMOTIC VOLUMES BY THE RABBIT GALL-BLADDER *IN VITRO*

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SUMMARY

1. Fluid transport rate and oxygen consumption (Q_{O_2}) were studied in rabbit gall-bladder preparations *in vitro* exposed on both sides to identical Ringer solutions with NaCl concentrations (and osmolarities) varying from 70 to 140 m-equiv Na^+/l. (and 173–313 m-osmole/l.).

2. The time sequence of acute effects on transport rate resulting from sudden changes in the NaCl concentration of the bathing solutions indicated that, (*a*) as a primary effect, fluid volume transfer rate remained unaffected whereas Na transport rate changed abruptly in direct proportion to the Na concentration of the bathing media; (*b*) a secondary, delayed and partly reversible depression of fluid transfer rate following elevation of the NaCl concentration was observed only when the rate of transport was relatively high initially.

3. A fixed, and highly significant, linear relationship between changes in transport-linked oxygen consumption (ΔQ_{O_2}) and measured net fluid volume transport (ΔT_{vol}) was found independent of the NaCl concentration of the bathing media, dQ_{O_2}/dT_{vol} being $0{\cdot}22 \pm 11\%$ and $0{\cdot}25 \pm 8\%$ in bladders incubated in solutions containing 140 and 70 m-equiv Na^+/l. respectively.

4. Oxygen consumption per equiv of Na^+ (calculated) transported varied in inverse proportion to the Na concentration of the bathing media, dQ_{O_2}/dT_{Na} being $0{\cdot}0016 \pm 11\%$ and $0{\cdot}0036 \pm 8\%$ in '140 R' and '70 R' solutions, respectively.

5. Removal of K from the bathing solutions was followed by a gradual and partly reversible depression of fluid transport rate to a minimum level (about 100×10^{-4} μl H_2O. min^{-1}.mg^{-1}) independent of the initial transport rate.

6. It is concluded that the range of absorption rates of isosmotic fluid

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from the gall-bladder lumen represents a range of energy requiring capacities for transfer of fluid volume units; the data suggest that the intracellular (cytoplasmic) ion composition, depending on the presence of external K, as well as hormonal action may influence the capacity of the transcellular fluid transport mechanism.

7. A model (a 'mechanical volume pump') for transcellular transfer of fluid volume units, allowing for flexible specificity with regard to the actively transported solutes, and requiring the presence of Na^+ and Cl^-, is proposed.

INTRODUCTION

Absorption or secretion of fluid isotonic to plasma in the absence of external driving forces is a characteristic feature of various epithelia such as, for example, those lining the proximal tubules of the kidney, the gall-bladder, small intestine and the stomach. This type of transcellular transport has been intensively investigated in recent years in attempts to clarify the mechanism underlying the transfer of solute and water. In particular the proximal tubule and the gall-bladder have revealed striking similarities regarding their functional characteristics, including transport capacities, as well as morphological aspects.

From an analysis of available data obtained in the renal proximal tubules it appeared that the major difficulty in explaining the observed rates of transport of water, Na^+, Cl^-, HCO_3^-, K^+, and urea arises at the probably rate-limiting step across the luminal membrane (cf. Leyssac, 1966, ch. II); and assuming active transport of Na^+ from the cytoplasmic Na pool (15–30 m-equiv/l.) across the peritubular (or lateral) membrane (p.d.: -80 mV) at the known rates of transport and suprabasal oxygen consumption the efficiency would have to be 80–90 % in order to supply the energy requirements of the calculated minimum thermodynamic work (Bojesen & Leyssac, 1965). It was therefore concluded, in opposition to current concepts, that solute transported across the cellular wall is confined to separate subcellular compartments allowing a high solute concentration locally, i.e. that the route of solute and solute-linked water transfer from the lumen to the lateral spaces is restricted to special subcellular organelles. Accordingly proximal reabsorption rates, under physiological conditions, might represent capacities for the transport of a volume of fluid (Tm_{vol}) rather than Na (or NaCl) transfer capacities (Tm_{Na}) (Leyssac, 1966).

Because of the great similarity between the proximal tubule and the gall-bladder one would *a priori* expect a similar transport mechanism in these two epithelia; thus, the above conclusions drawn from data obtained in the proximal tubule might be tested in the *in vitro* preparation of the

gall-bladder taking advantage of the experimental possibilities offered by the simplicity of this preparation. The purpose of the present study was therefore to investigate whether or not transcellular transport rates in the gall-bladder have the character of capacities for the transfer of a fluid volume or Na ions (or NaCl). The results confirmed that the observed range of absorption rates represent various capacities for volume transfer.

METHODS

White female rabbits weighing about 3 kg were sacrificed by a blow on the neck; the right lobe of the liver with the attached gall-bladder was quickly removed through a ventral incision and placed in a dish of Ringer solution at 37° C; the composition of this solution was generally the same as that used in the first experimental period. The further isolation and cannulation of the gall-bladder was performed as described by Diamond (1964*a*); the bile was withdrawn and the lumen washed out several times with Ringer solution. The experimental technique for measurement of net fluid absorption by weighing of the gall-bladder preparation closely followed that used by Diamond (1964*a*). In short, the gall-bladder preparation, filled with Ringer solution saturated with a mixture of 5% CO_2 and 95% O_2, was suspended in a beaker of Ringer solution maintained at 37·0 ± 0·2° C in a thermostatically heated water-bath. The outside bathing solution was continuously stirred and oxygenated by a stream of O_2–CO_2 bubbles (95% O_2–5% CO_2) saturated with water vapour at 37° C. Inside (mucosal) and outside (serosal) bathing media were identical in composition except where specifically stated; at intervals of 5 min or more the preparation was removed from the beaker, rapidly drained of superficial fluid by a standard procedure, suspended by the wire hook on the hook of a Mettler balance, and weighed to the nearest milligram. The weighing procedure involved the gall-bladder being exposed to air for about 20 sec. At regular intervals determined by the rate of weight loss (absorption rate) luminal fluid was supplemented to the original volume in order to avoid too great differences in the degree of distension during the course of an experiment. Care was taken not to overdistend the bladder. During an experiment the luminal solution could rapidly be changed; the lumen was emptied through the cannulated polyethylene tubing, washed and refilled with solution of a different composition. Simultaneously the outside solution was changed to maintain identical media on both sides of the gall-bladder wall. At the end of an experiment the gall-bladder was cut open and the drained bladder wall was weighed after surface fluid had been gently blotted off with filter paper. The transport rates are given per milligram of wet tissue.

Oxygen consumption by the gall-bladder preparation was measured as described by Martin & Diamond (1966). The decline with time in oxygen tension of the Ringer solution contained in a closed chamber (volume 38·0 ml.), in which the gall-bladder was suspended, was measured using a Clark oxygen electrode (Yellow Springs Instr. Co., Ohio, U.S.A.). The current output was read on a Kipp Micrograph recorder. The electrode was calibrated in each experiment by measuring the output when the chamber contained Ringer solution equilibrated with atmospheric air. The sensitivity of the circuit was preset to give a reading of 5 μA corresponding to 75% saturation. The experimental solutions were equilibrated with 95% O_2 and 5% CO_2 and oxygen saturation never declined below 30% during the experimental periods. Within this range of saturations the current output is a linear function of oxygen saturation (Martin & Diamond, 1966).

With a slope of current output versus time of X μA/hr oxygen consumption (in μl. O_2/hr mg wet weight of tissue) was calculated from the expression:

$$X \frac{20{\cdot}93}{Y} \alpha(38{\cdot}0) \frac{760 - P_{H_2O}}{760} \frac{1}{Z},$$

in which Y is current output at 20·93% oxygen saturation; α is the solubility coefficient of oxygen in water at 37° C (= 0·0244 ml. O_2/ml. water); P_{H_2O} is the water vapour pressure (47 mm Hg at 37° C); and Z is the weight of the bladder wall (in mg).

The chamber for measuring oxygen tension was similar to that described by Martin & Diamond (1966). The temperature of the solution was kept constant at 37·0 ± 0·1° C by means of a water jacket with circulating and thermostatically heated water.

The temperature was controlled before and after each experimental period.

Net fluid transport rates during measurements of oxygen uptake were determined by weighing the bladder preparation immediately before and after the measurement of oxygen consumption and registration of the intervening time period; experimental periods lasting 20–30 min were sufficient for a reasonably accurate estimate of the slope of the line through the record of oxygen saturation versus time. All measurements were carried out with the *in vivo* orientation of the gall-bladder wall. The different Ringer solutions used are given in Table 1.

TABLE 1. Composition (m-mole/l.) of experimental solutions

	'70-R'	'140-R'	'110-R'	K^+-free '110-R'
NaCl	52	121	91	91
$NaHCO_3$	17·5	17·5	17·5	17·5
KCl	7·0	7·0	7·0	—
$CaCl_2$	2·0	2·0	2·0	2·0
$MgSO_4$	1·2	1·2	1·2	1·2
Glucose	11·0	11·0	11·0	11·0
NaH_2PO_4	1·2	1·2	1·2	1·2
Osmolarity (m-osmole/l.) calculated	173	313	233	219

The numbers 70, 140 and 110 refer to the total Na^+ concentration in m-equiv/l.

RESULTS

In vitro preparations of rabbit gall-bladder with identical bathing solutions on both sides maintain absorption of a fluid approximately equal to a NaCl solution isosmotic with the bathing medium over a range of osmolarities from about 80–600 m-osmole (Diamond, 1964*b*; Whitlock & Wheeler, 1964). Thus, with known osmolarity of the bathing medium Na transport rates can be calculated from the rate of net fluid absorption, i.e. from the weight loss per unit of time.

I. *Control experiments*

In order to test the reproducibility and stability of the steady-state transport capacity of the rabbit gall-bladder bathed in experimental Ringer solutions of about 300 and 170 m-osmole, respectively, two control series were made; the osmolarity of the two bathing solutions was varied by varying the concentration of the principal solute, NaCl, to give Na concentrations of 140 m-equiv/l. ('140 R') and 70 m-equiv/l. ('70 R') respectively. A fairly stable, although slowly declining, absorptive capacity was usually maintained for 4–6 hr or more in '140 R' solution as well as '70 R' solution (cf. Fig. 1). Individual transport rates varied considerably

in both series but were generally higher in '70 R' than in '140 R' solutions; the average transport rate in the initial period was 180 ± 52 (S.D.) $\times 10^{-4}$ μl. min^{-1} mg^{-1} in '140 R' (seven expts) as opposed to 500 ± 196 (S.D.) $\times 10^{-4}$ μl. min^{-1} mg^{-1} in '70 R' (nine expts). A typical control experiment with '70 R' bathing solution is shown in Fig. 1. These findings are in complete agreement with those reported by Diamond

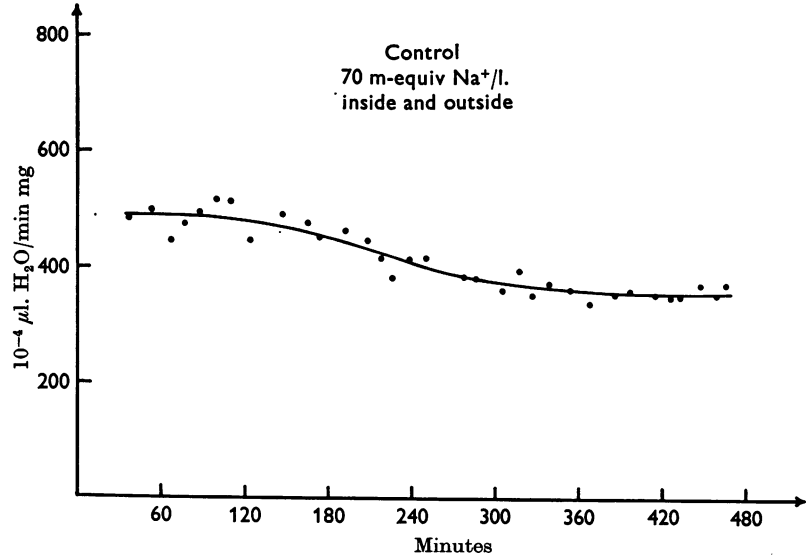

Fig. 1. Representative control experiment illustrating fluid absorption rates during the course of an experiment with an isolated rabbit gall-bladder prepared and incubated in '70 R' solutions on both sides.

(1964*b*). Further, it was observed that the absolute decrease in transport rate over a period of 3–4 hr was greater in bladders bathed in '70 R' than in those bathed in '140 R', while the decline expressed in relative terms was greater in experiments with '140 R' (8–20 %) than in those with '70 R' solution (5–10 %). Thus, a low NaCl concentration (or osmolarity) in the bathing solution (but still at, or above, the saturation limit below which the rate of transfer of volume is reduced) seems to be a favourable condition for the preservation of a high transport capacity of the *in vitro* preparation of the gall-bladder.

II. *Influence of acute changes in sodium concentration (osmolarity) on transport rate*

In experiments previously reported by Diamond (1964*b*) and present control experiments, average fluid transport rate (in μl. min^{-1} mg^{-1}) in 300 m-osmole solutions was about half of that measured in 150–170

m-osmole solutions. Thus, with isosmotic absorbates transport rates of NaCl in the two groups were equal as would be predicted from the current concept of a rate limiting capacity for the transfer of Na^+ (or NaCl). In view of the inference from data on proximal tubules of a limiting capacity for transfer of a fluid volume (Tm_{vol}) it might have been anticipated that the rate of water transport would have remained unchanged while Na transport rate was halved. However, these results are less conclusive because they were average values obtained in several bladders with a large range of individual variations.

More definite conclusions can be drawn from experiments, in which each single gall-bladder serves as its own control and permits the study of the time sequence of immediate effects resulting from sudden changes in the experimental conditions. Hence, the rate of net fluid transport in each single gall-bladder was measured in several consecutive periods, in which the concentration of NaCl in the bathing solutions was changed, in most experiments from '140 R' to '70 R' (*a*) or vice versa (*b*); in a few experiments NaCl concentrations were chosen to give Na concentrations of 130, 100, and 75 m-equiv/l. in the Ringer solutions used in successive periods (e.g. in Fig. 2, no. 45). This difference did not affect the consistency of the results.

(*a*) When '140 R' (or '130 R'), used as incubating medium during preparation and in the first experimental period (i.e. for more than 1 hr), was replaced by '70 R' the rate of fluid transfer remained completely unchanged (five gall-bladders), also in the following periods in which the bathing solutions were changed back to '140 R' or '100 R' (e.g. in Fig. 2, no. 45). A similar result was obtained in a few gall-bladders initially incubated in '70 R' solution, but in which the transport rate was relatively low (below 300×10^{-4} μl. min^{-1} mg^{-1}), i.e. in the same range as that obtained in bladders initially incubated in '140 R'. The fluid transport rate remained unaffected by shifting from '70 R' to '140 R' (Fig. 2, GB no. 76).

These results were unexpected in view of the evidence of a fairly stable Na transport capacity suggested from the average values of the above-mentioned experiments. Assuming isosmotic transport the data obtained in these single bladders, each exposed to solutions of varying NaCl concentrations, indicate that the Na transport rate varied in direct proportion to its concentration in the bathing medium, and further, that it must have changed abruptly in response to replacements with the different Ringer solutions.

(*b*) A somewhat different pattern was apparent in a series of seventeen gall-bladders incubated first in '70 R' solution and having high transport rates initially (above 300×10^{-4} μl. min^{-1} mg^{-1}) (e.g. in Figs. 3 and 4). Following replacement with '140 R' solutions on both sides net fluid

transport rate remained unchanged initially and then decreased slowly and gradually over a period of about 60 min to reach finally a value equal to that encountered in bladders incubated in '140 R' from the beginning of the experiment (*ca.* 200×10^{-4} μl. min^{-1} mg^{-1}). This final steady-state value was independent of the initial transport rate in '70 R' solution (cf.

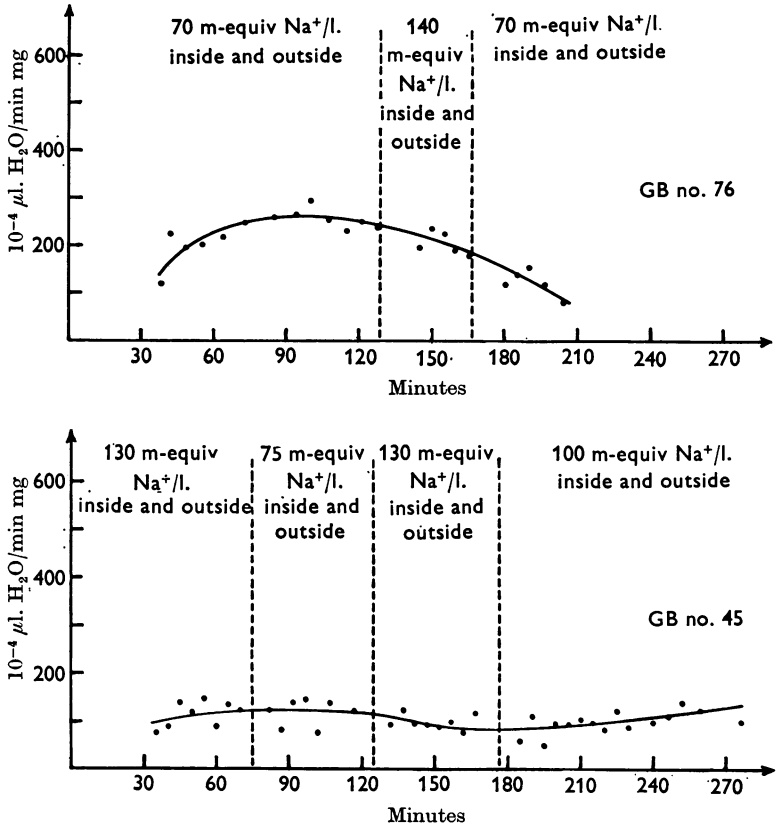

Fig. 2. Effect of acute changes in composition of the bathing solutions on fluid transport rate by two gall-bladders having a low rate of absorption initially (cf. section II (*a*)).

Figs. 3 and 4). If, in the following (third) period '140 R' was substituted by '70 R', fluid transport rate gradually increased again, but usually it did not reach the initial level. The ability to regain high transport rate after having transported in '140 R' was directly related to the initial transport rate and inversely related to the duration of the intervening period with '140 R' as bathing solution.

Sodium transport rate. When calculated from the rate of fluid transport and the Na concentration of the bathing solutions, Na transport rate again

changed in jumps in response to abrupt changes in the composition of the Ringer solution. Further, the change in Na transport rate in the first interval following a shift in the Na concentration of the bathing medium was directly proportional to the change in concentration; subsequently the rate slowly declined over a period of about 60 min in parallel with the

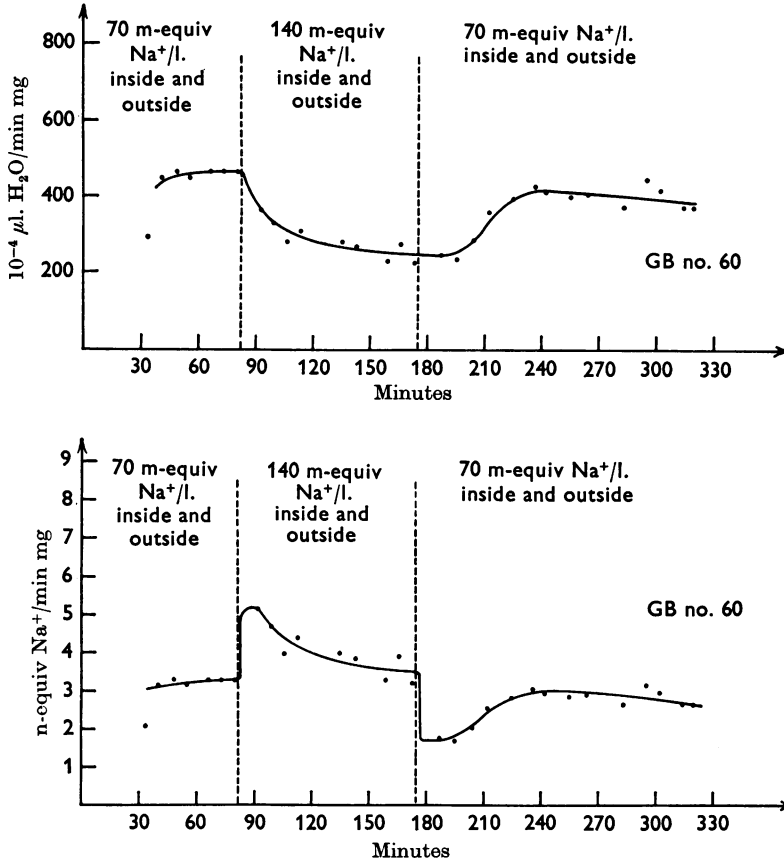

Fig. 3. Effect of acute changes in the composition of the bathing solutions on transport rate by a gall-bladder with initial absorption rate in the '70 R' solutions at an average level (cf. section II(*b*)).

water transfer rate, usually to reach a final value different from the initial transport rate (cf. Fig. 4).

The main conclusion drawn from these experiments, (*a*) and (*b*), is that an acute change in NaCl concentration (and osmolarity) of the incubating media as a primary effect induces a proportional jump in Na transport capacity while the capacity of fluid volume transfer remains unaffected. A secondary and delayed effect on the rate of volume transport may occur

depending on the initial transport rate and the change in composition of the bathing solution.

III. *Relationship between oxygen consumption and transport rates*

According to current concepts the energy-requiring step in the process of transcellular transfer of solute and water is the active transport of Na^+ (or NaCl), implying a fixed relationship between the change in transported

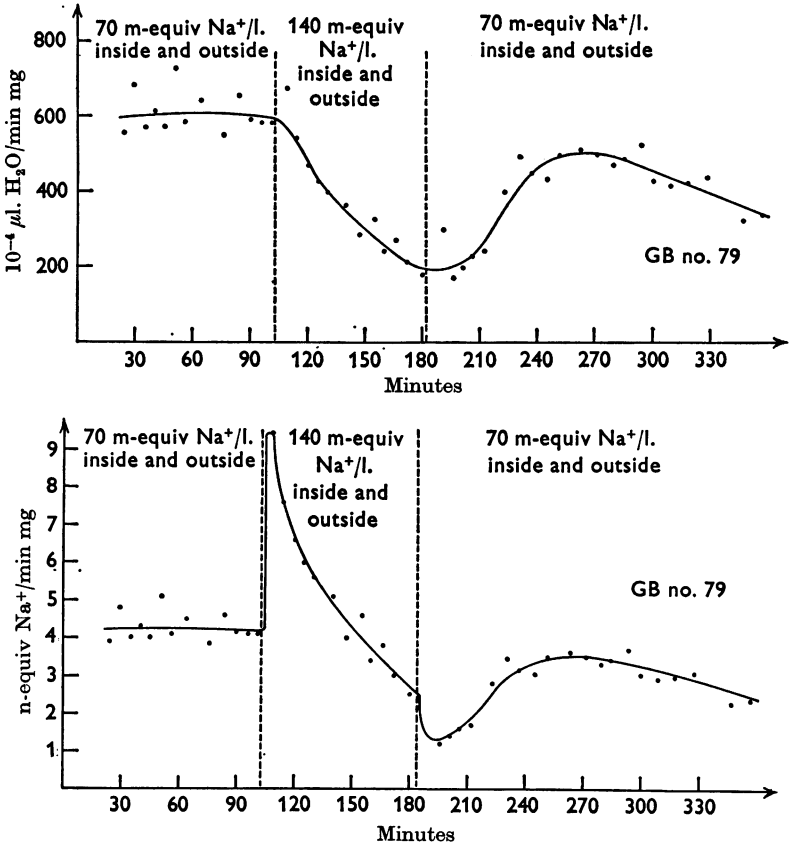

Fig. 4. Effect of acute changes in the composition of the bathing solutions on transport rate by a gall-bladder with initial absorption rate in '70 R' solutions at a high level (cf. section II(*b*)). Upper graph shows measured fluid transport rates. Lower graph represents calculated Na^+ transport rates of the same experiment.

equivalents of Na^+ (ΔT_{Na}) and the concomitant change in volume of oxygen consumed (ΔQ_{O_2}). From the concept of a capacity for the transfer of volumes ('volume pump') a fixed relationship between changes in transported volume (ΔT_{vol}) and ΔQ_{O_2} would be predicted. Therefore, in order further to distinguish between these alternatives oxygen consump-

tion and corresponding fluid volume transport were measured in two series of experiments. In one series ten gall-bladders were exposed to '140 R' solutions on both sides; in the other series ten bladders were studied when transporting in '70 R' solutions during the entire experiment. Corresponding values of transport rate and oxygen consumption were obtained in 3–6 periods of 15–30 min duration in each gall-bladder in

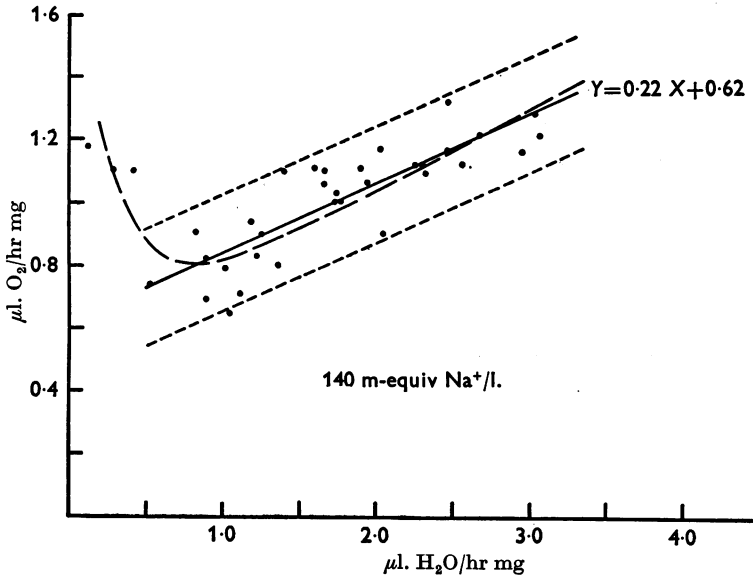

Fig. 5. Relationship between oxygen consumption (ordinate) and fluid volume transport (abscissa) in gall-bladders incubated in '140 R' solutions on both sides. ——, Regression line calculated by the method of least squares; – – –, limits of ±2 S.E. of estimate; — —, curve drawn by eye to the best fit with all the data.

order to obtain a representative spontaneous range of values in each series. The results are presented in Figs. 5 and 6 (cf. also Fig. 10). A highly significant correlation between fluid transport rate and oxygen consumption exists in both series (correlation coefficients of 0·85 and 0·90, respectively). The regression lines shown in Figs. 5 and 6 were calculated by the method of least squares, excluding the few values obtained at extremely low transport rates (i.e. below 0·8 μl. hr^{-1} mg^{-1}, or 130×10^{-4} μl. min^{-1} mg^{-1}). The slope of the regression line relating oxygen consumption (in μl. O_2 hr^{-1} mg^{-1}) to rate of net fluid volume transport, dQ_{O_2}/dT_{vol}, was $0{\cdot}22 \pm 11\%$ S.D. for bladders transporting in '140 R' solutions and $0{\cdot}25 \pm 8\%$ S.D. for those transporting in '70 R' solutions. The difference is insignificant, indicating a fixed relationship between the volume of fluid transferred and utilization of energy independent of the Na concentration (or osmolarity) of the bathing media.

Further, the data suggest that at any given transport rate oxygen consumption tends to be higher in gall-bladders transporting in '140 R' solutions than in bathing media with a low Na concentration ('70 R'), the intercept of the regression lines with the ordinate being 0·62 and 0·38 respectively.

The absence of a fixed ratio of oxygen consumed per Na^+ ion transported transcellularly ($\Delta Q_{O_2}/\Delta T_{Na}$) in the same two series of bladders transporting in solutions with different NaCl concentrations is most clearly illustrated

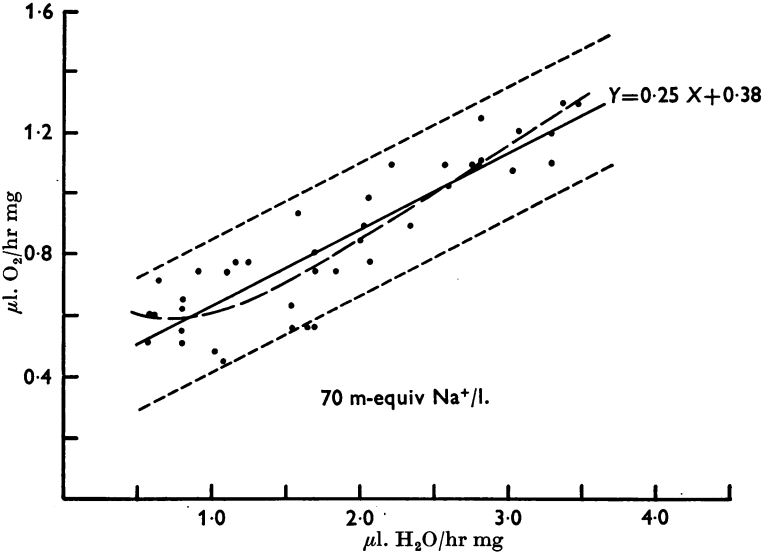

Fig. 6. Relationship between oxygen consumption (ordinate) and fluid volume transport (abscissa) in gall-bladders incubated in '70 R' solutions on both sides. ——, Regression line calculated by the method of least squares; – – –, limits of ± 2 S.E. of estimate; — —, curve drawn by eye to the best fit with all the data.

in Fig. 7. In this figure oxygen consumption is plotted versus the calculated net Na transport rate (in n-equiv. Na^+ hr^{-1} mg^{-1}). The slope of the two regression lines, (dQ_{O_2}/dT_{Na}), calculated by the method of least squares, differed significantly, being 0·0036 ± 8 % for bladders transporting in '70 R' solutions and 0·0016 ± 11 % for those transporting in '140 R' solutions, apparently indicating that it is two times more expensive, energetically, to pump 1 mole of Na ions (or NaCl) isosmotically across the cell bathed in Ringer solutions containing 70 m-equiv Na^+/l. than in solutions containing 140 m-equiv Na^+/l.

In conclusion, these experiments demonstrate that the amount of transport linked oxygen consumed primarily depends upon the net volume of fluid transported, whereas the oxygen consumption per equivalent of net Na transport varies with the Na concentration of the bathing media.

IV. *Effect of potassium*

The concept of a transcellular volume transport process, strongly supported by the present results, implies that the transferred solute and accompanying water move along separate pathways subcellularly, spatially separated from the active transport processes responsible for the maintenance of high K^+, low Na^+ concentrations intracellularly (i.e. in the cytoplasm). Thus, these latter processes (Na^+- and K^+ pumps) should be similar to those described, e.g. in nerve cells and erythrocytes. Absence of extracellular K inhibits the active Na efflux from these cells with the consequence that intracellular Na concentration increases; ouabain has the same effect (cf. e.g. Glynn, 1956). Evidence of an effect on fluid transport capacity by elevated Na concentration intracellularly might therefore be obtained by removal of K from the external bathing solutions. Conditions providing a relatively low intracellular Na concentration in the control period would be of significance for a clear response.

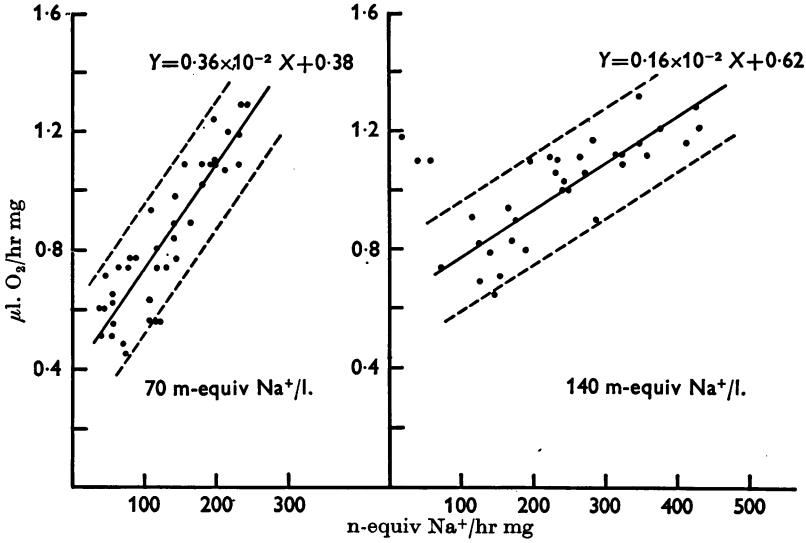

Fig. 7. Relationship between oxygen consumption (ordinate) and calculated net Na^+ transport (in n-equiv Na^+ hr^{-1} mg^{-1}) in gall-bladders incubated in '70 R' (left) and '140 R' solutions (right). The regression lines, calculated by the method of least squares (continuous lines), are given with ± 2 S.E. of estimate (interrupted lines).

In this series of experiments four gall-bladders were prepared and incubated in Ringer solutions containing a relatively low Na concentration ('110 R').

Following removal of K from both sides fluid transport rate invariably decreased gradually over a period of about 45–60 min to reach a level almost equal to, or slightly below that obtained in bladders incubated in '140 R' solutions. Two representative experiments are shown in Fig. 8. The effect of removal of K from the bathing media much resembled that of increasing the external Na concentration. The final transport level was

independent of the initial transport rate; thus, the inhibitory effect was directly proportional to the initial rate. Further, the ability to regain high transport rate following addition of K^+, in the third period, was related to the initial transport rate, and recovery was impaired by repeated exposures to K-free media (and/or the duration of the experiment).

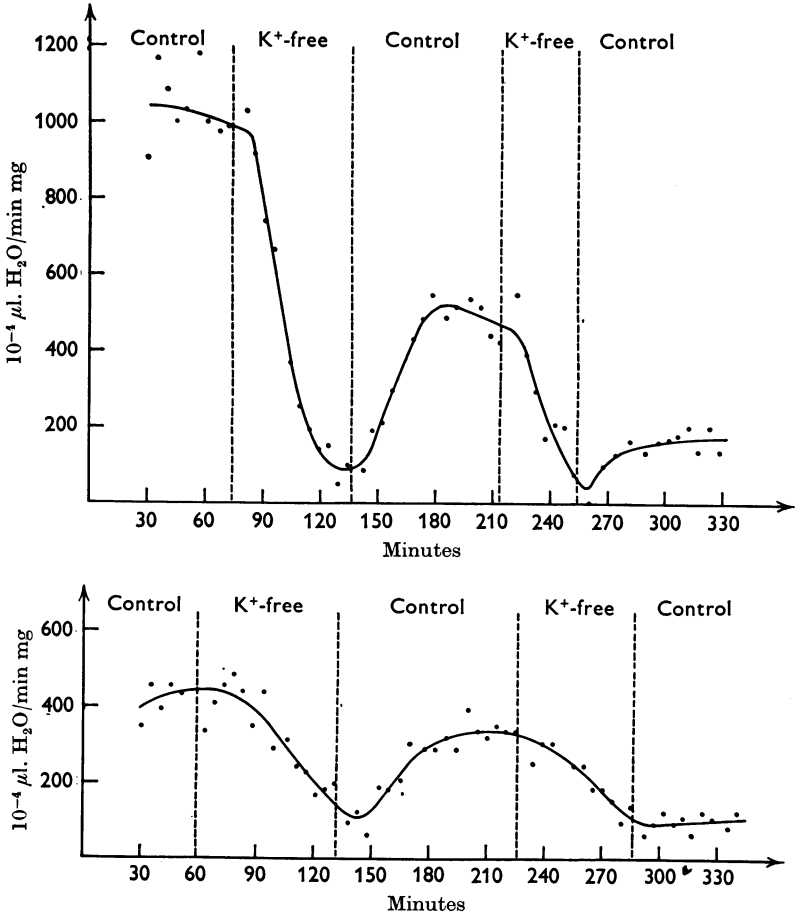

Fig. 8. Effect of K removal from the incubation media on the rate of fluid transport by two gall-bladders prepared and incubated in '110 R' solutions.

V. *Effect of angiotensin*

In order to investigate to some further extent the similarity in transport characteristics between the renal proximal tubule and the gall-bladder, the effect of angiotensin II was tested in a small series of experiments. Angiotensin was shown to inhibit the rate of fluid reabsorption in rat proximal tubules *in vivo* (Leyssac, 1964, 1965). A representative experi-

ment from the present preliminary results obtained in the gall-bladder *in vitro* is shown in Fig. 9. The results seem to indicate that angiotensin in relatively low concentrations has a moderate and reversible inhibitory effect on gall-bladder fluid transport.

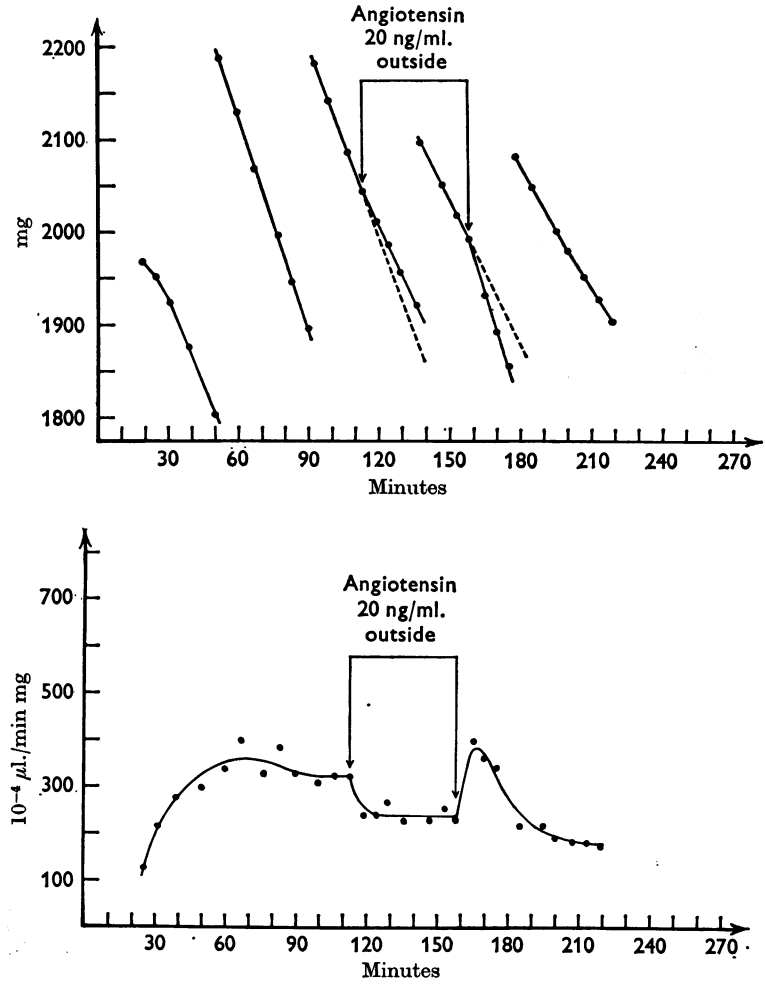

Fig. 9. The effect of angiotensin-II-amide (20 ng/ml.) upon fluid absorption by a rabbit gall-bladder when added to the serosal bathing solution. In the upper graph the ordinate is the weight of the gall-bladder preparation in mg. Before each period (i.e. series of weighings) the bladder lumen was refilled with fresh solution. In the lower graph the ordinate gives the rate of fluid transport ($\times 10^{-4}$ µl. min^{-1} mg^{-1}) in the same experiment.

DISCUSSION

A valid interpretation of the present data must explain, first the difference of the transport pattern observed between '140 R–70 R' replacements and '70 R–140 R' replacements; secondly, the difference between the level of non-transport linked oxygen consumption (i.e. basal oxygen consumption) in '140 R' and '70 R' solutions; and, finally, how the present results may be compatible with apparently conflicting data obtained from studies on the gall-bladder and other similarly functioning epithelial membranes by other investigators.

Previous studies

Summarizing the basic evidence established in earlier studies (Diamond, 1962*a*, *b*, *c*, 1964*a*; Wheeler, 1963; Dietschy, 1964; Whitlock & Wheeler, 1964) it can be stated that the gall-bladder epithelium from the lumen absorbs NaCl and water in isosmotic proportions between identical bathing solutions in the absence of impermeant solute. Net transports of both Na^+ and Cl^- are active, since they depend on metabolic energy and may proceed 'uphill' i.e. against their electrochemical potential gradients even when there is no net water movement; but without external concentration differences there is no net water movement in the absence of active salt transfer. The active transport of Na and accompanying anion is accomplished by an electrically neutral transfer mechanism indicating some sort of coupling between Na ions and anions; furthermore, under steady-state experimental conditions the relationship between absorption rates and varying concentrations of NaCl in the mucosal (luminal) bathing solution apparently indicated saturation kinetics (Diamond, 1962*a*).

Based on these results the problem of net water movement in the direction of solute transport has been analysed. With a given concentration of NaCl in identical Ringer solutions on both sides of the bladder wall the rate of net water movement is proportional to the rate of solute transport over a wide range of absorptive capacities, implying a close interaction between net solute and net water transfer. Of most significance is the evidence indicating that net water transport may occur against a considerable osmotic concentration difference both *in vivo* and *in vitro* (Grim & Smith, 1957; Diamond, 1962*c*; Grim, 1963). Based on the evidence of active salt transport and criteria derived from the irreversible thermodynamics of Kedem & Katchalsky (1958) it has been shown that active solute transport can account quantitatively for passive isosmotic water transport and can also carry water against osmotic gradients (Diamond, 1962*c*). Thus, it would seem that net water transport in the gall-bladder is the passive consequence of a primary active salt transport, as has also been accepted by most investigators.

In recent years attention has been focused on two very similar models proposed to explain isosmotic solute linked water absorption: the three-compartment system (Curran, 1960) and the theory of local osmosis (Diamond, 1964*b*); the latter may be considered a special case of the Curran model, as pointed out by Whitlock & Wheeler (1964), the difference being only the value of the reflexion coefficient for solute (σa) of the first barrier, a. Both of these models assume primary active solute transport into restricted compartments of the epithelium, in which osmotic equilibration may occur. This water flow then would generate a hydrostatic pressure forcing fluid across the second non-selective barrier, b. Attention has been drawn to the lateral intercellular spaces possibly representing such subcellular compartment, the dimensions and geometry of which would be suitable for osmotic equilibration (Diamond & Tormey, 1966; Diamond & Bossert, 1967). In the transporting gall-bladder epithelium these lateral spaces are widely distended, as has also been observed in other absorb-

ing epithelia (quoted from Tormey & Diamond, 1967, and personal unpublished observations). The degree of distension of the lateral spaces was quantitated and found to vary in proportion to the transport rate measured immediately before fixation, strongly suggesting that these spaces are part of the route of transcellular fluid transport (Tormey & Diamond, 1967). In view of these observations, Diamond & Bossert (1967) reformulated the theory of local osmosis in the model of a 'standing-gradient osmotic flow system'.

The most serious difficulties in accepting either of these two theories describing isosmotic fluid transport are, first, the generally accepted main assumption common to both of them, postulating that solute (salt) is pumped across the lateral cell membrane from the cytoplasm into the lateral space, making it hypertonic (cf. Fig. 11). Several pieces of evidence, including those obtained from studies on the renal proximal tubule and the present results, appear incompatible with this assumption. Furthermore, Fisher (1955) showed that when water (and NaCl) was being absorbed from a Ringer solution by the isolated small intestine, urea, creatinine and sorbitol were also absorbed at rates proportional to their concentrations in the luminal medium; in the absence of water transport these substances did not appear in the serosal medium, suggesting a solute-to-water coupling by a mechanical pump-mechanism (a 'solution-pump'); Grim's results (1963) from measurements of electrical potential differences (p.d.) and unidirectional fluxes of water, Na^+, and Cl^- across the wall of the canine gall-bladder *in vivo* gave the best fit to a 'solution-pump'. Secondly, both of these models require a similar, but unexplained, isosmotic water-to-solute coupling in the process of transfer across the luminal membrane into the cell interior, as also emphasized by Diamond & Bossert (1967).

These conflicting interpretations and apparently paradoxical results motivate the outline of a model, which eliminates the main difficulties and is consistent with the available data.

Interpretation of present results

Martin & Diamond (1966), in gall-bladder experiments, observed that the depression of oxygen consumption by ouabain exceeded the fraction linked to fluid transport (in NaCl Ringer solutions corresponding in composition to the present '140 R' solution). This finding suggested that the inhibition of net fluid transport in the gall-bladder caused by ouabain might be the secondary consequence of increased Na concentration intracellularly due to an impaired capacity of maintaining physiological intracellular cation distribution. This hypothesis anticipates that removal of extracellular K should cause a similar depression of the net fluid transport capacity. The present experiments confirm this prediction. A similar reduction in fluid transport rate in response to K removal from the serosal medium was previously observed in studies

on the fish gall-bladder (Diamond, 1962*a*), but this effect could not be reproduced in rabbit gall-bladders transporting in NaCl Ringer solutions containing 140 m-equiv Na^+/l. (Diamond, 1964*a*). However, the quantitative and kinetic similarity between the effects on fluid transport rate of K removal, addition of ouabain, and replacements of '70 R' with '140 R' solutions suggests a common underlying mechanism, namely the detrimental effect of elevated intracellular Na concentration. Exposed to a high extracellular Na concentration (140 m-equiv/l.) Na influx is also high; with a relatively reduced Na extrusion capacity (due to undefined *in vitro* conditions) the cells may not be able to maintain the physiological low intracellular Na concentration but reach a higher steady-state level. Some evidence from *in vitro* preparations of mammalian kidney cortex incubated in '140 R' solutions actually indicates that intracellular Na concentration under this condition is higher than *in vivo* (cf. Leyssac, 1966, p. 44). This interpretation explains why any effect of K removal failed to be established in Diamond's experiments (1964*a*) on bladders already depressed in this way by high extracellular Na concentrations during preparation; further, gall-bladders incubated in solutions with a low Na concentration ('70–110R') can presumably transport at high rates because a lower steady-state level of intracellular Na concentration is more readily maintained than in solutions of high Na concentration. Accordingly, replacement with '140 R' solutions was followed by a gradual and slow decline in transport rate to a fixed minimum level independent of the initial transfer capacity as a consequence of the gradual transition to a new and higher steady-state concentration of the intracellular Na pool determined by the extracellular concentration. The large individual variability in transport rates observed in gall-bladders incubated in identical bathing solutions might be explained by individual quantitative differences in maintaining such low intracellular Na concentrations; and the slow decline in fluid transport rate during the course of an experiment may likely be due to exhaustion of the Na extrusion capacity. The factor(s) responsible for this individual difference seen under *in vitro* conditions are unknown, but the gentleness of handling the preparation might be such a factor.

Further, one would expect that functional damage caused by the high (toxic) Na concentration increases with the duration of the exposure and finally may become irreversible. This is consistent with the present observations and will explain why gall-bladders prepared and initially incubated in '140 R' solutions were unable to respond with increasing fluid transport rate to replacements by solutions of low NaCl concentrations (cf. Fig. 2). Finally, in the steady state the rate of active Na extrusion (efflux) from the cytoplasm of cells incubated in solutions with 140 m-equiv Na^+/l. must be higher than that of cells bathed in media with 70

m-equiv Na^+/l., because the passive influx must be higher as long as the increase in intracellular Na concentration is less than 70 m-equiv/l. Consequently the fraction of over-all oxygen consumption which is unrelated to the mechanism of transcellular transfer (i.e. the basal oxygen consumption) should be greater in bladders incubated in '140 R' than in those incubated in '70 R' solutions. The present observations further

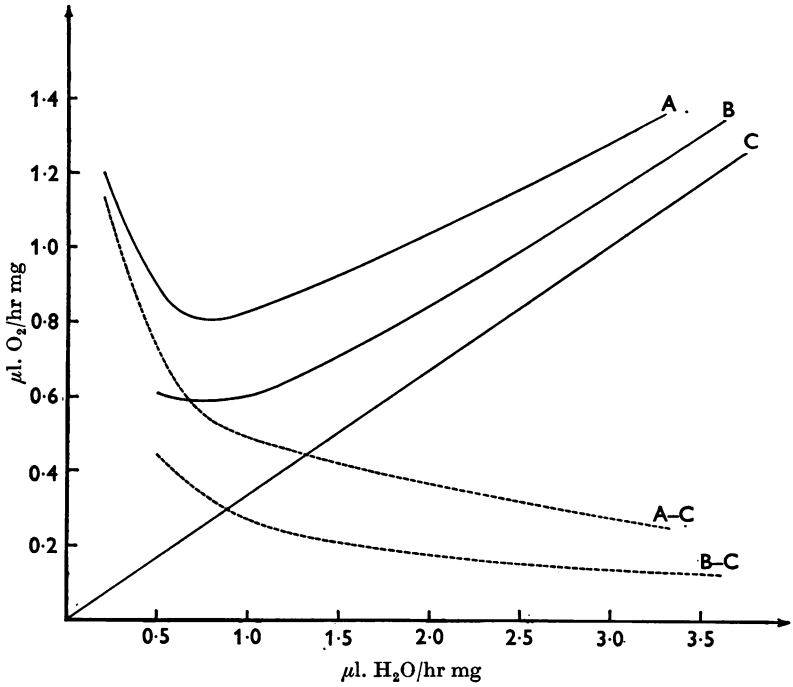

Fig. 10. Oxygen consumption (ordinate) related to rate of fluid transport (abscissa) by gall-bladder *in vitro* preparations incubated in '140 R' (curve *A*) and '70 R' solutions (curve B), taken from Figs. 5 and 6, respectively. C indicates the relationship between transport-linked (suprabasal) oxygen consumption and the rate of fluid transport. The interrupted curves (A–C) and (B–C) indicate the calculated basal oxygen consumption related to transport rate in '140 R' and '70 R' solutions, respectively. See text for further explanation.

support this interpretation (Fig. 10, curves A–C and B–C). It is also seen (from Fig. 10) that the curves (A and B), drawn to the best fit with the data, asymptotically approach lines parallel with the line C, representing the theory postulating a direct proportionality between volume transfer and oxygen consumption. Consequently the calculated basal consumptions (A–C) and (B–C) in '140 R' and '70 R' solutions, respectively, decline towards parallel baseline levels. According to the interpretation proposed above, this decline indicates decreasing passive Na influx, i.e. decreasing

permeability and, further, that this is associated with increasing capacity of the transcellular transfer process.

The present data on oxygen consumption disagree in certain respects with those reported by Martin & Diamond (1966), probably because of the difference in experimental technique. Martin & Diamond measured O_2 consumption in everted gall-bladders (mucosal side facing outwards), while fluid transport rates, to be compared with O_2 consumption, were measured on bladders with the *in vivo* orientation of the wall. In the present experiments fluid transport and oxygen consumption were measured simultaneously on the *in vivo* oriented bladders. Average oxygen consumption with '140 R' solution as the mucosal bathing medium was about two times higher in the everted bladders reported by Martin & Diamond (1966) than in the present experiments; but in two non-everted gall-bladders they found oxygen consumptions about 30 % and 40 % lower than in the same everted bladders, in reasonable agreement with the present data. The reason for the difference in oxygen consumption between non-everted and everted bladders is unknown; Martin & Diamond assumed that the longer diffusion path for oxygen to the transporting cells (about 300 μ) in the natural orientation than after eversion becomes rate-limiting. However, this assumption implies that their measurements of corresponding transport rates were carried out under anaerobic conditions; further, this assumption appears less valid for several reasons. First, a distance of 300 μ is on the border line for maintaining sufficient oxygen tension at the most distant cells only in oxygen consuming tissues such as liver and kidney (Umbreit, Burris & Stauffer, 1957), but it is probably insignificant when this layer is connective tissue, as in the gall-bladder, with a very low O_2 consumption. Secondly if O_2 diffusion were rate limiting the recorded O_2 tension would yield a curved line with time in contrast to straight lines actually obtained in our experiments; and, thirdly, the slope of the line relating O_2 consumption to transport rate should have been less steep in the *in vivo* oriented (rate-limited) bladders than in everted bladders. It appears, however, that this slope actually is more steep in the *in vivo* oriented than in the everted bladders, as seen when Diamond's results are compared with ours. Martin & Diamond (1966) calculated the oxygen consumed per mole of NaCl transported from the changes in O_2 consumption (everted) and transport rate (non-everted) observed in bladders alternately bathed in NaCl (140 m-equiv Na^+/l.) and Na_2SO_4. They calculated 24·6 moles NaCl to be transported per mole O_2 consumed. From the present data (cf. Fig. 7) a $NaCl/O_2$ ratio of 13·5 is calculated; since Na^+ as well as Cl^- are actively transported across the gall-bladder epithelium, 26–28 ions are actively transferred per mole O_2 consumed in bladders transporting in solutions containing 140 m-equiv Na^+/l. However, the difference in oxygen con-

sumption between everted and non-everted bladders raises the question whether or not it is justified to relate data obtained in the everted to data obtained in the *in vivo* oriented state. In view of the present results it remains possible that fluid transport rate as well as oxygen consumption were proportionally increased in the everted bladders.

The main conclusion drawn from the present results is that the range of absorption rates of isotonic fluid from the gall-bladder lumen represents a range of transfer capacities for fluid volume units, thus confirming previous suggestions inferred from studies on the proximal tubular reabsorption of the kidney. However, this conclusion apparently is inconsistent with previous interpretations of results reported in studies on the gall-bladder *in vitro* by several investigators.

A model

In an attempt to encompass the established phenomena within a unifying theory it may be postulated that, as a primary event, Na^+ and Cl^- (and/or any monovalent anions) are bound to coupled sites in localized areas of the luminal membrane in continuity with a helical filamentous substructure. One may imagine some sites specific for Na^+ and anion while other sites may be less specific to be occupied by other transported solutes according to their affinity. The saturation of sites induced somehow the formation of an isolated fluid compartment isosmotic with the luminal solution. Any detailed description of the mechanism by which such a volume unit may be formed cannot be given with our present knowledge; but tentatively one might suggest either a process involving invagination of this membrane area due to contraction of the helical substructure, i.e. a 'micropinocytosis' with dimensions of the 'channel' of the order of molecular sieves; or conformational changes of the helical protein structure may induce within the substructure ion movement and accumulation accompanied by water without involving surface membrane flow or invagination. The elementary unit volume thus formed is assumed then to be mechanically propulsed (peristalsis) through the cell along such a continuous substructural system ending in the lateral cell membrane to be pumped into the lateral intercellular space (cf. Fig. 11). The proposed model may be termed a 'mechanical volume pump', not to be confused with a 'solution pump'. The rate of transfer of solutes and solute-linked water, determined by the capacity for transferring unit volumes, thus, is independent of the osmotic concentration of the enclosed fluid but may depend upon the maintenance of the intracellular (cytoplasmic) cation distribution, as suggested by the present data. The structural outline of such a model has been presented by Sanborn (Sanborn, Szeberenyi, Messier & Bois, 1965; Sanborn, 1966) based on electron-microscopic evidence of continuous filamentous interconnexions between, and sub-

structure of, organelles such as microtubules, dense microtubules and microvesicles. The apical cytoplasm of absorbing cells is rich in these organelles; furthermore such a fibrillar, possibly microtubular structure constitutes the central core of microvilli in the proximal tubule cell (Hanssen & Herman, 1962) and intestinal epithelium (Laguens & Briones, 1965) extending to the level of the terminal web.

The kinetics of the present model will be similar to pinocytosis, but it differs from pinocytosis (or a 'solution pump', i.e. inclusion and bulk transfer of a droplet of luminal solution as such) by accounting for any

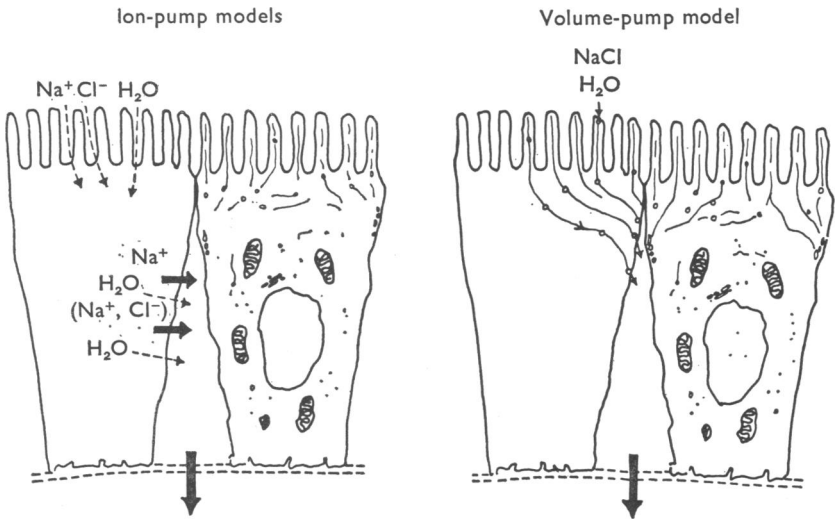

Fig. 11. Representation of main features in ion-pump models (left) and the present volume-pump model (right). The arrows at the basement membranes indicate water- and solute transport due to hydrostatic pressure.

specificity of transported solutes depending on the affinity characteristics of the luminal membrane sites, thus allowing for great variability between cells of different epithelia. It requires the presence of Na^+ and monovalent anions in the luminal solution. It is not electrogenic and it is compatible with net transfer of Na and Cl against their electrochemical gradients, even when net water movement is in the opposite direction in the presence of an osmotic concentration difference between the external bathing solutions, if passive water movement occurs in pathways ('channels') distinct from those for volume transport. Evidence of an osmotic permeability of the gall-bladder epithelium much too small to permit osmotic equilibration of the transported fluid (Diamond, 1964*b*) and the failure of solute-linked water transport to produce a streaming potential with identical bathing solutions on both sides while such an electrokinetic

potential is associated with osmotic water flow (Pidot & Diamond, 1964) indicated distinct pathways for water transport driven by external forces and that coupled to solute transfer. A similar conclusion was drawn by Clarkson (1967) from experimental observations on small intestine analysed according to the equations of irreversible thermodynamics as expressed by the 'friction model' of Kedem & Katchalsky (1961).

The mechanism of isosmotic water movement proposed in the present theory appears essentially consistent with previous concepts and results. Water movement is passive; formally it is analogous with 'local osmosis' only the location of the process is different: in the present model located at the luminal membrane whereas previous models suggested a location at the lateral cell membrane and intercellular space.

The theory accounts for the appearance of 'saturation kinetics' under steady-state conditions and postulates that the rate-limiting step in the process of transcellular solute transport is located at the luminal membrane, in accordance with results obtained in other epithelia, in which saturation kinetics has been demonstrated (cf. e.g. Frazier, Dempsey & Leaf, 1962).

The delay of ionic equilibration observed in efflux transients following addition of $^{24}Na^+$ and $^{82}Br^-$ to the luminal solution, most likely representing equilibration of the cytoplasmic pool specific activity, has been taken as an argument favouring the theory postulating that actively transported Na is taken from the intracellular pool (Diamond, 1962*b*). However, the delay in ionic transients does not necessarily invalidate the present concept, since it remains quite possible that this delay is due to equilibration by exchange between the cytoplasmic pool and the transported fluid compartment in the subcellular organelles and/or the lateral intercellular space.

The behaviour of K seems explicable in terms of the present model. It was observed by Diamond (1964*b*) that in the absence of impermeant solutes $[K^+]_{absorbate}/[K^+]_{lumen}$ increased with increasing fluid transport rate towards a value of about 0·8–0·9 at a transport rate of about 40 μl./hr cm^2; in the range between 40 and 130 μl./hr cm^2 the ratio remained unchanged. The fluid transport rates were varied by increasing the NaCl concentration (osmolarity) of the bathing media going from hypo- to hypertonic solutions. Although the passive permeability of the gall-bladder epithelium for K is of the same size as the value required to explain the observed K movement at a transport rate of 56 μl./hr cm^2, a permeability two times greater than the actual value would be required to explain the transport at fluid transport rates of 130 μl./hr cm^2. According to the present concept the K concentration ratio observed at high and intermediate fluid transfer rates (in bathing solutions of 100–300 m-osmole/l.) may represent the ratio deter-

mined by the normal occupation of sites with preferential affinity for K. At the low rates of transport (i.e. high concentrations of Na) competition between K and Na for these sites may become significant thereby lowering the K concentration of the absorbate. The finding by Diamond (1964*b*) that $[K^+]_{absorbate}$ may exceed that of the luminal solution in the presence of e.g. sucrose in the lumen may be explained, as suggested by Diamond (1964*b*) by addition of K to the absorbate by passive movement due to the diffusion potential difference (lumen positive) measured under this condition.

It would seem that the theory proposed above is capable of unifying the main results of the present as well as previous investigations including those hitherto considered controversial or inconsistent with current concepts. In this model the difficulties in explaining the apparently rate-limiting, 'carrier-mediated' transfer step across the luminal membrane as well as the high efficiency of the energy requiring process has been overcome, and the model is consistent with morphological evidence.

It remains unknown whether or not the level of intracellular Na concentration (or cation distribution) plays any part in physiological adjustments of net fluid absorption rate; the present results suggest that it may do so under *in vitro* conditions. Hormonal factors may more likely be of significance *in vivo*. Oxytocin applied to the serosal side in high concentrations (300–400 m-u./ml.) inhibited the transport completely and irreversibly in the fish gall-bladder (Diamond, 1962*a*). The present preliminary results obtained in the rabbit gall-bladder seem to indicate that angiotensin in relatively low concentrations has a moderate but reversible inhibitory effect. Experiments are being designed to examine this effect in greater details. It is possible, though, that intracellular cation distribution determines the gross level of absorptive capacity and that hormonal effects may be superimposed serving as a more rapid physiological regulating system.

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