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Temperature-Dependent Changes in Fluid Transport across Goldfish Gallbladder

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Summary. The temperature dependence of fluid transport across *in vitro* preparations of goldfish gallbladder was measured using a gravimetric technique. Fluid transport showed a direct dependence on incubation temperature when the adaptation temperature was kept constant. For constant incubation temperature, transport fell as the adaptation temperature rose. The width of intercellular channels varied with incubation and adaptation temperature as expected if fluid were to cross the tissue by this route. The structure of the gallbladder was otherwise unaffected by changes of temperature. Intracellular concentrations of Na, K and Cl also depended on the environmental temperature of the fish. The levels of Na and Cl increased and the level of K decreased, at constant incubation temperature, as the adaptation temperature rose from 8 to 30 °C. These changes took two to three weeks to become apparent while fluid transport regulated within 20 hours of raising the environmental temperature. The osmotic permeability of the gallbladder remained independent of both incubation and adaptation temperature.

The outcome of adaptation was to maintain constant both the ionic composition of the epithelium and the rate at which it could transport fluid, when these parameters were measured at incubation temperatures equal to the previous environmental temperature of the fish. The significance of these findings is discussed and a mechanism for regulation postulated which involves an initial regulation of salt entry into the mucosa followed by long term changes in the pumping ability of newly synthesized cells.

The mechanism whereby water is removed from bile held within the gallbladder is now understood in some detail, thanks largely to the work of Diamond and his co-workers (*see* the review of Diamond, 1968). The active extrusion of salt into intercellular channels creates a standing osmotic gradient which then provides the driving force for fluid movement (Kaye, Wheeler, Whitlock & Lane, 1966; Diamond & Bossert, 1967; Tormey & Diamond, 1967). Such a mechanism for fluid transport might be expected to be particularly susceptible to temperature and, indeed, changes in

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temperature have already been used successfully to provide morphological evidence of the temperature dependence of fluid transport, the extent to which intercellular channels remain open being shown to depend on the temperature at which gallbladders are incubated (Tormey & Diamond, 1967; Smulders, Tormey & Wright, 1972). This effect of temperature on transport has in most cases little physiological significance, since the temperature of the animal remains constant. This is not true for the goldfish however, where the body temperature can be made to vary from 0 to 40 °C. Changing the environmental temperature of goldfish is already known to produce both short and long term effects on the way the intestinal mucosa transports salts and nonelectrolytes (Smith & Ellory, 1971; Smith, 1972). The main object of the present work was therefore to test whether the gallbladder epithelium, with its many structural similarities to the intestinal mucosa and with a common embryological origin, might also show adaptational changes in the transport of fluid and salt. The gallbladder is a better model than the small intestine in this respect, the metabolism and transport of nonelectrolytes by the intestine affecting fluid movement in a separate and not always predictable fashion (Munck, 1972).

Fluid appearing at the serosal surface of transporting epithelia can be isotonic or hypertonic depending on the geometry and osmotic permeability of the tissue in question. The osmotic permeability of gallbladder is high compared with other epithelia so that the transported fluid reaches osmotic equilibrium before it leaves the intercellular channels. Gross stimulation of fluid transport however, achieved by varying both the environmental temperature of the fish and the temperature at which gallbladders are incubated, could result in this tissue also secreting a hypertonic solution of sodium chloride. If this did occur it would give valuable information concerning the limiting factors controlling secretion generally. A part of the present work was therefore designed to test for such a possibility.

Materials and Methods

Fish

Goldfish (*Carassius auratus*) weighing about 100 g were obtained from Italy *via* Robinsons Fisheries Ltd., London. They were kept for two days in aerated water at room temperature in a large aquarium before being transferred to smaller tanks maintained at one of a series of constant temperatures for at least three weeks before use. During this time they were fed daily with Duffields Anglers groundbait (Buxton Distributors Ltd., Norwich, U.K.). All experiments were completed within the period July to February. Groups of experiments were randomized to minimize any seasonal variation.

Electron-microscopy

Fish were anesthetized in 0.1% w/v MS 222, (Sandoz Ltd., Basle) and fixative was injected into the gallbladder *in situ*. The fixatives used were either 4% glutaraldehyde (Koch-Light, Colnbrook, U.K.) in 0.1 M NaH_2/Na_2HPO_4 buffer, pH 7.0 or 1% osmium textroxide in 0.1 M veronal/acetate buffer, pH 7.0. After the content of the gallbladder had been thoroughly washed out by the fixative, the bladder was removed from the fish, cut up and left in the fixative for 1 to 2 hr. Gallbladders were also fixed after incubation for 2 hr by injecting fixative into the bladder lumen and dropping the bladder into fixative. The fixatives were the glutaraldehyde solution detailed above or a mixture of the glutaraldehyde and osmium fixatives at 4 °C mixed immediately before use and used for 1 to 24 hr at 4 °C. All other processing and fixations were at room temperature. Where necessary, postfixation was with the above osmium solution followed by 2% uranylacetate in aqueous solution, both for 1 hr. The tissue was dehydrated in alcohol, embedded in Araldite (Ciba, Duxford, U.K.). Sections were stained with uranylacetate and/or lead citrate and examined in an AEI EM 6B at 60 kV.

All three fixatives glutaraldehyde or osmium or a mixture of both produce equivalent results with respect to preservation of the width of the intercellular spaces. Glutaraldehyde alone preserved the cellular fine structure of endoplasmic reticulum and golgi apparatus far better than the osmium-based fixatives. The simplification of ground cytoplasm produced by the latter fixatives made simpler the visualization of the complex foldings of the plasmalemma.

Measurement of Fluid Transport

Gallbladders removed from fish killed by decapitation were opened and washed at room temperature in medium containing (in mM): NaCl 138, KCl 5.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, buffered with 5 mM-Tris to pH 7.4. The sodium concentration in this medium closely resembled that found in the blood of goldfish kept in fresh water (Lahlou, Henderson & Sawyer, 1969). Cleaned gallbladders were cannulated and filled with 50 to 100 μliters of physiological saline and then incubated in identical medium gassed with O_2. A steady-state rate of fluid transport became established during the first hour of incubation. The gallbladder was removed and weighed at the end of this equilibration period and incubation then continued for a further hour. The gallbladder was reweighed, and the wet weight of the tissue determined separately on a Stanton balance to an accuracy of ± 0.1 mg. Fluid transport, calculated from the weight lost during this second incubation, was expressed per mg wet weight of tissue.

Weight loss during a 60-min incubation period could vary from 1 to 20 mg depending on the conditions of the experiment. To check the accuracy of these measurements, ^{3}H-inulin, 8 μC/ml, was sometimes incorporated into the fluid bathing the mucosa at the start of an experiment and samples were taken for counting 60 and 120 min later. Fluid transport measured by weight difference was found to equal that calculated assuming inulin to be an impermeant molecule in this tissue (0.57 ± 0.07 and 0.52 ± 0.06 μliter mg^{-1} hr^{-1}, respectively; 13 comparisons, means $\pm$ SE).

Variation of transport rate with time was measured in separate experiments using an initial 30-min equilibration period followed by several 30-min periods of measurement. In these cases the amount of fluid transported per unit time was calculated by weight difference only.

Measurement of Osmotic Permeability

To determine osmotic permeabilities, gallbladders were first equilibrated in medium for 30 min and the control rate of transport then measured over the following 30-min

period. Bladders were next transferred to fresh medium containing an additional 50 mM of sucrose, the original saline being left in contact with the mucosal surface throughout the experiment, and a new rate of fluid transport determined 30 and 60 min later. The gallbladders were then replaced in the original medium and the final control rates of transport measured after a further 30- and 60-min incubation. When the final rate was less than the initial rate of transport, the control corresponding to the time when osmotic flow was being measured was calculated graphically. Only the second period of osmotic flow measurement was used to calculate osmotic permeabilities.

Measurement of Intracellular Ion Concentrations

Gallbladders were incubated with medium in contact with both mucosal and serosal surfaces for a period of 2 hr. ^{3}H-inulin (8 μC/ml) was present in the mucosal fluid and ^{14}C-sucrose (1 μC/ml) was added to the fluid bathing the serosa at the start of incubation. Samples of mucosal and serosal fluid were taken at the end of incubation to determine the specific activities of the radioactive markers. The gallbladder was cut open, the mucosa blotted lightly and separated from the underlying tissues with the aid of a coverslip cleaned previously in concentrated H_2SO_4. The wet weight of mucosa was recorded immediately and 2 ml of distilled water added to liberate cell contents. This suspension was frozen and thawed, boiled for 5 min and then assayed for Na and K on a flame photometer (Evans Electroselenium Ltd.). Chloride was determined using the diphenyl carbazone method of Schales & Schales (1941) modified to allow the detection of small amounts of chloride. Microliter quantities of $Hg(NO_3)_2$ were added to the test chloride solution using a micrometer driven syringe, optical densities being recorded at 540 nm on a Unicam SP 500 spectrophotometer. The amount of chloride determined by this method varied from 10 to 200 nmoles.

Separate samples of extracted tissue were taken to determine ^{3}H and ^{14}C in a Tricarb scintillation spectrometer. These counts were then used to correct total ion content for serosal extracellular space (^{14}C-sucrose) and residual fluid contaminating the mucosal surface (^{3}H-inulin). The dry weight of the mucosa was determined in each case and the amount of each ion divided by cell water content to give its final intracellular concentration.

Materials

Inulin – ^{3}H, 300 mC/mmole, and sucrose – ^{14}C(U), 600 mC/mmole, were obtained from The Radiochemical Centre, Amersham. Reagents for the determination of chloride were purchased from The Sigma Chemical Company, St. Louis, Missouri. All other reagents were of analytical grade.

Results

Fine Structure of the Goldfish Gallbladder

Gallbladders taken from fish adapted to cold or hot conditions after fixation *in situ* were used for this study. In cold-adapted fish there were no intercellular spaces visible between the cells. In hot-adapted fish the usual finding was closed intercellular spaces but occasionally in restricted areas some basal separation of the cells was to be seen. The cytoplasmic fine structure is equivalent in both cold- and warm-adapted gallbladders. The

dimensions and shape of the cells are in the same range as those found for mammalian gallbladder (Tormey & Diamond, 1967; Hayward, 1968). Microvilli are present at the luminal surface and the mitochondria are collected in a subapical zone as in mammals. The features which seem to be peculiar to goldfish gallbladder are the cytoplasmic bodies which are present and the architecture of the lateral plasmalemma.

Cytoplasmic bodies ranging in size from 0.3 to 3 μ are found immediately above or below the nucleus (Fig. 1*a*, *b*). They consist of single rows of 200 to 300 Å particles lying between flat or concentric membranes which are continuous with cisternae of the rough endoplasmic reticulum (Fig. 1*c*, *d*). The particles are extracted by aqueous 2% uranylacetate used as a block stain (Fig. 1*c*). If the uranylacetate soak is omitted the particles stain with the lead grid stain more densely than the surrounding membranes and cytoplasm (Fig. 1*d*).

The intercellular space is a straight-sided channel 300 to 400 Å wide down past the mitochondrial zone (Fig. 1*a*). The channel then becomes very complex, with both simple microvilliform foldings and blind ending invaginations of the plasmalemma forming fenestrated sheets within the cytoplasm of the individual epithelial cells (Fig. 2*a*, *b*). These blind invaginations are usually more uniform in width than the intercellular space proper although sometimes it is not possible to distinguish the two (Fig. 1*a*, *b*).

The plasmalemma invaginations can still be seen when the intercellular spaces are widely swollen by incubation *in vitro* (*see below*). Successive sections show that they form flat fenestrated sheets in the cytoplasm, each connected to the plasmalemma at several points (Fig. 2*c*, *d*, *e*).

Fluid Transport by the Goldfish Gallbladder

The dependence of fluid transport on both the temperature of incubation and on the previous environmental temperature of the fish is shown in Table 1. The transport of fluid by gallbladders taken from fish adapted to 15 °C measured at 15 °C increased threefold on raising the incubation temperature to 30 °C (0.23 to 0.79 μliter mg^{-1} hr^{-1}, respectively). This immediate effect of temperature on transport was maintained throughout the three periods of measurement, but transport at the relatively high temperature of 30 °C decreased somewhat with time using gallbladders taken from 15 °C-adapted fish, so that by the end of 90 min the final rate of transport was only some 50% higher than that found for the corresponding period at 15 °C. Adapting fish to 30 °C for three weeks reduced the transport of fluid measured subsequently, the rate now being only half that found for the cold-adapted gallbladder incubated at 30 °C. The transport of fluid by

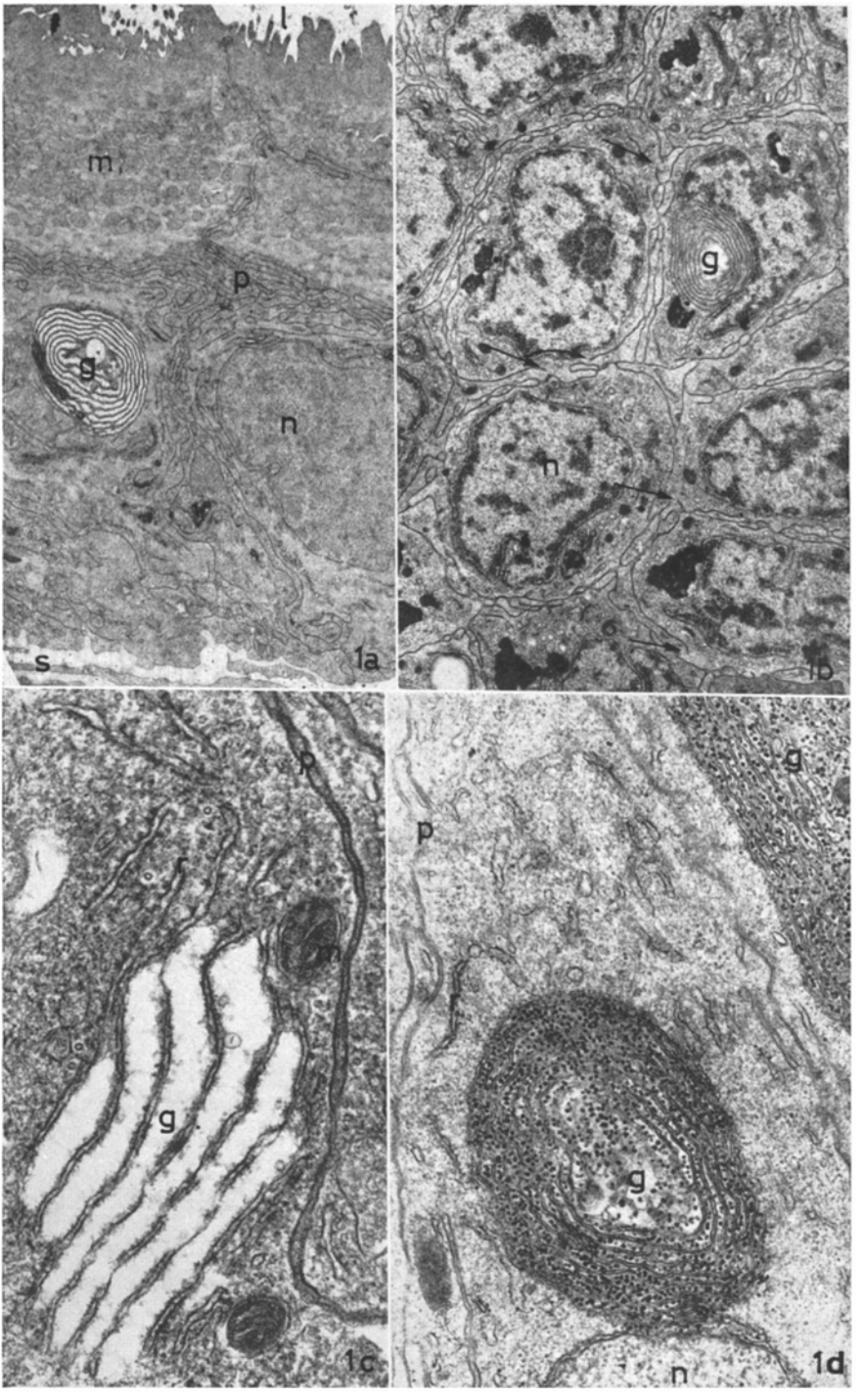

Fig. 1

Table 1. Temperature dependence of fluid transport by goldfish gallbladder

Temperature (°C)		Fluid transport (μliter mg^{-1} hr^{-1})		
Adaptation	Incubation	I	II	III
15	15	0.23 ± 0.06 (4)	0.29 ± 0.09 (6)	0.27 ± 0.08 (3)
15	30	0.79 ± 0.14 (8)	0.64 ± 0.16 (3)	0.44 ± 0.14 (3)
30	30	0.38 ± 0.06 (3)	0.26 ± 0.08 (4)	0.37 ± 0.13 (4)

Gallbladders taken from fish adapted to 15 or 30 °C were incubated at 15 or 30 °C and fluid transport determined by weight difference. Periods I, II and III refer to consecutive 30-min periods of incubation following an initial equilibration period of 30 min. Values give means ± SE (No. of observations).

gallbladders taken from 30 °C-adapted fish incubated at 15 °C was too small to be measured.

Further experiments were carried out to study these changes in detail. Five groups of fish adapted to 8, 15, 20, 25 and 30 °C were used at three different incubation temperatures. Results are shown in Fig. 3. The transport of fluid at 30 °C depended on the previous adaptation temperature of the fish, falling from 0.823 to 0.370 μliter mg^{-1} hr^{-1} as the adaptation temperature increased from 8 to 30 °C. The difference between these two rates of transport was highly significant ($p < 0.001$). Results obtained using gallbladders taken from fish adapted to temperatures between 8 and 30 °C incubated at 30 °C lay between these two extremes, the regression analysis giving a straight line with a correlation coefficient of 0.67 (DF 42, $p < 0.001$) and a slope of -0.021 ± 0.004 μliter mg^{-1} hr^{-1} $°C^{-1}$.

The transport of fluid by gallbladders taken from fish adapted to 8 °C and incubated at 8 °C was not different from that of gallbladders taken from fish adapted to 30 °C and incubated at 30 °C ($p > 0.2$). The immediate

Fig. 1. Electron-micrographs of gallbladder epithelium taken from 30 °C-adapted goldfish and fixed *in situ* to show different aspects of cytoplasmic bodies and invaginated plasmalemma. The following abbreviations have been used on this and other figures describing gallbladder structure: *g*, glycogen body; *l*, lumen of gallbladder; *m*, mitochondrion; *n*, nucleus; *r*, rough endoplasmic reticulum; *s*, serosal side of gallbladder epithelium; *p*, plasmalemma.

(*a*) Oblique section through epithelium showing the great increase in convolution of the plasmalemma at about nuclear level (×7,500). (*b*) Transverse section at the nuclear level, showing blind ending invaginations of the plasmalemma (arrows) (×4,500). (*c*) Section block stained with uranylacetate and stained on the grid with uranyl acetate and lead to extract glycogen (×47,000). (*d*) Similar section but with no uranylacetate or grid stain. The lead stain contrasts the glycogen particles more than cytoplasmic membranes (×27,000)

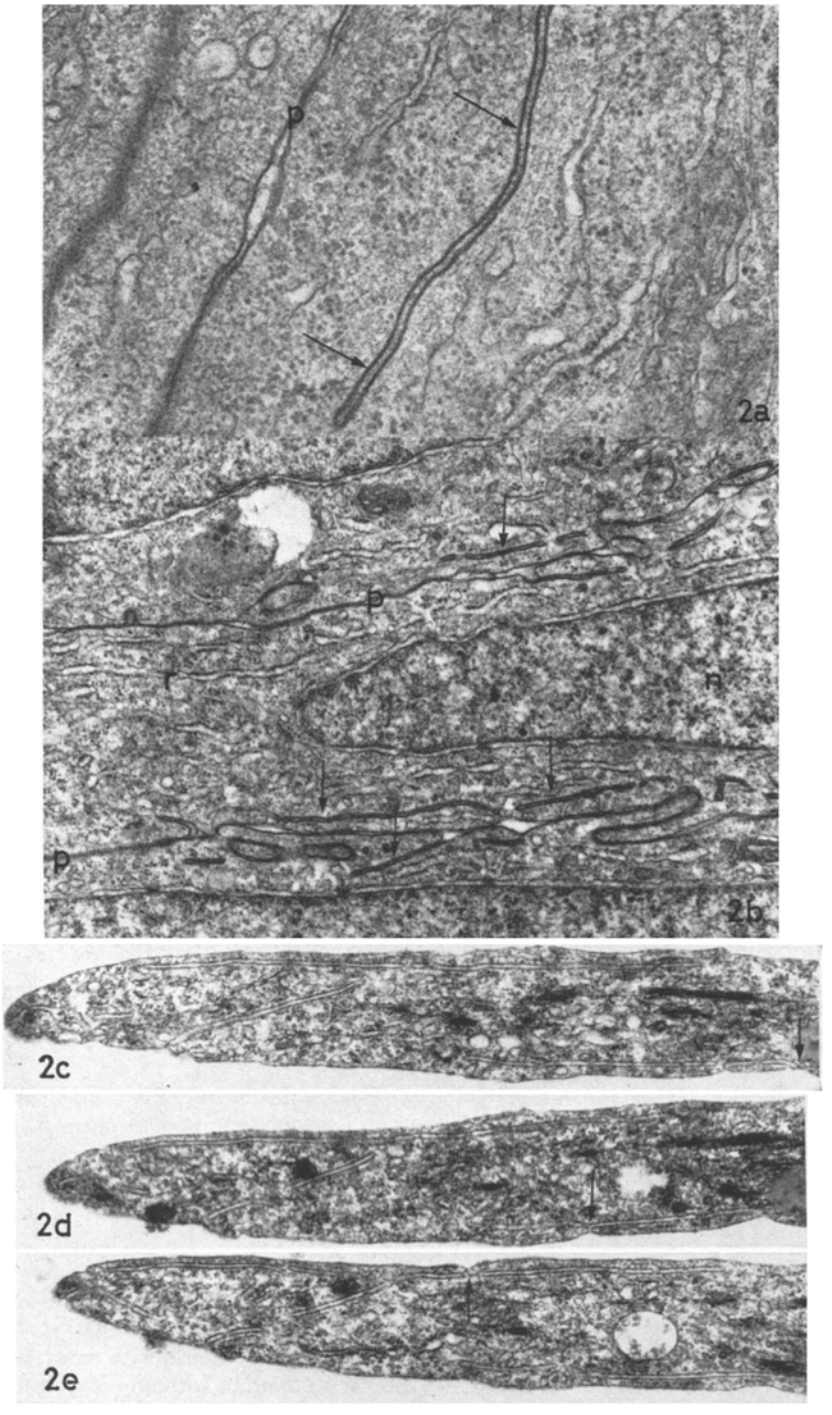

Fig. 2

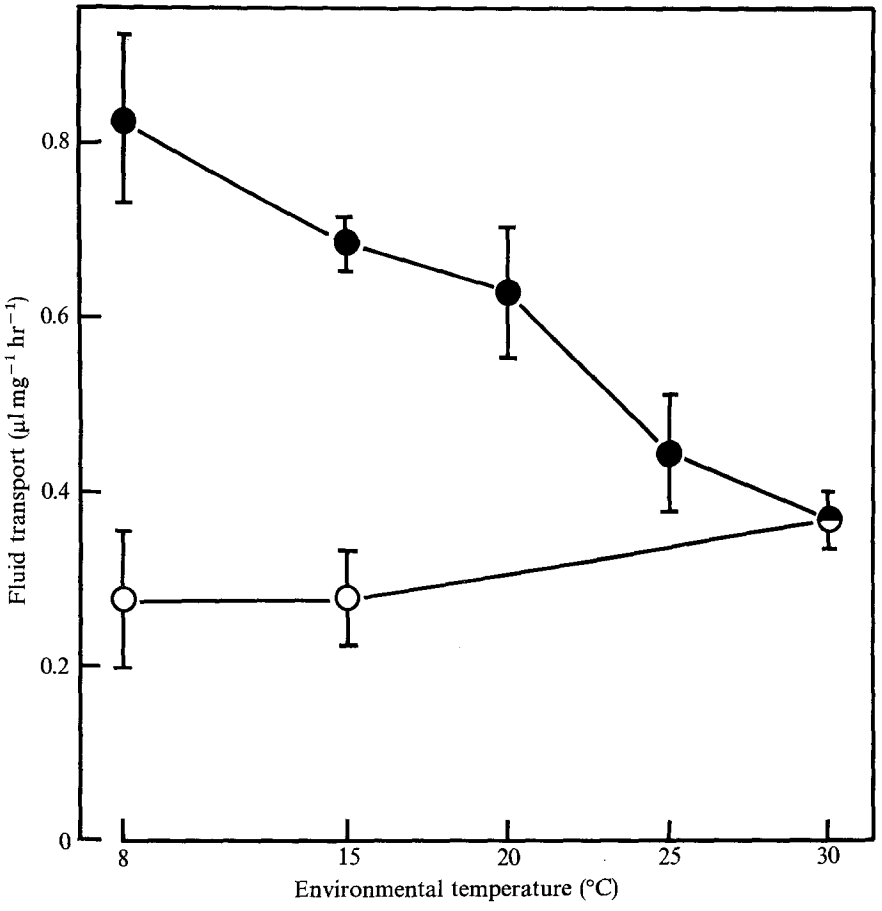

Fig. 3. Fluid transport across goldfish gallbladders. Gallbladders taken from fish adapted to temperatures ranging from 8 to 30 °C were incubated in medium gassed with O_2 for 2 hr, fluid transport being measured during the second hour of incubation. —•—, Transport measured at 30 °C; —○—, transport measured at temperatures corresponding to the previous environmental temperature of the fish. Values give means ± SE of from 6 to 13 determinations

Fig. 2. (*a* and *b*) Electron-micrographs of gallbladder taken from 8 °C-adapted goldfish to show plasmalemma invaginations. Longitudinal sections show the uniformity of the width of the plasmalemma invaginations (arrows) compared with the plasmalemma itself. (*a*) × 46,000; (*b*) × 30,000.

(*c, d and e*) Electron-micrographs of gallbladder taken from 15 °C-adapted goldfish showing successive longitudinal sections through a crest cell in a wide intercellular space. Plasmalemma invaginations can be seen to be fenestrated sheets with occasional connections to the lateral spaces (arrows); all × 15,000

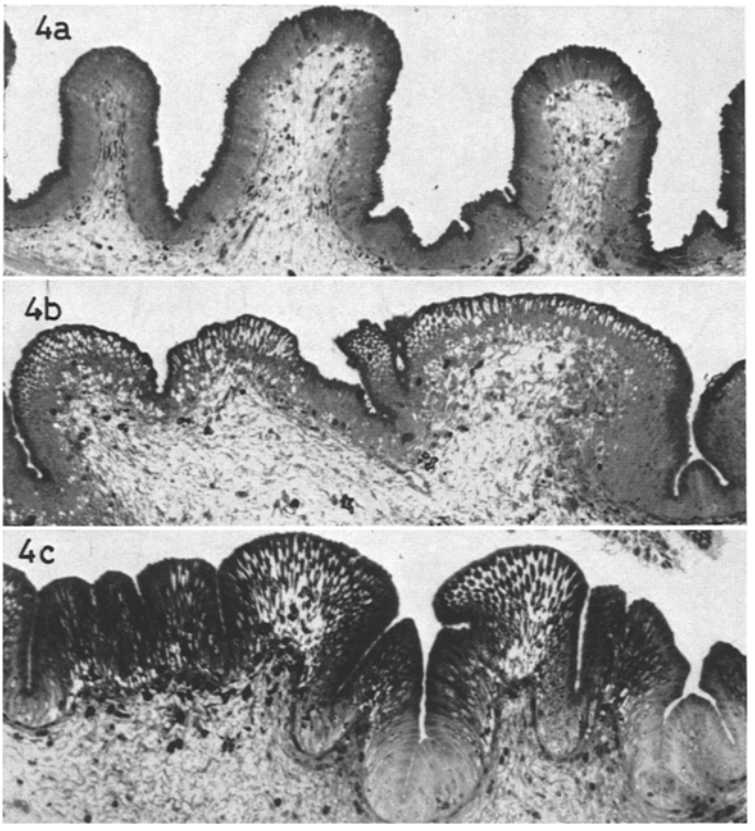

Fig. 4. Light micrographs of goldfish gallbladder epithelium (araldite sections stained with toluidine blue). (*a*) 30 °C-adapted gallbladder incubated at 4 °C. No spaces are visible between the cells in the troughs or at the crests of the folds. (*b*) 30 °C-adapted gallbladder incubated at 30 °C. Small spaces present between the cells except in the troughs of the epithelium. (*c*) 15 °C-adapted gallbladder incubated at 30 °C. Spaces between the cells at the crests appear wider than in (*b*); (all × 140)

increase in transport, seen when gallbladders taken from fish adapted to 8 °C were incubated at 30 °C (0.276 to 0.823 µliter mg^{-1} hr^{-1}), was abolished completely by adaptation.

Gallbladder Morphology after in vitro Incubation

Incubation does not grossly affect the fine structure of the goldfish gallbladder. Fish adapted to 8, 15 or 30 °C have equivalent cytoplasmic organelles whether incubated at 4, 8 or 30 °C. A considerable difference is seen however in the width of the intercellular spaces. Gallbladders incubated at 4 °C taken from fish adapted to 30 °C always have very narrow intercellular spaces, never more than 400 to 600 Å wide (Fig. 4*a*). Gallbladders

taken from 15 °C or 30 °C-adapted fish, incubated at 30 °C, show wide intercellular spaces (Fig. 4*b*, *c*). 15 °C-adapted gallbladders incubated at 30 °C have very wide spaces at crests of the folds in the epithelium, with little or no separation at the troughs between the folds, while 30 °C-adapted gallbladders show a smaller cellular separation, but it is more uniform from crest to trough (Fig. 4*b*, *c*). There are also differences between 15 and 30 °C-adapted gallbladders in the way intercellular channels widen during incubation at 30 °C. This can best be seen at higher magnification (Fig. 5*b*, *c*). A view of completely closed intercellular channels in an 8 °C-adapted gallbladder (Fig. 5*a*) is given for comparison. The spaces in the 15 °C-adapted gallbladder are more nearly continuous than those in the 30 °C-adapted gallbladder.

The observation that the degree to which intercellular spaces widened depended on the previous environmental temperature of the fish was verified by measuring the height of the cells and the width of the intercellular channels halfway up the cells, restricting the measurements to the crests of the epithelial folds. The results, with the number of measurements in parentheses, were as follows: 15 °C-adapted gallbladder, cell length 54.7 ± 2.4 μ (25), channel width 2.7 ± 0.1 (38); 30 °C-adapted gallbladder, cell length 52.2 ± 1.4 μ (16), channel width 1.8 ± 0.1 (16); all measurements were made after incubation of the gallbladders at 30 °C.

The invaginations of the plasmalemma can be clearly seen in cells which are widely separated and there are very few cell processes, the cell outline appearing smooth. No cell organelle has been observed to be associated consistently with the invaginations, which seem completely unaffected by the incubation conditions which cause the swelling of the intercellular space.

Distribution of Fluid within the Goldfish Gallbladder Epithelium

Changes in the intramucosal and submucosal spaces of the goldfish gallbladder, which can be seen to depend upon the conditions under which the tissue is incubated and adapted, should be reflected as changes in extracellular space. The results of experiments where extracellular water was measured directly, *in vitro* after a 2-hr incubation in medium containing ^{14}C-sucrose, is shown in Table 2. Extracellular spaces remained constant in different groups of fish provided the gallbladders were incubated at temperatures equal to their previous environment. There was a pronounced increase in extracellular space when the incubation temperature exceeded that of the environment (gallbladders from fish adapted to 8 or 15 °C incubated at 30 °C). The proportion of intracellular water to tissue dry weight remained constant under all conditions.

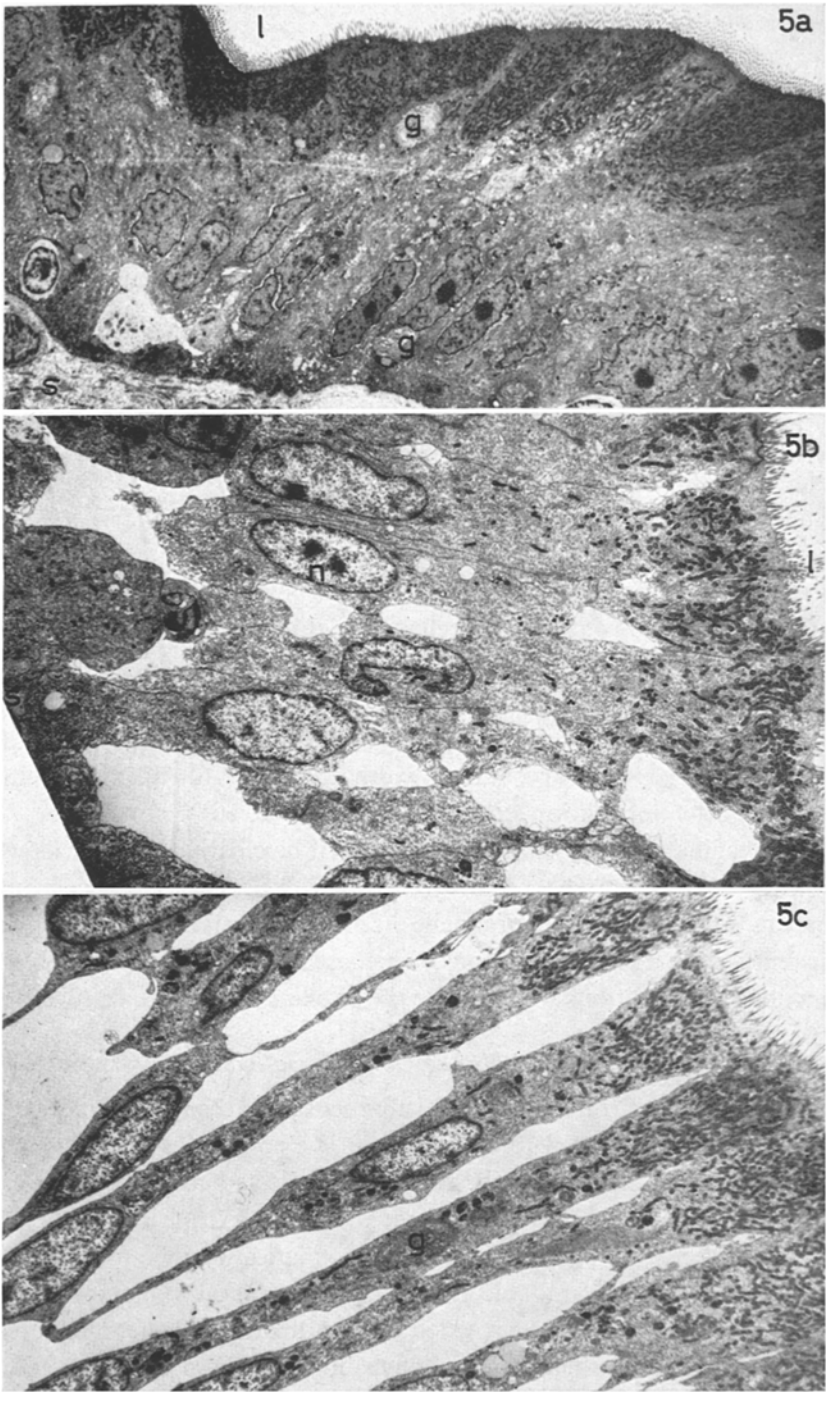

Fig. 5. Electron-micrographs from the crests of gallbladder epithelium. Note the mitochondrial zone and the glycogen bodies. (*a*) 8 °C-adapted gallbladder fixed *in situ* showing no intercellular spaces (× 1,200). (*b*) 30 °C-adapted gallbladder incubated at 30 °C showing irregular separation of the cells (× 3,000). (*c*) 15 °C-adapted gallbladder incubated at 30 °C showing virtually complete cell separation, the sides of the cells being without projections (× 2,500)

Table 2. Tissue water of goldfish gallbladder

Temperature (°C)		Tissue water/dry weight (μliters mg^{-1})	
Adaptation	Incubation	Intracellular	Extracellular
8	8	3.1 ± 0.3 (5)	2.3 ± 0.2 (5)
8	30	2.9 ± 0.3 (6)	4.3 ± 0.5 (6)
30	30	2.6 ± 0.4 (4)	2.8 ± 0.6 (4)
15	30	2.3 ± 0.5 (3)	3.6 ± 1.3 (3)
15	15	2.5 ± 0.3 (3)	2.2 ± 0.2 (3)

Gallbladders taken from fish adapted to 8, 15 or 30 °C were incubated at 8, 15 or 30 °C for a period of 2 hr. Tissue wet and dry weights were determined at the end of incubation. Total tissue water was divided into intracellular water and extracellular water (labeled with ^{14}C-sucrose). Values related to tissue dry weight give the means ± SE of from 3 to 6 determinations.

Transport of Sodium by the Goldfish Gallbladder

Fish gallbladders normally transport an isotonic solution of NaCl so that, as incubation proceeds, these two ions become depleted in comparison with other nontransportable ions present in the luminal fluid (Diamond, 1962). Knowing the amount of fluid transported by goldfish gallbladders, and assuming this fluid to consist of an isotonic solution of NaCl, one can calculate the fall in luminal NaCl concentration produced during incubation and compare this with experimentally determined values. The results of such experiments, where gallbladders were taken from fish adapted to widely different temperatures, are shown in Fig. 6. The Na concentration of luminal fluid, taken after 1 and 2 hr of incubation, was always less than that determined in the original medium. There was, moreover, a close correspondence between the experimentally determined and predicted falls in luminal Na concentration. This was in spite of the fact that gallbladders had been incubated at temperatures ranging from 5 to 22 °C above their previous environmental temperatures. This congruity between fluid and Na transport allows the calculation of Na transport from the measurement of fluid transport at different environmental temperatures.

Intracellular Concentration of Ions

The following experiments were carried out to determine whether changes in fluid transport, induced through alterations of environmental temperature, would be accompanied by changes in the ionic composition of the gallbladder epithelium. Gallbladders taken from fish fully adapted

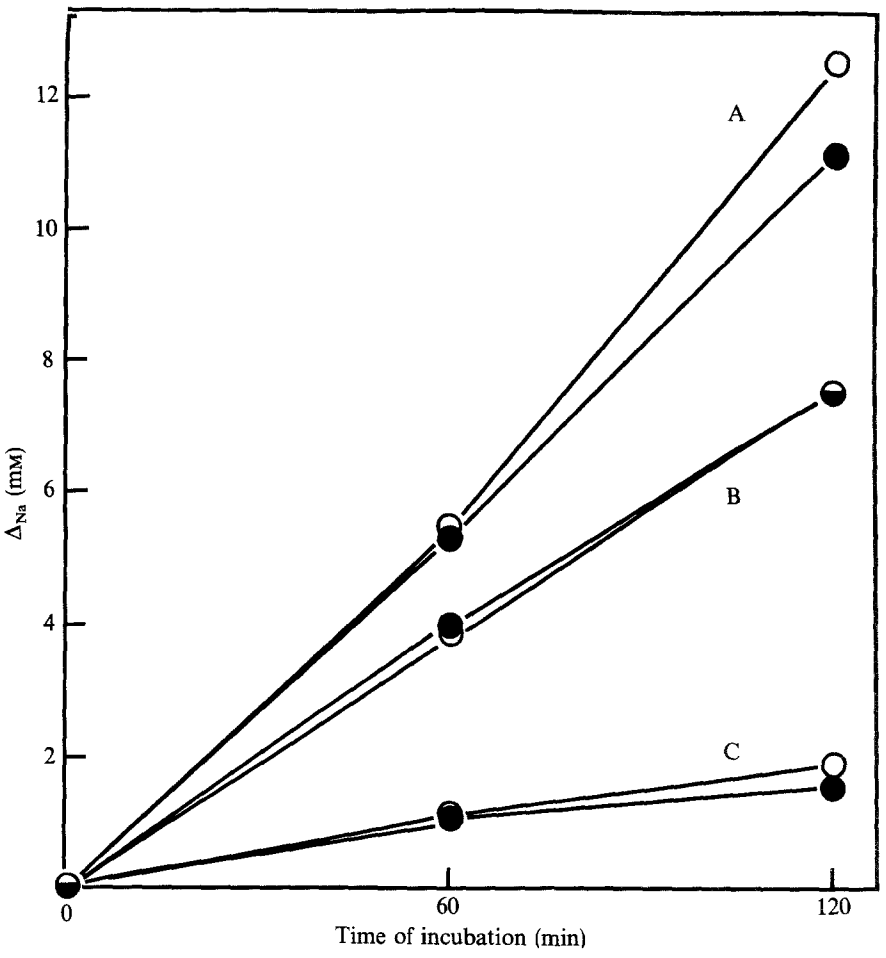

Fig. 6. A comparison of experimental with predicted falls in Na concentration in luminal fluids held within gallbladders incubated *in vitro*. Conditions of incubation were as described for Fig. 3. Samples of luminal fluids were analyzed for Na after 1- and 2-hr incubation. —•—, Predicted fall in Na concentration assuming gallbladder to transport an isotonic solution of NaCl; —○—, experimentally determined falls in Na concentration. *A*, *B* and *C* give the results of three experiments using gallbladders taken from fish adapted to 8, 15 and 25 °C, respectively. All incubations were at 30 °C

to different temperatures were incubated for 2 hr and cellular K, Na and Cl determined in mucosal scrapings as described above. The results are shown in Fig. 7. Cellular K, measured after incubation at 30 °C, fell from 131 ±2 to 94 ±6 mM as the environmental temperature rose from 8 to 30 °C. There was a reciprocal rise in intracellular Na, from 43 to 78 mM, the Na + K content remaining constant at about 170 mM. This is some 15 mM in excess of the total cation content of the incubation medium. Some of the intra-

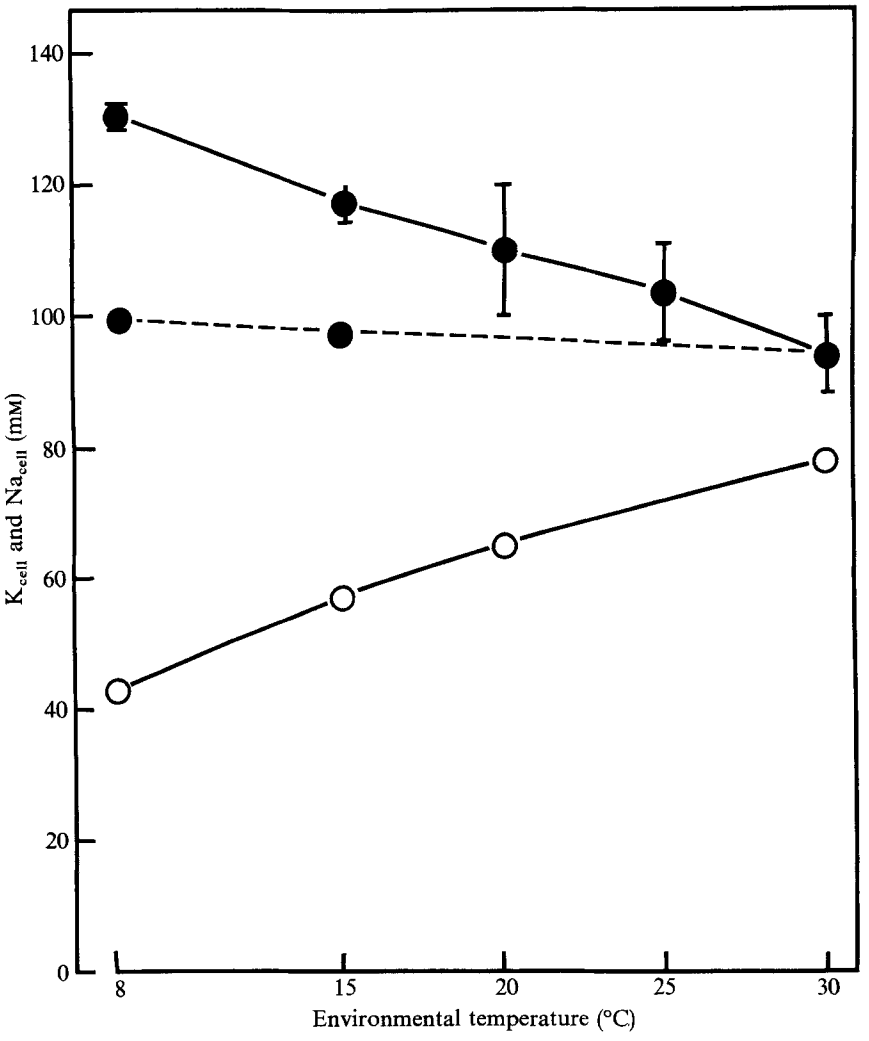

Fig. 7. Dependence of goldfish gallbladder intracellular Na and K concentrations on the previous environmental temperature of the fish. Conditions of incubation as described for Fig. 3. ^{3}H-Inulin and ^{14}C-sucrose were used to correct total cation content for its extracellular component. —•— and —○—, Incubation at 30 °C; – –•– –, incubation at temperatures equal to the previous environmental temperature of the fish. —•— and – –•– –, Potassium; —○—, sodium. Potassium values give means ± SE of from four to eight determinations. Sodium values give means of up to three determinations

cellular cations must be present in a nonosmotically active form. Gallbladders incubated at the previous environmental temperature of the fish (8 at 8 °C, 15 at 15 °C and 30 at 30 °C) had the same intracellular concentration of K. Probably the Na concentration would also be equal in the three cases but this was not determined directly.

Table 3. Intracellular chloride concentrations in goldfish gallbladder

Temperature (°C)		Chloride concentration (mM)
Adaptation	Incubation	
8	30	79 ± 11 (3)
15	30	89 ± 19 (3)
20	30	91 ± 9 (3)
25	30	107 ± 19 (2)
30	30	122 ± 7 (7)
8	8	118 ± 6 (5)

Gallbladders were incubated for 2 hr at 8 and 30 °C in medium containing ^{14}C-sucrose and ^{3}H-inulin. Tissue water was determined in mucosal scrapings as a wet weight-dry weight difference. Total Cl content was corrected using ^{14}C-sucrose to measure serosal and ^{3}H-inulin to measure mucosal extracellular space. Final values give means ± SE (No. of observations).

Intracellular concentrations of Cl were also determined under these different conditions. Results obtained are shown in Table 3. Chloride measured after incubation of gallbladders at 30 °C behaved like Na, the intracellular concentration increasing from 79 to 122 mM as the environmental temperature rose from 8 to 30 °C. The scatter within these groups was quite large but the difference was statistically highly significant ($p < 0.01$). The Cl concentration of gallbladder mucosa taken from fish adapted to 8 °C and incubated at 8 °C was not different from that of the 30 °C-adapted gallbladder incubated at 30 °C ($p > 0.7$).

The rise in intracellular Na and fall in net Na movement, seen at high adaptation temperatures, might reflect part of the same control mechanism. As a test for this the time needed for fluid transport (i.e., Na transport) to adapt was compared with that needed for the mucosa to change its ionic composition.

Time Course of Adaptation

Mucosal K concentrations were determined at different times after raising the environmental temperature of 15 °C-adapted fish to 30 °C, the same gallbladders being used to first measure fluid transport. Tissue K was preferred to Na as a measure of changes taking place within the cells since this could be determined with greater precision, the correction applied for extracellular K being only a small proportion of the total tissue K. Results are shown in Fig. 8. The transport of fluid measured 12 hr after raising the environmental temperature was not different from that measured at zero time ($p > 0.4$). Eight hours later, however, regulation was complete (dif-

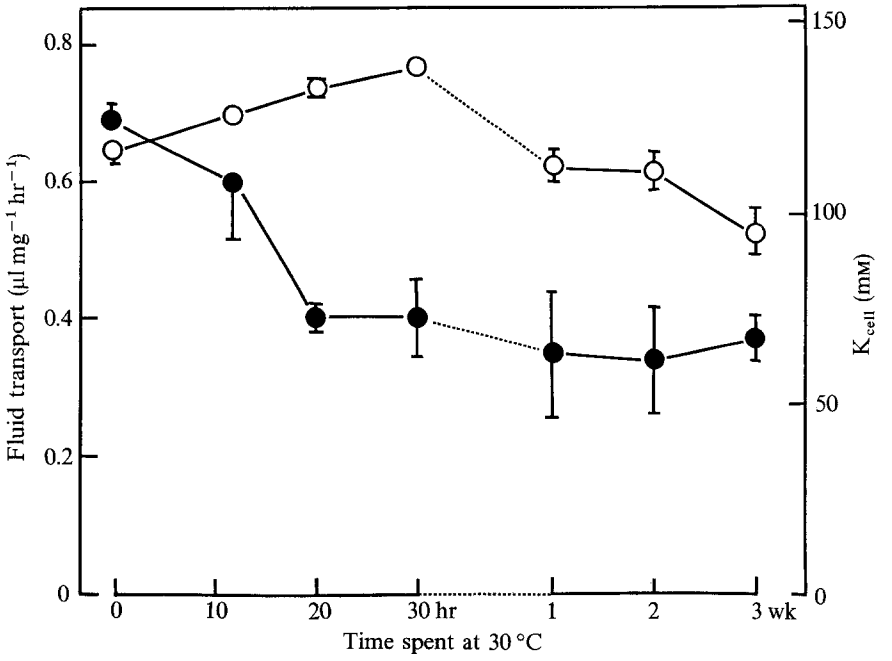

Fig. 8. Time course of adaptive changes taking place within the mucosa of the goldfish gallbladder. Fish adapted to 15 °C for three weeks were placed in water at 30 °C and their gallbladders removed at fixed times later. The transport of fluid across these gallbladders was measured *in vitro* at 30 °C and the intracellular concentration of K determined in mucosal scrapings as described previously. —•—, Fluid transport; —○—, intracellular concentration of K. Values give means ± SE of from four to nine determinations

ference from value measured at zero time, $p<0.001$; difference from value measured three weeks later, $p>0.3$). The intracellular concentration of K remained high until between two and three weeks after raising the environmental temperature; the value after 20 hr at 30 °C was significantly *higher* than that determined at zero time (133 ± 2 and 118 ± 3 mM, respectively; $p<0.001$). The fall in Na transport could not therefore be linked directly to the reciprocal changes taking place in the mucosal levels of Na and K.

Osmotic Permeability of Goldfish Gallbladder

To determine whether water permeability *per se* was changing with adaptation, fluid movement across gallbladders of fish adapted to 15 and 30 °C was measured using medium containing an additional 50 mM sucrose in contact with the serosal surface. No change was made in the osmolarity of the luminal fluid since this has been shown to produce anomalous effects

Table 4. Osmotic permeability of goldfish gallbladders

Temperature (°C)		Apparent permeability constant ($cm\ sec^{-1} \times 10^3$)
Adaptation	Incubation	
15	15	2.1 ± 0.6 (4)
15	30	2.3 ± 0.5 (6)
30	30	2.8 ± 0.5 (6)

Fluid movement across goldfish gallbladders was determined in normal medium and with 50 mM of sucrose added to medium bathing the serosal surface. Values of P_{osm} were calculated from measurements made after a preliminary 30-min incubation in the hypertonic medium. Goldfish gallbladders incubated at 30 °C are virtually impermeable to sucrose. This is true whatever the previous environmental temperature of the fish. The mean permeability to sucrose, determined using gallbladders taken from fish adapted to 8, 15, 20 and 30 °C was only $2.5 \pm 0.4 \times 10^{-6}$ cm sec^{-1} (8 observations).

on the subsequent movement of water (Smulders *et al.*, 1972; Wright, Smulders & Tormey, 1972). The apparent permeability constant for water was shown to remain independent of the previous environmental temperature of the fish (Table 4). Neither could one distinguish between water permeability measured at 15 and 30 °C using gallbladders taken from fish adapted to 15 °C.

Discussion

The structure of the goldfish gallbladder is very similar to that found in mammalian gallbladder (Tormey & Diamond, 1967; Hayward, 1968) and the swelling of the intercellular space during transport is also largely confined to the crests of the epithelial folds as in mammals (Kaye, Maenza & Lane, 1966). However, the membranous cytoplasmic bodies and plasmalemma invaginations have not been reported previously in gallbladder structural studies.

The extraction of the particles in the cytoplasmic bodies by aqueous uranyl acetate and their size and preferential staining with lead indicates that they are probably β particles of glycogen (Vye & Fischman, 1971). Glycogen has been reported in mouse gallbladder (Hayward, 1968) and similar glycogen particle-membrane associations ('glycogen bodies') have been documented in several mammalian tissues (*see* Pannese, 1969 for a list) but not previously in fish. The mammalian gallbladder epithelial cells have numerous slender processes which interdigitate and are closely apposed when the bladder is not transporting. Such structures vastly increase the intercellular channel surface area. When these channels swell they are

traversed by these fine prolongations. In goldfish gallbladder, however, the cell surface remains smooth and the cells are connected laterally only by occasional desmosomes. The surface of the lateral plasmalemma is extensively invaginated and these fenestrated intracytoplasmic sheets of membrane may be analogous to the microvilliform processes of the mammalian gallbladder. Plasmalemma invaginations have been reported previously in renal tubular cells and in the epithelial layer of the salt gland in birds. In these cases invaginations were usually closely associated with mitochondria but this was not so for the gallbladder of the goldfish.

The present work showed that the ability of the goldfish gallbladder to transport fluid depended upon its previous environmental history. The only morphological changes accompanying these changes in transport were in the degree to which intercellular spaces widened during incubation. Increasing temperature might 'turn on' previously dormant cells, the number of spaced cells in warm-adapted being somewhat greater than in cold-adapted gallbladders, but this can not explain a *fall* in transport at high environmental temperatures. Neither does it seem likely that the production of new cells could occur fast enough to explain these adaptational changes (20 hr to change transport compared with >9 days to renew the mucosa of rabbit gallbladder; Kaye *et al.*, 1966). The conclusion is that it is the existing cells in the crests of the epithelial folds which are able to modify their transport at different environmental temperatures.

The importance of the micro-architecture of the gallbladder in determining both the amount and osmolarity of transported fluid has been emphasized by Diamond and Bossert (1967). The present demonstration that the goldfish gallbladder transports an isotonic solution of sodium chloride, whatever the conditions of temperature manipulation, rules out the possibility that the radius or length of the intercellular channels have any part to play in adaptation. It has, however, been suggested that some fluid gains access to the intercellular channel through the tight junctional complexes and that this fluid pulls sodium after it by a process of solvent drag (Frömter, 1972). In this case, the transported fluid will remain isotonic with that bathing the luminal surface of the gallbladder and an adaptational change in the permeability of these junctions to water could change net fluid movement while maintaining osmolarity constant. If this did happen, however, one might also expect the permeability of sucrose to change, since this molecule is said to cross the gallbladder by a purely extracellular route (Smulders & Wright, 1971; Smulders *et al.*, 1972). In fact no such change took place, the permeability to sucrose being 2.1×10^{-6} cm sec^{-1} for both 8- and 30 °C-adapted gallbladders incubated at 30 °C. The osmotic permea-

bility of the gallbladder was likewise uninfluenced by long-term changes in the adaptational temperature of the fish.

The possibility remains that it is the primary driving force for fluid transport, the pumping of salt into intercellular channels, which changes as fish adapt to different environmental temperatures. How this can be achieved is a matter for conjecture. Fluid transport might be inhibited by limiting the availability of high energy substrates to the pump, by interfering directly with the efficiency at which the pump operates, or by decreasing the passive permeability of the microvillar membrane to salt. Some distinction can be made between these three possibilities through the measurement of intracellular sodium. Inhibition of the pump with passive entry unchanged would cause the intracellular sodium to rise *at the same time* as fluid transport fell. If the passive entry were restricted however, and the pump allowed to remain fully operational, one would predict no change or a fall in intracellular sodium to accompany the fall in fluid transport. Both predictions are made on the assumption that the transport pool for sodium constitutes a significant proportion of the total sodium found within the cell. Experiments on the time course of adaptation showed the cellular potassium to increase as fluid transport fell, both changes being complete 20 hr after raising the environmental temperature of the fish. Since cellular sodium and potassium always change in a reciprocal fashion, we can conclude that both the net transport of sodium and the level of sodium within the mucosal cells fall at the same time following a sudden increase in body temperature. The early stage of adaptation, therefore, probably involves a control over the passive entry of salt into, rather than the active extrusion of salt from, the gallbladder epithelium. This is similar to the effect seen previously in the goldfish intestine, where adaptation to salt has been shown by direct measurement of uptake, to decrease the rate at which sodium enters the mucosa (Ellory, Lahlou & Smith, 1972; Ellory, Nibelle & Smith, 1973).

The cellular levels of sodium and potassium revert to normal after two to three weeks at the high environmental temperature. We assume, but have no direct evidence, that this coincides with the gradual appearance of new cells having a reduced capacity to pump salt into the intercellular spaces. The fact that fluid transport remains constant throughout these later stages of adaptation would suggest that the passive permeability of these new cells to salt has again increased to something approaching the pre-adapted level. Similar changes in the active transport of sodium have been seen in the goldfish intestine, the turnover of the sodium pump decreasing but only after fish have spent three weeks at a high environmental temperature (Smith & Ellory, 1971).

Results obtained with goldfish gallbladder provide an opportunity to test predictions made from the theory of Diamond and Bossert (1967). The first prediction, that fluid transport should take place through intercellular channels, has been verified through direct morphological observation. The intercellular channels widen at high rates of fluid transport in a way very similar to that reported previously for the rabbit (1.8 μ for the 30 °C-adapted gallbladder incubated at 30 °C; 1.5 μ for the rabbit gallbladder incubated at 37 °C; Tormey & Diamond, 1967). With the length of the intercellular channels not too different in the two preparations and with osmotic permeabilities similar in both cases, 2.4×10^{-3} cm sec^{-1} in the goldfish, 3.3×10^{-1} cm sec^{-1} in the rabbit (Wright *et al.*, 1972), one would anticipate that the goldfish gallbladder would, like the rabbit, produce an isotonic absorbate when incubated at its previous environmental temperature; and this was in fact the case. A further prediction made from the theory of Diamond and Bossert (1967) for a tissue of high osmotic permeability is that the osmolarity of the absorbate will remain relatively independent of the transport rate for solute. The present finding that fluid absorbed by the goldfish gallbladder remains isotonic when the transport rate is increased, by adaptation, from 1.3 to 2.9 mOsm cm^{-2} hr^{-1} (1 mg wet wt being equivalent to 2.5 mm^2 bladder surface), is fully in agreement with this prediction.

Finally, it is interesting to speculate about the significance of the present findings to the physiology of the fish. The first adaptive change involves a change in function. Only later does the epithelium modify its intracellular ionic composition. It appears from this that control of absorption is the prime need of adaptation. The result is to maintain constant the rate at which fluid is removed from bile, *irrespective* of the temperature at which the fish is living. This would be reasonable if the need to concentrate bile were also independent of the environmental temperature of the fish, but we have no way of determining if this is so. The close similarity between adaptive changes in the gallbladder and the anterior intestine of the goldfish (Smith & Ellory, 1971) would be consistent with their common embryological origin. It suggests that adaptation in the gastrointestinal tract is concerned primarily with the regulation of sodium and fluid transport, secondary effects on nonelectrolyte transport possibly resulting from the changed parameters of sodium transport.

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