CONCENTRATION GRADIENTS OF Na^+ AND Cl^- IONS BETWEEN THE EXTERNAL MEDIUM AND GALL BLADDER WALL TISSUES IN RABBITS

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During concentration of the bile, water together with Na^+ and Cl^- ions are absorbed from the lumen of the gall bladder in the form of an isotonic [4] or near-isotonic [10] fluid. The nature of isotonic transport of ions and water through the epithelial barrier has been inadequately studied. Several hypotheses have been put forward to explain this process [5-9]. The model of transport suggested by Diamond and his colleagues [5, 6], is widely known. According to their model, the moving factor transporting water from the lumen of the gall bladder outside is the local osmotic gradient, which is supported between the external medium and the region of the lateral intercellular spaces of the epithelial layer of the wall. This gradient is created by active transport of ions by the eipthelial cells, and its magnitude depends on the rate of absorption of fluid by the gall-bladder epithelium [6]. The existence of such a gradient has also been demonstrated in experiments with direct measurement of the Na^+ and Cl^- ion concentrations in the liquid of the rabbit gall-bladder wall collected after separation of the mucous and serous layers [1, 2]. It was decided to study the concentration of Na^+ and Cl^- irons not only in the interstitial fluid, but also in the tissues of the gall-bladder wall, during absorption of water and ions by the epithelium at different rates. The investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

The gall bladder, isolated in the rabbit, was tied onto a polyethylene cannula. Its lumen was then washed out several times and filled with isotonic Krebs' solution (in mM): NaCl 120.4, KCl 5.9, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.5; pH 7.4. The resulting preparation was immersed with its serous surface in a vessel with the same Krebs' solution, through which oxygen was bubbled, and incubated in it at 37°C for 2 h. Every 15 min the preparations were weighed on torsion scales, and the intensity of absorption of fluid by the gall bladder was estimated from the decrease in their weight. The rate of absorption was expressed in µl/h/100 mg wet weight of gall-bladder wall. After the end of the incubation, the remains of fluid were removed from the lumen of the gall bladder, after which it was turned inside out so that the mucous membrane was outside, and was fitted onto a glass rod. In this position the gall bladder was stretched a little to straighten the folds of the mucous membrane, and fixed with a ligature. Visible remains of fluid were removed from the surface of the mucous membrane, and it was separated from the gall-bladder wall under a stereoscopic binocular loupe (magnification 25×). During separation of the tissues into layers, small quantities (2-10 µl) of transparent fluid appeared, and were collected in a graduated capillary tube. The mucous and serous membranes of the gall-bladder wall were placed in Teflon crucibles, weighed, and then dried to constant weight at 105°C. For extraction of electrolytes the dried tissue was immersed in 5 ml demineralized water, boiled for 1 h, and allowed to stand in the cold for a further 48 h [3]. The sodium concentration (by flame photometry) and chloride concentration (potentiometrically) were determined in all the media studied. The concentration of electrolytes in the tissues was expressed as the ratio between the total content of ions in the tissue and the total content of tissue water.

EXPERIMENTAL RESULTS

The results of these experiments showed that the absorptive activity of isolated rabbit

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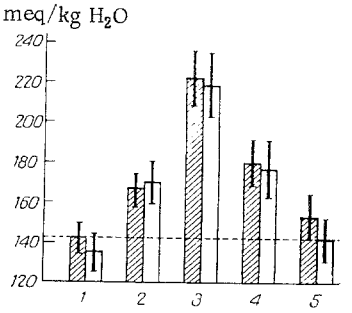

Fig. 1. Concentrations of sodium (shaded columns) and chlorides (unshaded columns) in external incubation solutions, in tissues, and in interstitial fluid of isolated rabbit gall-bladder wall after incubation for 2 h. 1) Mucosal solution; 2) mucosal tissue; 3) interstitial fluid; 4) serosal tissue; 5) serosal solution.

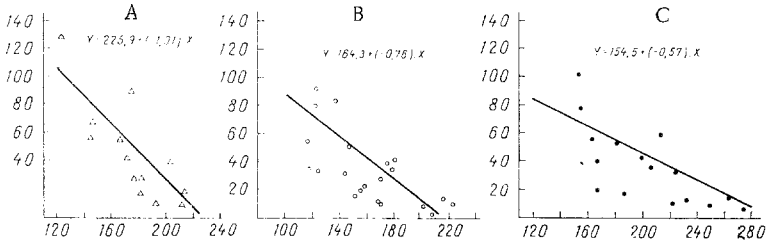

Fig. 2. Rate of transport of fluid through gall-bladder wall as a function of sodium concentration in its serous membrane (A), epithelial layer (B), and interstitial fluid (C). Abscissa, sodium concentration (in μeq/kg tissue water); ordinate, rate of transport of fluid through gall-bladder wall (in μl/h/100 mg wet weight of wall).

gall bladders shows well-marked individual differences. The rate of absorption in the different gall bladders varied from 10 to 120 μl/h. During 2 h of the experiment the gall bladder absorbed a mean volume of 108.1±52.8 μl water/100 mg wet weight of gall-bladder wall.

The results showed that absorption of fluid was accompanied by a decrease in the concentrations of Na^+ and Cl^- ions in it. The Na^+ concentration fell (from 154 mM) by 9.6 mM, and the Cl^- concentration (from 137.9 mM) by 14.8 mM after contact for 2 h between the isotonic Krebs' solution and the gall-bladder epithelium. These findings indicate that transport of ions from the lumen of the gall bladder takes place somewhat more intensively than the excretion of water. Calculations showed that in the course of 2 h the gall-bladder epithelium transported 22.2 μeq Na and 21.3 μeq Cl, calculated per 100 mg wet weight of gall-bladder wall. The epithelium transported only 82% of the total quantity of Na and 73% of the Cl ions in the form of isotonic fluid, and the rest of the ions were transported against the gradient. With this ratio between the rates of transport of water and ions through the gall-bladder wall the Na^+ and Cl^- concentrations in the absorbed fluid should have been slightly hypertonic. In fact, according to the calculation the Na^+ concentration in this fluid was 205.4 mM and the Cl^- concentration 197 mM. These figures were obtained by dividing the quantity of Na and Cl by the quantity of water transported through the gall-baldder wall in 2 h.

Analysis of the water—electrolyte composition of the gall-baldder wall showed that the level of Na^+ and Cl^- concentration in all its layers was higher than in the external medium. This is clear enough from the data given in Fig. 1. The highest concentration of these ions was found in tissue fluid extracted from the gall-bladder wall during separation of the mu-

cous membrane. The averaged data show that the Na^+ concentration in this fluid was 220.8 mM and the Cl^- concentration 218.6 mM. The concentrations of these ions in the serous membrane (Na^+ 181.7 mM, Cl^- 174.0 mM) was a little lower, and their concentration in the mucous membrane lower still (Na^+ 166.6 mM, Cl^- 167.7 mM).

The tissue concentrations of Na^+ and Cl^- changed depending on the rate of absorption of fluid, i.e., on the intensity of the transepithelial flow of water and electrolytes. In all layers of the gall-bladder wall there was a regular increase in the Na^+ and Cl^- concentration as the absorptive activity of the organ diminished (Fig. 2). The results of regression analysis show that with a decrease in the absorption by 10 µl/h the concentration of, for example, Na^+ increased in the serous membrane by 9.7 mM, in the epithelium by 13.2 mM, and in the interstitial fluid of the gall-bladder wall by 17.5 mM. Coefficients of correlation between these values are significant.

On the basis of the results of regression analysis the optimal level of absorption of fluid from the lumen of the gall bladder, i.e., the level at which retention of ions in the transporting tissue does not take place, can be determined. According to calculations, if the rate of absorption was 60 µl/h/100 mg gall-bladder wall, the Na^+ concentration in the epithelial layer of the gall-bladder wall was 140 mM, so that, naturally, it did not exceed the concentration of this ion in the external solution. However, even at that rate of absorption, the Na^+ concentration in the interstitial fluid should have been 166 mM, or 22 mM higher than in the external solution. Consequently, even with optimal absorptive activity of the organ, the concentration gradient for Na^+ and Cl^- was preserved between the fluid of the gall-bladder wall and the external incubation medium.

These investigations thus showed that concentration gradients for Na^+ and Cl^- exist between the fluids in the wall of the isolated gall bladder and the external incubation medium. The magnitude of these gradients increases with a decrease in the intensity of absorption of water and electrolytes from the gall-bladder lumen. It was also shown that Na^+ and Cl^- ions are absorbed together with water as a slightly hypertonic solution, confirming previous observations [10].

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