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Fluid Transfer in the Everted Human Gallbladder *

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Summary. The rates of fluid transfer across human gallbladders obtained at cholecystectomy for cholelithiasis were determined by the measurement of weight changes of everted preparations under controlled conditions. Active transport of fluid from the mucosal to the serosal surface was indicated since weight gain occurred with the same solution on both sides of the membrane and against hydrostatic, osmotic, and potential differences. With respect to sodium, the fluid transferred was isotonic to the bathing solutions. Metabolic inhibitors and temperature extremes inhibited weight gain.

In addition, muscle contractions in this *in vitro* preparation were related to the rates and direction of fluid movement. Cholecystokinin increased muscle activity and caused weight loss in preparations that previously had gained weight. Norepinephrine caused weight gain or increased weight gain in all preparations tested.

The direction of net fluid movement in the isolated everted human gallbladder was determined by the opposing forces of active mucosal transport and a filtration pressure generated by muscle contractions.

Introduction

The gallbladder selectively absorbs water and electrolytes from hepatic bile (1–3). This concentrating mechanism has been studied extensively in various animal preparations (4–15). Recently, a practical *in vitro* technique has been reported for the study of transfer mechanisms in the gallbladder of the fish, rabbit, and guinea pig (4, 7). This technique was used to study fluid movement in human gallbladders that had been removed because of cholelithiasis. The absorption of salt and water has been postulated to be increased with induced gallstones (16) but was absent in some species with spontaneous cholelithiasis (4). In the present study, net fluid movement in the everted human gallbladder resulted from the opposing forces of active mucosal transport and a filtration pressure generated by muscle contractions.

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Methods

Procedure. Gallbladders obtained at cholecystectomy from patients having chronic cholecystitis with cholelithiasis were immediately immersed in warm isotonic saline. Since the cystic duct had been ligated, bile did not leak from the gallbladder. Within 5 minutes of removal, each gallbladder was transferred into a modified Ringer's bicarbonate bathing solution at 37° C or 4° C.

The serosal covering was removed by sharp and blunt dissection because the human gallbladder is only partially covered by this membrane. This was usually accomplished easily, and care was taken to remove as little subserosal tissue as possible. This side of the preparation will, however, for convenience be referred to as the serosal surface. The neck of the gallbladder was cut, and the bile and stones were poured out. Bile contamination of the serosal surface was carefully avoided. The preparation was everted by gently reflecting the wall over a glass rod (11). Even though the mucosal surface was then lavaged repeatedly with the bathing solution, the mucosa remained slightly stained with bile pigment.

A Y-shaped Teflon cannula was tied securely in the everted gallbladder. The preparation, filled with bathing solution, was suspended in a beaker also containing the

bathing solution so that the levels of fluid of the inner and outer baths were equal. These procedures required 15 to 30 minutes from the time of cholecystectomy. The outer bathing solution was bubbled with 95% oxygen and 5% carbon dioxide, and the preparation was maintained at 37° C by a water bath. The modified Ringer's bicarbonate solution contained (in mmoles per L) Na^+ 150, K^+ 4.5, Ca^{++} 2.5, Mg^{++} 0.5, Cl^- 130, HCO_3^- 25, $HPO_4^=$ 1.2, $SO_4^=$ 0.5, and dextrose 10.

Water movement. After an equilibration period of 15 to 20 minutes, both inner and outer bathing solutions were replaced and the preparation was weighed at 5-minute intervals. Transferring the gallbladder to an analytical balance, weighing it, and returning it to the outer bathing solution required 15 to 20 seconds. Water adhering to the mucosal surface was drip drained by suspending the preparation above the outer bath for an additional 15 seconds before each weighing. Changes in weight were attributed to water movement (4). Experiments were performed to determine the movement of water and salt in relation to electrochemical, osmotic, and hydrostatic differences.

Determinations. Sodium and potassium concentrations were measured with a flame photometer (Beckman DU) and chloride concentrations by electrometric titration (17). Osmolality was estimated by determining the freezing point depression with a Fisk osmometer. The pH was measured with an expanded-scale Beckman pH meter.

Potential differences were measured with calomel half-cells, saturated KCl agar bridges, and an expanded-scale Beckman pH meter and were recorded with a Honeywell Servorecorder. One bridge was placed inside the gallbladder and the other in the outer bathing solution.

Isotope studies. Radioactive sodium (^{24}Na) and deuterium (D_2O) were placed in the inner bathing solution to determine unidirectional sodium and water movement according to the method of Grim (10). The outer bathing solution was changed at 20- to 30-minute intervals; back flux was therefore assumed to be negligible. Radioactive sodium was measured in a well-type gamma scintillation counter and D_2O by mass spectrometry.[1] In eight experiments, 100% (range, 95 to 106%) of the ^{24}Na and 93% (range, 85 to 100%) of the D_2O were recovered.

Pressure recordings. A fluid-filled open-tipped catheter, strain gauge, and modified Honeywell recorder were used to graph pressure changes within the everted gallbladder. A closed system was maintained while the catheter was in the gallbladder.

Pathology. Tissue was studied by means of light and electron microscopy. Light microscopic specimens were fixed in formalin and stained with hematoxylin and eosin. Specimens for electron microscopy were fixed in Dalton's solution and then embedded in an epoxy resin (Epon 812) after the technique of Luft (18) for study with an electron microscope (RCA EMU-3).

[1] In the laboratory of Dr. C. F. Code with a Consolidated Nier mass spectrometer, model 21-201.

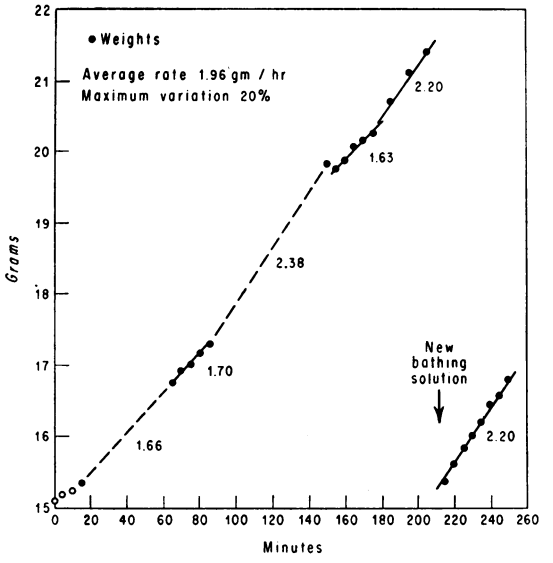

FIG. 1. WEIGHT CHANGE OF AN EVERTED HUMAN GALLBLADDER. If weighings were made at intervals of 5, 10, or 60 minutes without the bathing solution being changed or if new bathing solution was substituted, the rate of weight gain in this preparation did not significantly change over 4 hours.

Results

Weight gain

Of the 47 gallbladders studied, 27 preparations gained weight and 20 lost weight. The average gain in weight of 27 preparations was 1.00 g per hour (SE, 0.123; range, 0.24 to 2.88) or 13% of the initial intraluminal volume per hour when identical solutions were placed on both sides of the gallbladder wall. The combined weight of gallbladder and cannula was about 45%, and the initial intraluminal volume was about 55% of the total weight of the preparation. Weighing reproducibility, determined in poisoned preparations, was 0.3% (SD) of the total weight of the preparation. The greatest variation among hourly rates of weight gain determined up to 4 hours for any single preparation was 20%, when weighings were made at intervals of 5, 10, or 60 minutes (Figure 1). Thus, fluid movement rather than the weighing procedure per se was responsible for the observed weight changes, and in the everted gallbladder, this weight gain represented movement of fluid from mucosal to serosal surface. Adapting this same procedure for the gallbladder of the rabbit, we have found weight changes similar to those reported by others (7, 11, 13, 15).

TABLE I

Fluid transfer in everted human gallbladders during weight gain

Bathing solution	Initial solution		Inner solution		Outer solution		No. pairs	p value (*t* test)
	Mean	Range	Mean	Range	Mean	Range		
Osmolality, *mOsm*	293	280–310	297	285–313	301	293–317	11	<0.01
pH	7.56	7.30–7.75	7.35	7.04–7.52	7.55	7.49–7.72	10	<0.01
Na^+, *mEq/L*	151	142–164	149	138–164	153	145–164	13	<0.01
Cl^-, *mEq/L*	131	123–142	134	128–143	134	130–142	13	>0.6
K^+, *mEq/L*	4.5	3.8–6.6	4.4	3.0–8.0	4.3	2.9–5.0	11	>0.7

Evidence for active transport

Electrolytes, pH, osmolality. Table I shows the mean and range of Na^+, K^+, Cl^-, pH, and osmolality of initial bathing solutions and of inner and outer bathing solutions sampled simultaneously from preparations after periods of weight gain ranging from ½ to 4 hours. The differences in Na^+ concentrations and osmolalities between the inner and outer bathing solutions (paired observations) were significant. This was so because the differences were practically always in the same direction when simultaneously sampled solutions were compared (even though the mean values were not significantly different). Also, the observed differences were not greater than the error of the respective measurements. The pH of the inner solution was significantly less than that of the outer solution sampled at the same time ($p < 0.01$).

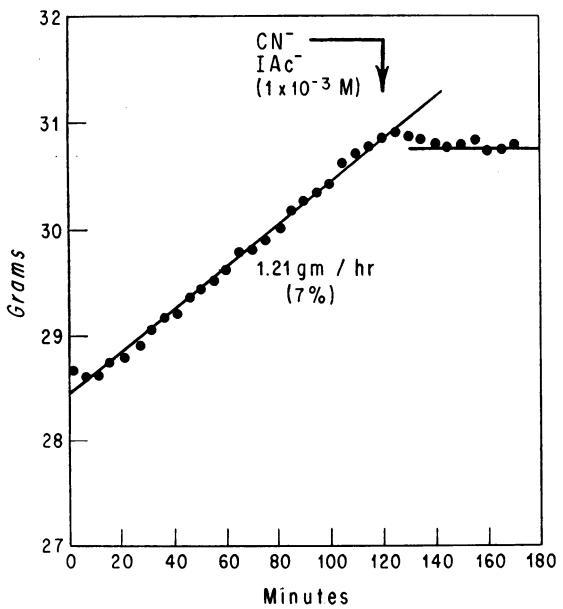

FIG. 2. EFFECT OF METABOLIC INHIBITORS ON FLUID MOVEMENT IN AN EVERTED HUMAN GALLBLADDER. Addition of cyanide and iodoacetate ions (1×10^{-3} M) to the outer solution stopped weight gain within 10 minutes.

Metabolic inhibitors. In 23 preparations, weight gain stopped within 20 to 30 minutes after addition to the outer bathing solution of sodium cyanide and iodoacetic acid, each in final concentration of 1×10^{-3} mole per L (Figure 2), or dinitrophenol, final concentration 1×10^{-4} mole per L. When fresh bathing solution was then substituted for that containing the poisons, no weight gain occurred, thus indicating that the effect was irreversible.

Potential differences. In ten preparations, the average potential difference across the gallbladder wall determined after 30 to 40 minutes of weight gain was 6 mv (SE, 1.0; range, 0.8 to 15), with the mucosal surface negative to the serosal surface. There was no significant difference between the mean potential difference measured during weight gain and that determined during weight loss (also ten preparations). The potential differences measured at intervals during an individual experiment lasting up to 4½ hours did not change more than ± 2.0 mv. As previously noted by Grim (10), the potential difference increased when bile was added to the mucosal bathing solution. Thirty minutes after the preparation had been poisoned, the potential difference across each of five gallbladders decreased to a mean of 0.9 mv (range, 0 to 3.8), with the mucosa being negative.

Fluid transfer against osmotic differences. The

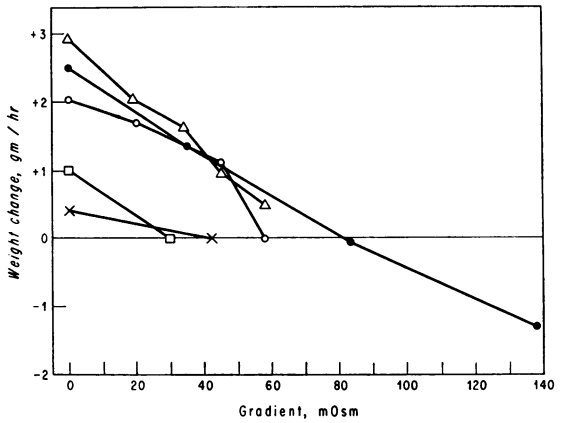

FIG. 3. EFFECT OF OSMOTIC DIFFERENCES ON FLUID MOVEMENT IN FIVE EVERTED HUMAN GALLBLADDERS. Osmotic differences were established by addition of mannitol to mucosal solution. Fluid movement occurred against differences of 30 to 80 mOsm.

osmolality of the mucosal solution was increased by the addition of mannitol. Figure 3 illustrates the relationship between fluid movement and osmotic differences across five gallbladders. Fluid movement was observed against differences up to an extrapolated 30 to 80 mOsm; with further increase in osmolality, the direction of net flow was reversed.

Fluid transfer against hydrostatic differences. Hydrostatic pressure differences were produced by the addition of bathing solution to the inside of the preparation and were measured in centimeters of water in the Teflon cannula. Figure 4 illustrates the relationship between weight change and hydrostatic pressure differences maintained for 25 to 45 minutes across four gallbladders. Fluid movement occurred against differences up to an extrapolated 1.5 to 2.5 cm of water; with further increments of pressure, a reversal of net flow occurred. When zero pressure differences were reestablished, the net fluid movements approximated initial rates.

Fluxes of water and sodium. Fluxes of water and sodium were determined in five preparations during weight gain (Table II). The average net flux of water was 1.10 g per hour (range, 0.09 to 2.20) and of sodium was 0.181 mEq per hour (range, 0.010 to 0.469). Figure 5 shows the relationship between the net movement of sodium and that of water ($y = 0.008 + 0.157x$). The concentration of sodium determined from this regression equation (157 mEq per L) was not significantly different from that measured in the inner and the outer bathing solution. Sodium fluxes may be influenced by solvent drag. The rapid bidirectional movement of both sodium and water suggests a high degree of passive permeability to both substances. Net movements of both sodium and water occur that cannot be accounted for by external forces alone.

Measurement of temperature coefficients. Rates of water movement were measured in three gallbladders at 37° C and then at 43° C. At 43° C the rates for the three preparations were, respectively, 1.5, 1.6, and 2.8 times those at 37° C. The calculated temperature coefficients (Q_{10}, 37 to 43° C) were 2.5, 2.7, and 4.7. Net fluid movement decreased and then stopped after 30 to 40 minutes at 43° C.

Weight loss

The 20 preparations that did not gain weight lost weight at a mean linear rate of 7% per hour (range, 2 to 17). Among the gallbladders that lost weight, weight loss did not necessarily correlate with the pathologic findings or with the results of preoperative cholecystographic studies. Some gallbladders that lost weight had been visualized, whereas some that gained weight had not. Also the fluid levels of inner and outer bathing solutions at the beginning of each experiment

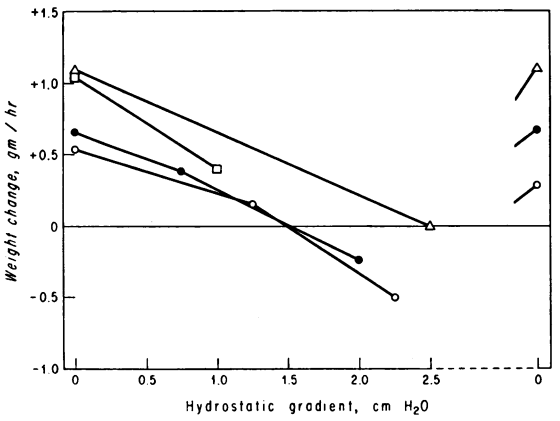

FIG. 4. EFFECT OF INDUCED HYDROSTATIC PRESSURE DIFFERENCES ON FLUID MOVEMENT IN FOUR EVERTED HUMAN GALLBLADDERS. Weight gain occurred against pressure differences up to 1.5 to 2.5 cm water. With further increases, weight loss occurred. Weight gain was again observed when zero differences were reestablished.

TABLE II

Unidirectional and net fluxes of radioactive sodium and deuterium in five everted human gallbladders

Gall-bladder no.	Minutes of weight gain	Unidirectional fluxes: Serosa to mucosa H_2O	Unidirectional fluxes: Serosa to mucosa Na^+	Unidirectional fluxes: Mucosa to serosa H_2O	Unidirectional fluxes: Mucosa to serosa Na^+	Net fluxes: Mucosa to serosa H_2O	Net fluxes: Mucosa to serosa Na^+
		ml/hour	*mEq/hour*	*ml/hour*	*mEq/hour*	*ml/hour*	*mEq/hour*
70	15		2.545		2.888	2.20	0.343
	30		1.427		1.683	1.64	0.256
	45		0.841		0.971	0.84	0.131
	60		0.596		0.821	1.44	0.224
71	17	13.13	1.470	14.08	1.658	0.95	0.188
78	37	1.44	0.196	2.46	0.361	1.02	0.165
	67½	1.08	0.084	1.86	0.133	0.78	0.125
79	61	14.64	1.007	17.48	1.476	2.84	0.469
80	32½	7.48	0.776	7.57	0.786	0.09	0.010
	68½	5.35	0.289	5.50	0.311	0.15	0.022
	96	4.84	0.249	4.97	0.303	0.13	0.054

were equal. When periodic fluctuations of the fluid level in the cannula were noted, muscle contractions were postulated to be responsible, in part at least, for the lack of weight gain in some of these preparations.

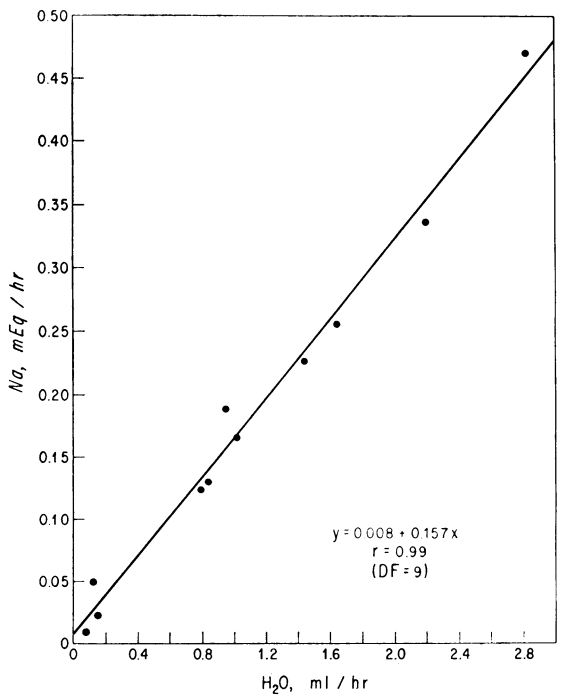

FIG. 5. RELATIONSHIP BETWEEN THE NET FLUXES OF SODIUM AND WATER IN FIVE EVERTED HUMAN GALLBLADDERS. With respect to sodium, the fluid transferred was isotonic to the bathing solutions used.

Pressure recordings were made in eight preparations before and after the addition of cholecystokinin to the inner solution (average final concentration, 0.50 dog U per ml; range, 0.23 to 2.94). As was found with preparations gaining weight, the weight loss (spontaneous or stimulated) was stopped by metabolic inhibitors (16 gallbladders).

These gallbladders exhibited both sustained rises in pressure (persisting for 10 minutes or more) and rhythmic pressure increases occurring up to three per minute. In preparations that lost weight, the magnitude of the sustained pressure increase was greater than in those that gained weight (Table III). After the addition of cholecystokinin, increases in both sustained and rhythmic pressures were recorded; all preparations either lost weight or showed an increased rate of weight loss. An inverse correlation between weight changes and mean pressure increases attributed to muscle contractions is shown in Figure 6 ($r = -0.84$, $df = 11$, $p < 0.01$). This regression indicates that at an increase of about 2.0 cm water pressure in the gallbladder, net movement of fluid does not occur.

Norepinephrine (levarterenol bitartrate, Levophed) was added to the outer or inner bathing solutions of 18 preparations (average final concentration, 0.052 mg per ml; range, 0.008 to 0.169). In nine preparations that had been losing weight (including four after addition of cholecystokinin),

TABLE III

Pressure changes in eight everted human gallbladders during weight gain or loss and after cholecystokinin

Gall-bladder no.	Spontaneous contractions: Vol.*	Weight change	Rhythmic: Mean increase	Rhythmic: No. per min	Sustained mean increase	After cholecystokinin: Vol.*	Weight loss	Rhythmic: Mean increase	Rhythmic: No. per min	Sustained mean increase
	ml	*g/hour*	*mm H_2O*		*mm H_2O*	*ml*	*g/hour*	*mm H_2O*		*mm H_2O*
53	15.30	+0.66	0	0	8	16.30	3.78	6	1.5	40
56	10.01	+0.53	6	0.5	0			21	0.7	24
84	17.96	+1.61	0	0	0	20.80	1.28	14	3.0	42
86	15.85	+0.35	6	0.5	4			20	2.0	40
54	10.65	−0.50			27	10.80	1.33	12	1.0	72
70	10.00	−2.04	6	0.75	22					
72	30.51	−0.89	6	0.5	44	31.92	7.44	6	1.5	137
89	14.22	−2.18	12	1.0	12	12.95	4.20	16	0.9	40

* Initial intraluminal volume.

in five showing no weight change, and in four gaining weight, a weight gain or an increase in the rate of weight gain occurred after norepinephrine use. Thus, in 18 preparations, including all 5 of the 20 nonabsorbing gallbladders tested, the effect was an increase in absorptive function. This weight gain stopped within 10 to 20 minutes after metabolic inhibitors had been added. In three of the four preparations that had been gaining weight, the potential difference across the membrane decreased after the addition of norepinephrine; in the fourth, no change was observed.

Pathology

The hematoxylin and eosin sections were examined by an investigator [2] without previous knowledge of the results of the transport studies (Table IV). Although all gallbladders had contained gallstones, of those examined, 9 of 11 that had gained weight and 4 of 19 that had lost weight were normal histologically. Chronic inflammation was present only in the submucosal and muscle layers (Figure 7). The assumption that a single tissue specimen was representative of the entire gallbladder is not completely valid since microscopic findings were different in 2 of the 12 preparations from which 2 specimens had been obtained. Interpretations were recorded as being normal on one specimen and as being inflamed on the other in both instances. The abnormal interpretation was used in both. The mean thickness of the mucosa plus muscle was 0.86 ± 0.06 mm (SE) in preparations that gained weight and 1.54 ± 0.10 mm (SE) in those that lost weight ($p < 0.001$). This

TABLE IV

Histologic findings in 30 everted human gallbladders

Weight change	No.	Normal	Abnormal: Thick wall	Abnormal: Inflammation
Gain	11	9	1	1
Loss	19	4	9*	6

* Associated chronic inflammation in six preparations.

[2] Dr. M. B. Dockerty of the Section of Surgical Pathology.

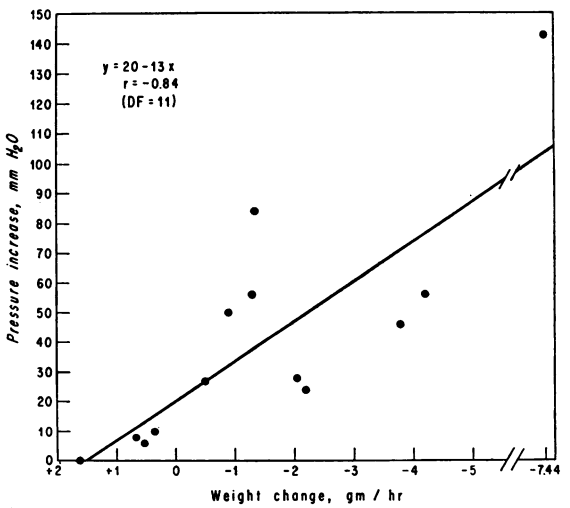

FIG. 6. RELATIONSHIP BETWEEN WEIGHT CHANGES AND MEAN PRESSURE INCREASES ATTRIBUTED TO MUSCLE CONTRACTIONS IN EVERTED HUMAN GALLBLADDERS. At pressure increments of less than 2 cm of water, weight gain occurred; at those greater than 2 cm, weight loss occurred.

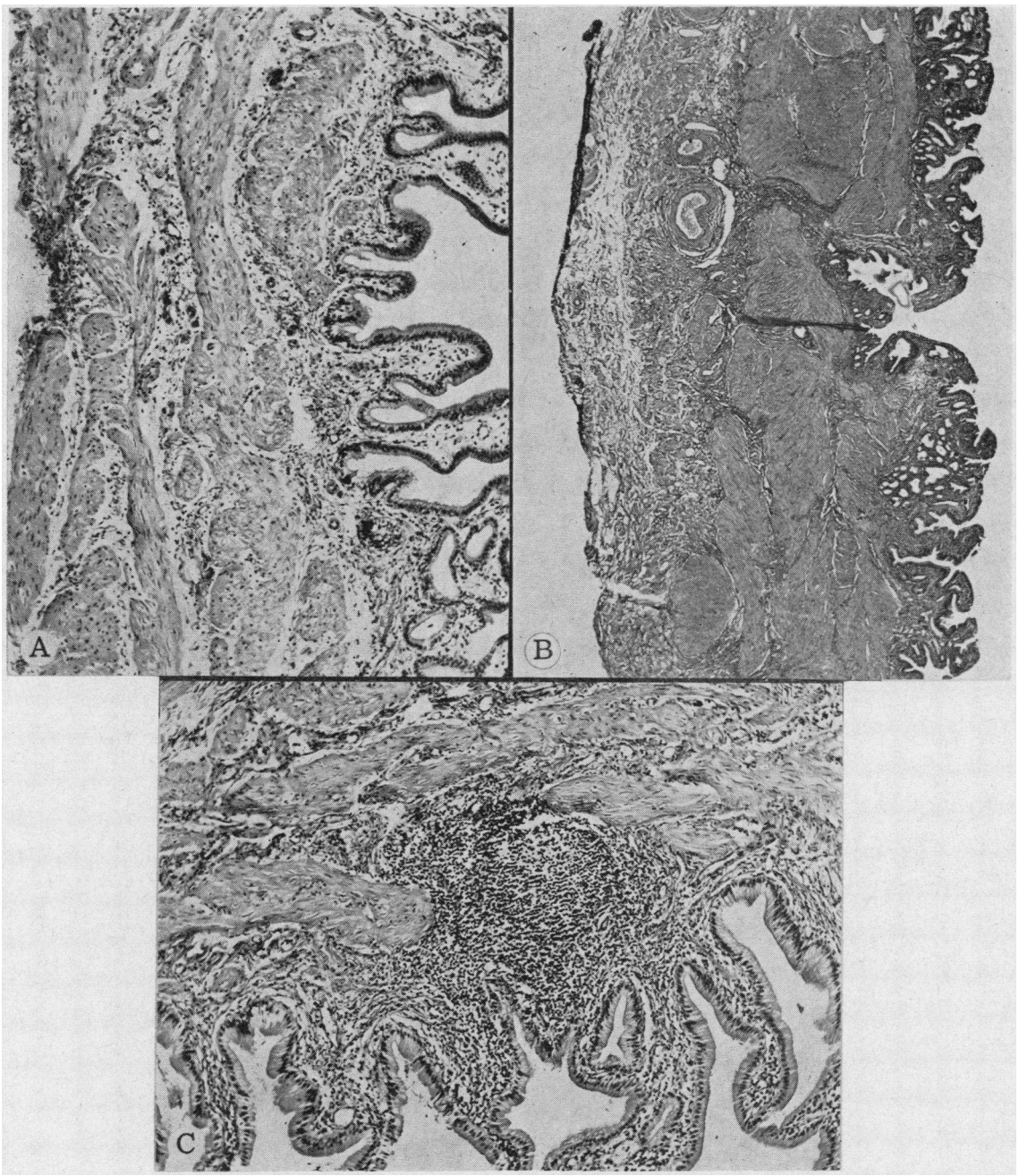

FIG. 7. PATHOLOGIC FINDINGS IN RELATION TO WEIGHT CHANGE IN EVERTED HUMAN GALLBLADDERS. *A*) Normal gallbladder that gained weight. Hematoxylin and eosin; × 86. *B*) Thick wall in gallbladder that lost weight. Hematoxylin and eosin; × 22. *C*) Chromic inflammation in gallbladder that lost weight. Hematoxylin and eosin; × 85.

difference in thickness may represent a difference in contractility of smooth muscle of the gallbladder, since in two other experiments, muscle thickness approximately doubled after the addition of cholecystokinin.

Mucosa from seven gallbladders, obtained after 50 to 150 minutes of weight gain, was examined with the electron microscope and showed ultrastructural detail similar to that of the normal human gallbladder (19).

Discussion

In 1921 Rous and McMaster (20) reported that 49.8 ml of bile placed in the *in vivo* gallbladder of the dog was reduced to 4.6 ml (concentrated 10.8 times) after 22½ hours. Fresh hepatic bile, when added in increments to the gallbladder, was concentrated three to ten times in 22 hours (average, 8.9). Our mean rate of absorption is comparable and, assuming a constant gallbladder volume of 50 ml, accounts for a threefold concentration of nondiffusible substances. In studies of standard and everted animal gallbladder preparations, rates of absorption of 10 to 25% per hour have been reported (4, 7, 10, 11, 13, 20). In the human gallbladder, noneverted preparations were not so satisfactory as the everted preparations, perhaps, as with the rat small intestine (21), because of inadequate oxygenation of the mucosal surface. In the everted preparation, fluid transported by the epithelial cells is measured at once, whether or not it escapes the gallbladder wall. In the noneverted preparations the only fluid transport measured is that which crosses the entire thickness of the gallbladder wall. If the fluid collects in the connective tissue and muscle tissue layers in the noneverted preparation, a delay in the measurement of weight change occurs.

According to Diamond (6, 8), Dietschy (11), and Whitlock and Wheeler (15), active transport of fluid across the animal gallbladder is associated with an energy–requiring electrically neutral sodium chloride pump, the water movement resulting from local osmosis (bulk flow). Grim (10) concluded, however, that active transport of luminal solution does not likely include passive movement of water. The present study demonstrated active transport of solution across human gallbladders. With respect to sodium, the fluid transferred was isotonic to the bathing solutions; the data do not permit further elaboration on the mechanism of this transport.

In animal gallbladders, potential differences smaller than those found herein have been reported (5, 10, 11). The presence of anions in the residual bile salt on the mucosal surface of our preparation may be responsible for the potential differences. An alternative explanation is that, unlike other preparations, a neutral sodium chloride pump is not the only mechanism for fluid transport. The possibility that this potential difference resulted from glucose transport as has been reported for the small intestine of the rat (22) was not investigated.

In our everted preparation, as with Dietschy's (11), the osmotic force was five to ten thousand times greater than the hydrostatic force when these forces were each sufficient to stop fluid movement from the mucosal to the serosal surface. The method by which these forces were created may have been responsible for the observed differences, since the osmotic force was established by adding mannitol to the mucosal solution, whereas the hydrostatic force was established by adding fluid to the serosal solution.

In the noneverted gallbladder of the fish, little effect was observed when hydrostatic pressure was applied to the mucosal surface (5), but in the everted gallbladder of the rabbit, a marked decrease of fluid transfer occurred when hydrostatic pressure was applied to the serosal surface (11). In the latter, the hydrostatic force caused irreversible mucosal damage; in our experiments under similar conditions, the decrease in fluid movement was reversible.

Muscle contractions were observed and recorded; this activity correlated with the rate of weight gain or weight loss. Stimulation with cholecystokinin increased the magnitude and frequency of contractions and the rate of weight loss. Stimulation with norepinephrine, a known inhibitor of smooth–muscle contraction (23), resulted in an increased rate of weight gain or reversal of weight loss in all preparations tested. The effect of cholecystokinin or of norepinephrine on the mucosal activity of the gallbladder is unknown. Other studies have shown that epinephrine decreased fluid absorption in the *in vitro* noneverted gallbladder of the fish (4) and in the *in vivo* gallbladder of the dog (24). Norepinephrine increased secretion and permeability of the submaxillary glands of the cat (25) but inhibited gastric secretion in dogs (26, 27).

That the gallbladder muscle produces a hydrostatic pressure *in vivo* is supported by the observations of spontaneous contractions in animal gallbladders (28) and by the existence of a physiologic sphincter between the gallbladder and the common bile duct in the unanesthetized dog (29).

However, as previously noted, experiments in other species suggest very limited hydrostatic filtration when pressure is applied to the mucosal side of the membrane. Whether a filtration force generated by muscle contractions is operative as an absorptive mechanism *in vivo* is unknown; the tissue need not necessarily behave symmetrically in response to differences in hydrostatic pressure on the mucosal or serosal surfaces. The postulated mechanisms for solute–water coupling in other species (8, 15) might be more sensitive to hydrostatic pressure elevation on the serosal side than to comparable osmotic gradients.

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