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ELECTRICAL POTENTIAL DIFFERENCES IN THE BILIARY TREE

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SUMMARY

1. Electrical potential differences (p.d.'s) have been measured between the major bile ducts and blood (or vascular perfusion fluid) in perfused guinea pig livers and in livers of unanesthetized dogs. The bile duct lumen was always electrically negative by 2 to 23 mV.

2. In the absence of the hormone secretin larger p.d.'s were correlated with higher bile concentrations of Na^+, K^+, and bile acid anions and with lower concentrations of Cl^- and HCO_3^-. In taurocholate choleresis the p.d. either increased, decreased, or remained unchanged, mirroring changes in bile composition.

3. Secretin choleresis was associated with an increased duct-negative p.d. in the dog.

4. Calculation from the Nernst equation shows that K^+ in bile is at equilibrium but that Na^+ and Cl^- concentrations are lower than equilibrium values.

5. These results suggest that distal reabsorption in the biliary tree involves active absorption of both Na^+ and Cl^-, possibly by a coupled, electrically neutral pump; that the hormone secretin activates an active anion secretory mechanism; and that the p.d. in the absence of secretin is simply an NaCl (or $NaCl$–$NaHCO_3$) diffusion potential.

INTRODUCTION

In analyzing ion distribution across biological membranes, knowledge of the electrical potential difference (p.d.) is as essential as that of ion concentration gradients in order to determine whether ions are distributed passively or subject to active absorption or secretion. While p.d.'s were measured in most other important epithelia many years ago, it is surprising that there have been no studies of transepithelial p.d.'s in the liver, despite the liver's importance and the many other recent advances in understanding biliary secretion (summarized in TAYLOR[1]). The present paper reports p.d.'s measured in the major bile ducts of isolated, perfused guinea pig livers and of livers of surgically prepared dogs.

As background, it may be recalled that secretion of bile is thought to involve at least three processes: (1) At a proximal site, probably in the bile canaliculi them-

Abbreviation: p.d., electrical potential difference.

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selves, secretion of organic anions (notably bile acid anions such as taurine and glycine conjugates of cholic acid derivatives) forms a primary bile into which inorganic ions and small non-electrolytes diffuse or are filtered osmotically (SPERBER[2]). (2) At some distal site, probably in the bile duct system, some water and inorganic ions are absorbed (SCHANKER AND HOGBEN[3], FORKER[4]). (3) At some distal site, probably also in the bile duct system, the hormone secretin stimulates an outpouring of NaCl, $NaHCO_3$, and water (WHEELER AND RAMOS[5], WHEELER AND MANCUSI-UNGARO[6], O'MAILLE, RICHARDS AND SHORT[7], FORKER[4]). From electrical recording in the major bile ducts one may hope to learn something about the two distal steps but not about the proximal step. Wherever the operationally defined distal site of bile formation is located anatomically, the recording of p.d.'s associated with the hormone secretin in the dog (p. 512) shows that the electrical manifestations of this site were being observed in our experiments.

METHODS

Male guinea pigs weighing 400–550 g were anesthetized intraperitoneally with sodium pentobarbital (Diabutal). The cystic duct was ligated and cut; the common duct was cannulated, with the tip of the cannula lying at the junction of the cystic and common ducts; the portal vein was cannulated; and the hepatic vein was cannulated *via* the inferior vena cava. The liver was excised, briefly washed in saline, transferred to a thermostatically controlled box (generally at 37° except where stated otherwise), and perfused *via* the portal vein by the method of MILLER *et al.*[8] as used by KATZ *et al.*[9]. The perfusion fluid had a total volume of 100 ml, was gassed with O_2–CO_2 (98:2, v/v) and was a Ringer's solution with the following composition (in mM): NaCl, 110; $NaHCO_3$, 25; KCl, 6; $CaCl_2$, 2; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; and glucose, 11. In two experiments the perfusion fluid was replaced with heparinized guinea pig blood after 60 or 90 min, but no change in the rate of bile flow resulted. The perfusion fluid was changed several times in quick succession at the beginning of each experiment to wash out residual blood, and thereafter every 30 min to prevent appreciable increase in its $[K^+]$ and decline in its $[HCO_3^-]$. Bile was collected from the duct cannula for periods of 30 min each in tared glass bottles covered with parafilm to prevent evaporation. Na^+ and K^+ were analyzed flame photometrically, Cl^- by a direct-reading Beckman Chloridometer, and HCO_3^- gasometrically by the Van Slyke method.

Potential differences were measured with a Keithley 610B electrometer and calomel half-cells. One half-cell was connected by an agar-saturated-KCl bridge to the hepatic vein (the p.d. reading was found to be the same whether this bridge led to the hepatic vein or to small pools of fluid in contact with various parts of the liver). This bridge was periodically re-immersed in saturated KCl to assure a fresh junction. The other calomel half-cell led by a similar agar-saturated-KCl bridge to a beaker of saturated KCl into which the common duct cannula dipped during p.d. measurements; this procedure assures a flowing junction. This attention to junction potentials and to the grounding of accessory electrical equipment (perfusion pump, heater, and circulating fan) was found to be essential for obtaining valid p.d. measurements, as artifacts due to junction potentials and electrical pick-up were otherwise significant.

Experiments were carried out *in vivo* on conscious dogs that had been surgically

prepared by cholecystectomy and ligation of the minor pancreatic duct and that had a duodenal fistula opposite the opening of the bile duct into the gut (THOMAS[10], WHEELER AND RAMOS[5]). For the experiment a length of polyethylene tubing was inserted through the fistula about 5 cm into the common bile duct. Measurement of p.d.'s was as in guinea pig experiments, except that one bridge led to a cannula in a leg vein and the other bridge led either to the common duct cannula or else to the bile in the collecting tube, into which the common-duct cannula dipped. A cannula leading to another leg vein was used for infusion of saline or administration of secretin or taurocholate. In one experiment p.d.'s were measured in the perfused left hepatic duct of an anesthetized dog while bile was being collected from the right hepatic duct in the usual fashion. The left hepatic duct was ligated (taking care to preserve its blood supply intact), cannulated distal to the ligature, and this distal segment of the duct was perfused with saline or Ringer's solution by means of a perfusion pump.

RESULTS

Perfused guinea pig liver

Since WHEELER AND RAMOS[5] had found that bile composition in conscious dogs remained more constant with time when bile salt was infused at a constant rate than when the bile salt supply to the liver was interrupted, we added sodium taurocholate to the perfusion fluid at 1 g/l. Under these conditions bile flow rate, bile composition, and p.d.'s were quite stable for up to 4 h (longer durations were not tried). Bile flow rate in 11 livers was 1.0 ± 0.6 ml/h (average value ± S.D.; all errors quoted are S.D.'s). Bile $[K^+]$ (abbreviated $[K^+]_b$; the subscript b will be used to refer to bile) was always higher than perfusion fluid $[K^+]$ (abbreviated $[K^+]_{pf}$; the subscript pf will be used to refer to perfusion fluid), the ratio being 1.21 ± 0.12 for 18 collection periods in ten livers and the actual range of $[K^+]_b$ being 6.4 to 11.0 mM. $[Na^+]_b$ was slightly greater than $[Na^+]_{pf}$ in 16 out of 19 samples, the ratio being 1.07 ± 0.09 for 19 periods in five livers and the range of $[Na^+]_b$ being 135 to 180 mM. $[Cl^-]_b$ was always lower than $[Cl^-]_{pf}$, the ratio being 0.71 ± 0.08 for 51 periods in 10 livers and the range of $[Cl^-]_b$ being 64 to 105 mM. $[HCO_3^-]_b$ was always higher than $[HCO_3^-]_{pf}$, the ratio being 1.9 ± 0.4 for 25 collection periods in eight livers and the range of $[HCO_3^-]_b$ being 26 to 52 mM, comparable to FORKER's[4] average value of 40 mM during dehydrocholate infusion. The bile duct lumen was always electrically negative to the perfusion fluid, with little variation in a given experiment or between different preparations. For the eight livers tested all p.d.'s fell between 2.7 and 4.2 mV (average value, 3.3 ± 0.5 mV). Cooling the liver and perfusion fluid by 14° in three experiments produced only a small decrease in p.d., to an average value of 2.4 mV.

Addition of 5 units of the hormone secretin (manufactured by the Gastrointestinal Hormone Research Group, Kemiska Institutionen II of the Karolinska Institute, Stockholm, Sweden) to the 100 ml of perfusion fluid in five experiments increased bile flow on the average by 53 %. The principal change in bile composition was an increase in $[HCO_3^-]_b$ to 58–84 mM, comparable to FORKER's[4] average value of 76 mM. $[K^+]_b$ increased to 10.6–12.5 mM (on the average, 1.68 times $[K^+]_{pf}$). $[Cl^-]_b$ decreased slightly to 66–79 mM, but total Cl^- output still increased, because of the increase in flow rate. The bile became notably less colored, possibly due simply to

the dilution of a constant bile pigment output. Secretin had no discernible effect on the p.d., which remained constant to within at least 0.5 mV.

Dog liver

In conscious dogs either a 0.9% NaCl solution or a 4% sodium taurocholate solution was infused into a leg vein at 74 ml/h. The range of variation in bile ionic composition and in the p.d. was much greater than in our guinea pig experiments, but it was immediately obvious that ionic concentrations and p.d.'s under these circumstances were closely correlated. The bile duct lumen was always electrically negative to the blood by 4 to 23 mV. The p.d. increased as the bile became more 'concentrated' (*i.e.*, as bile $[Na^+]$, bile salt concentration*, and $[K^+]$ increased and as bile $[Cl^-]$ and $[HCO_3^-]$ decreased (see Fig. 1)). With the possible exception of

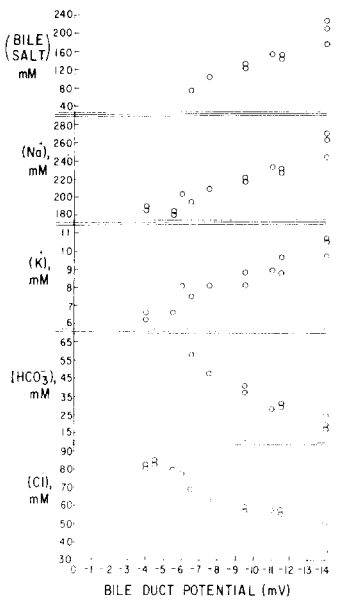

Fig. 1. Relation between bile composition and bile duct p.d. in conscious, unanesthetized dogs. The ordinate gives the measured concentrations of Na^+, K^+, Cl^-, and HCO_3^- and the calculated (see footnote) concentration of bile acid anions in bile samples. The abscissa is the potential difference, measured during collection of each sample, between the bile duct lumen and a leg vein. The bile duct was always electrically negative. Samples were obtained during saline infusion and during taurocholate choleresis.

$[HCO_3^-]$, the bile compositions and p.d.'s measured in the guinea pig all fall close to the patterns of Fig. 1 extrapolated in the low-p.d. direction, suggesting that the low p.d. and dilute bile composition we observed in the guinea pig reflect the same correlation. The intercorrelations among the concentrations of different ions which we observed ($[Na^+]$ and $[K^+]$ higher, $[Cl^-]$ and $[HCO_3^-]$ lower, in more concentrated biles, *i.e.*, those with higher bile salt concentrations) agree with those described in the dog and subjected to detailed analysis by WHEELER AND RAMOS[5]. Average dog plasma composition (in mM) was $[Na^+]$, 147; $[K^+]$, 4.5; $[Cl^-]$, 106; and $[HCO_3^-]$, 24.

* Calculated as $[Na^+]_b + [K^+]_b - [Cl^-]_b - [HCO_3^-]_b$.

$[K^+]_b$ was always higher than plasma $[K^+]$, ranging from 6.2 to 13.3 mM. $[Na^+]_b$ was always higher than plasma $[Na^+]$, ranging from 172 to 265 mM. $[Cl^-]_b$ was always lower than plasma $[Cl^-]$, ranging from 30 to 93 mM. $[HCO_3^-]_b$ ranged above and below plasma $[HCO_3^-]$ from 0 to 57 mM.

Changing the venous infusion from saline to taurocholate increased the rate of bile flow by 5 to 7 times. Bile composition became either more concentrated (higher bile salt concentration) or more dilute (lower bile salt concentration) or did not change, depending upon how long the bile salt supply had previously been interrupted. The p.d. either increased, decreased, or did not change. The effect on p.d. was closely correlated with the effect on composition: taurocholate increased the p.d. if it caused the bile to become more concentrated, and decreased the p.d. if it caused the bile to become more dilute.

Injection of 25 to 100 units of secretin (approx. 1–4 units per kg) in the absence of taurocholate infusion transiently increased the rate of bile flow to about 9 times its previous value, the effect on flow being visible within at least a few minutes of injection. At the same time the p.d. rapidly increased, *i.e.*, the bile duct went more negative by between 3.5 and 8.5 mV, sometimes doubling the existing p.d. An increase in p.d. was visible within a fraction of a minute; the maximum value was reached at about 4 min after injection; and the p.d. returned to its original level in 8–14 min. The most striking effect on bile composition was an increase in $[HCO_3^-]_b$ to 64–74 mM, coupled with a more modest increase in $[Cl^-]_b$ and decrease in $[Na^+]_b$. Because of the great increase in flow rate the total outputs of HCO_3^-, Cl^-, Na^+, and K^+ were all much increased. These effects of secretin on composition of dog bile agree with those described by WHEELER AND RAMOS[5]. The short time span of the secretin effect *in vivo* has been previously noted and is presumably due to rapid destruction of the injected hormone dose.

In one anesthetized dog the left hepatic duct was perfused with 0.9 % NaCl solution or with Ringer's solution, while flow of bile continued in the right hepatic duct. The p.d. in the perfused left duct remained within 1 mV of zero at all perfusion rates tested (0.16 to 25 ml/h). The range of p.d.'s in the right duct (5.8 to 9.6 mV, duct lumen negative) fell in the same range as for conscious dogs.

DISCUSSION

The p.d.'s described here were measured in the larger bile ducts. Because the resistance of intervening solution is slight, it seems likely, though unproved, that the same p.d.'s exist distally in the smaller bile ducts, a probable major locus of the reabsorptive mechanism and the secretin mechanism. There would be no justification, however, for assuming the p.d. in more proximal parts of the biliary system (*e.g.*, the bile canaliculi) to be the same. The measured p.d.'s are therefore relevant only to the most distal stage of bile formation. They permit one to assess two problems: whether the distribution of K^+, Na^+, and Cl^- at this distal stage is passive or involves active transport; and what the ionic mechanisms involved in bile reabsorption and secretin choleresis are.

K^+ distribution

$[K^+]_b$ was always higher than plasma or perfusion fluid $[K^+]$ in both dog and guinea pig, a fact that could be a passive consequence of bile always being electrically

negative. This hypothesis can be tested quantitatively by the Nernst equation. It is necessary to take into account activity coefficients (γ's) in bile, since these differ significantly from γ's in NaCl or KCl (DIAMOND[11], MOORE AND DIETSCHY[12,13]). For the calculations in the present paper we shall use γ's measured by MOORE AND DIETSCHY[12,13] in bile and in bile salt solutions, or γ's interpolated from their measurements. Some uncertainties in interpolation exist but are not large enough to affect our conclusions.

In the guinea pig under conditions of constant taurocholate concentration in the perfusion fluid, the p.d. was found to be -3.3 ± 0.5 mV. γ_K, γ_{Na}, and γ_{Cl} in the Ringer's solution used as perfusion fluid should be close to 0.75 (MOORE AND DIETSCHY[12], ROBINSON AND STOKES[14]). Guinea pig bile is sufficiently dilute ($[Cl^-] + [HCO_3^-]$ near 125 mM, calculated taurocholate concentration near 30 mM) that γ's in it should be only slightly lower than in the Ringer's solution, and a value of $\gamma_K =$ approx. 0.72 is interpolated from γ's measured by MOORE AND DIETSCHY[12] in cholate or glycocholate solutions (conjugation appeared to have little effect on γ's). Substituting in the Nernst equation to find the predicted value of $[K^+]_b/[K^+]_{pf}$ if K^+ is in equilibrium in bile, one obtains for E, the p.d. in mV at 37°: $E = 61.5 \log [(\gamma_K[K^+])_{pf}/(\gamma_K[K^+])_b]$, $(0.72[K^+]_b)/(0.75\ [K^+]_{pf}) =$ antilog $(3.3/61.5)$, $[K^+]_b/[K^+]_{pf} = 1.18$. The experimental value of 1.21 ± 0.12 is equal to 1.18 within experimental error, suggesting that K^+ is distributed passively in guinea pig bile.

In dog bile $[K^+]$ was found to increase with an increase in the bile-negative p.d., as one would expect for a passive Nernst-type distribution. γ_K's were taken or interpolated from measurements in dog bile samples by MOORE AND DIETSCHY[12] and ranged from 0.43 to 0.68, depending upon the bile salt concentration. Insertion of these γ_K's and measured values of $[K^+]_b$, plasma $[K^+]$, and the p.d. into the Nernst equation indicates that experimentally observed $[K^+]_b$'s remain close to the Nernst value over the range of p.d.'s encountered and are on the average only 1 % lower.

Thus, there is no need to postulate distal active transport mechanisms for K^+ in bile, and its raised concentration may be attributed to the p.d. This is the same conclusion which DIETSCHY AND MOORE[13], using γ's and p.d.'s measured on the same samples, reached for the very similar problem of K^+ distribution in gall-bladder bile.

Na^+ distribution

If distal Na^+ distribution is passive, the predicted Na^+ distribution ratio for guinea pig bile is the same as the predicted K^+ ratio, 1.18. The experimental value was smaller, 1.07 ± 0.09. 16 of the 19 measured values lay below the Nernst value.

For dog bile values of γ_{Na} may be interpolated with little uncertainty, since MOORE AND DIETSCHY[12] measured γ_{Na} in 10 samples, and values in even the most concentrated samples (0.68–0.70 in all but one case) were not far below values in blood or Ringer's solution (0.75). Substitution of these interpolated values, *plus* measured p.d.'s and values of $[Na^+]_b$ and plasma $[Na^+]$ in the dog, into the Nernst equation shows that $[Na^+]_b$ is lower than the Nernst equilibrium value by, on the average, 14 %, 15 of the 18 measured values falling below the Nernst value.

Cl^- distribution

If distal Cl^- distribution is passive, the predicted Cl^- distribution ratio for guinea pig bile is the reciprocal of the predicted Na^+ and K^+ ratio, namely, $1/1.18 =$

0.85. The experimental value was 0.71 ± 0.08, with 50 of the 51 measured values lying below 0.85. Even if γ_{Cl} were the same in dog bile as in dog blood, measured values of $[Cl^-]_b$ in the dog would be lower than the Nernst equilibrium value by, on the average, 12 %, with 22 of the 25 measured values falling below the Nernst value. The actual discrepancy between Nernst and observed values must be greater, since γ_{Cl} in dog bile will be some unknown amount below γ_{Cl} in dog blood.

Thus, in both guinea pig bile and dog bile the distal concentrations of both Na^+ and Cl^- are lower than those expected for passive distribution. This means either that distal bile is in a steady state (*N.B.*, steady state, not equilibrium) and that both Na^+ and Cl^- must be actively absorbed distally in the biliary tree; or else that distal bile is not in a steady state because the biliary tree is too impermeable, and the equilibration times too brief, for Na^+ and Cl^- to have diffused in up to their equilibrium concentrations. The latter hypothesis seems highly improbable, and the former hypothesis probable, for three reasons: (1) Studies of clearance of inert non-electrolytes have already provided convincing evidence for the existence of a distal reabsorption step (SCHANKER AND HOGBEN[3], FORKER[4]). (2) Rapid rates of exchange of radioactive tracers between bile and blood specifically indicate high permeability to ions (BRAUER[15]). (3) During controlled taurocholate infusion WHEELER AND RAMOS[5] found that biliary outputs of Na^+ and Cl^- are linear functions of taurocholate excretion rate over a considerable range of flow rates. This suggests that under those conditions $[Na^+]$ and $[Cl^-]$ in distal bile were in fact steady-state values, set by the balance between rates of bile-to-blood active transport and blood-to-bile diffusion down the resulting concentration gradients. The same conclusion is indicated by FORKER's[4] finding of a direct proportion between the rates of proximal bile production and distal reabsorption.

The secretin-dependent p.d.

The hormone secretin, which stimulates secretion of NaCl and $NaHCO_3$ at a distal site, causes in the dog an increased bile-negative p.d. approximately in phase with the choleresis. This strongly suggests that the effect of secretin is to activate a transport mechanism for the secretion of anions (HCO_3^-, Cl^-). The alternative explanation would have been that secretin modifies a diffusion potential across the biliary tree by modifying its permeability characteristics; but the secretin-induced changes in concentrations of the major ions are all in the wrong direction to explain an increased bile-negative p.d. ($[Cl^-]_b$ and $[HCO_3^-]_b$ increase, $[Na^+]_b$ decrease); and the measured p.d. in the presence of secretin is more negative than the equilibrium potentials of any of these ions in secretin-stimulated bile. Our failure to observe a secretin-dependent p.d. in the guinea pig *in vitro* is probably due to the much smaller effect of secretin on bile flow in these experiments (secretin stimulated flow by 800 % in the dog *in vivo*, by 53 % in the guinea pig *in vitro*).

The p.d. in the absence of secretin

The question remains whether the p.d. in the absence of secretin is simply a diffusion potential due to the bile-to-blood ion concentration gradients or whether distal active Na^+ and Cl^- reabsorption makes a contribution. The following four arguments favor the diffusion potential hypothesis and make a direct contribution from active transport mechanisms unlikely. (1) During perfusion of the bile duct,

when ion concentration gradients were negligible, there was no p.d. (2) Cooling guinea pig liver by 14° decreased the p.d. by only 27 %, a change that could easily have resulted by the diffusion potential hypothesis from a modest dilution of bile. An active transport mechanism with even as low a Q_{10} as 2.0 would have been reduced 62 % by cooling 14°. (3) When bile flow in the dog was stimulated 5- to 7-fold by taurocholate infusion, the p.d. either increased, decreased, or remained unchanged reflecting changes in bile composition, and there was no consistent change in p.d. associated with taurocholate choleresis *per se*. The primary effect of taurocholate infusion, taurocholate secretion at a proximal site, may well produce a lumen-negative p.d. in the bile canaliculi, but this would not be detected by an electrode in the major bile ducts. However, this increased primary output must also be accompanied by increased distal reabsorption (*cf.* FORKER[4]), and if distal reabsorption had involved, say, an active Na^+ pump capable of contributing to measured p.d.'s, taurocholate choleresis should have led consistently to an increased bile-negative p.d. (4) The observed correlations between bile composition and p.d.'s in the dog suggest that the p.d.'s are diffusion potentials resulting from bile-to-blood NaCl activity gradients established by distal reabsorption. The Cl^- gradient is always, and the Na^+ gradient almost always, oriented in the right direction to give a bile-negative p.d. ($[Cl^-]_b <$ plasma $[Cl^-]$, $[Na^+]_b >$ plasma $[Na^+]$). In the absence of secretin the observed p.d.'s in both guinea pig and dog were almost always intermediate between the sodium and chloride equilibrium potentials, suggesting that the p.d.'s are NaCl (or NaCl–$NaHCO_3$) diffusion potentials and that the biliary tree is permeable to both Na^+ and Cl^-.

The absence of a p.d. directly associated with active Na^+ and Cl^- reabsorption in the biliary tree may suggest that this reabsorption involves an electrically neutral, coupled NaCl pump, as has been demonstrated for the further reabsorption of bile carried out in the gall-bladder (DIAMOND[16], WHEELER[17], DIETSCHY[18], PIDOT AND DIAMOND[19]). The distal reabsorption site in the biliary tree also resembles the gall-bladder in permitting equilibration of K^+ (DIETSCHY AND MOORE[13]) and in its relative impermeability to small water-soluble non-electrolytes such as mannitol. Since the epithelia of the gall-bladder and distal biliary tree have a common embryological origin and a common physiological role (concentration of bile by selective reabsorption of water and electrolytes), it would not be surprising if they achieved this goal by the same mechanisms.

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