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Role of inorganic phosphate in total biliary phosphorus determination[1]

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Summary. Total phosphorus, inorganic phosphate, and phospholipids were measured in bile of rats and guinea pigs during choleresis and cholestasis produced by taurocholate and taurolithocholate, respectively. Under either experimental condition, total biliary phosphorus concentrations increased significantly in both species, due primarily to an increase in inorganic phosphate. These studies indicate that, if total phosphorus is taken as an estimate of biliary phospholipid concentration, correction for inorganic phosphate is essential under conditions associated with changes in bile secretory function.
Key words. Biliary phospholipids, bile flow; taurocholate; glycochenodeoxycholate; taurolithocholate.

Phospholipids (lecithins, lysolecithins, sphingomyelins, and cephalins) are, together with cholesterol, the major lipids present in bile of laboratory animals and man. Determination of phospholipid content of bile is of practical importance in studies of cholesterol gallstone formation and dissolution, and of bile secretory physiology and pathophysiology. For several years, biliary phospholipids have been measured by the Fiske and Subbarow reaction[2], following acid digestion of their molecules to inorganic phosphorus[3]. Recently, an enzymatic assay has been developed[4,5], but it measures only choline-containing phospholipids. Thus, determination of total biliary phosphorus is still commonly used for quantitating total biliary phospholipids. Although it has been demonstrated that inorganic phosphate is present in bile[6], it is assumed that the contribution of the latter to the total phosphorus content is minimal compared to that made by phospholipids. Hence, extraction of phospholipids from bile is not commonly carried out, and the total phosphorus concentration is often taken as an estimate of biliary phospholipid content. In research settings, however, biliary phospholipids are frequently measured under experimental conditions associated with changes in bile secretory function, yet no studies have ever examined the inorganic phosphate content of bile during changes in bile secretion rate. In the present report, our objective was to quantitate biliary inorganic phosphate in laboratory animals during choleresis and cholestasis, and to determine its contribution to total biliary phosphorus concentration.

Methods. Male Sprague-Dawley rats (270–320 g) were anesthetized with pentobarbital (50 mg/kg, i.p.), whereas albino male guinea pigs (400–500 g) with urethan (500 mg/kg, i.p.) and pentobarbital (20 mg/kg, i.p.). Animals (fasted for 24 h) were surgically prepared for bile collection by cannulating one jugular vein, one carotid artery, and the common bile duct. In the guinea pigs, the cystic duct was ligated, after the bile in the gallbladder was aspirated. Choleresis was produced by infusing 180 μmoles/kg/30 min of sodium taurocholate (rats, n = 5) or 120 μmoles/kg/30 min of sodium glycochenodeoxycholate (guinea pigs, n = 6) through the jugular vein cannula. Taurocholate and glycochenodeoxycholate are the physiological bile salts for these respective species. Cholestasis was induced by an i.v. injection of sodium taurolithocholate at 15 μmoles/kg to both rats (n = 4)

Bile flow and biliary concentrations of inorganic phosphate, total phosphorus, and phospholipids in rats and guinea pigs

Bile	Animal species	Spontaneous[a] secretion	Choleresis During[b]	Choleresis After[c]	Cholestasis During[b]	Cholestasis After[c]
Flow (μl/min/kg)	Rat	72.5 ± 4.9	121.3 ± 9.7*	66.4 ± 5.7	26.2 ± 3.7*	64.4 ± 5.3
	Guinea pig	193.6 ± 11.7	252.4 ± 13.5*	190.8 ± 9.9	72.4 ± 6.9*	184.5 ± 10,8
Inorganic phosphate	Rat	0.88 ± 0.17	0.79 ± 0.16*	2.14 ± 0.31*	1.49 ± 0.23*	1.27 ± 0.24*
(mmoles/l)	Guinea pig	0.79 ± 0.24	0.72 ± 0.21	3.39 ± 0.85*	2.75 ± 0.52*	2.94 ± 0.76*
Total phosphorus	Rat	6.75 ± 1.33	6.52 ± 1.15	8.26 ± 1.38*	8.39 ± 1.64*	7.19 ± 1.57
(mmoles/l)	Guinea pig	1.33 ± 0.41	1.27 ± 0.43	4.23 ± 0.77*	3.61 ± 0.72*	3.54 ± 0.56*
Phospholipids (mmoles/l)	Rat	5.15 ± 0.76	4.85 ± 0.59	5.47 ± 0.83	6.95 ± 1.13*	5.96 ± 1.22
	Guinea pig	0.27 ± 0.05	0.24 ± 0.05	0.34 ± 0.07*	0.56 ± 0.08*	0.43 ± 0.12*

Values are means ± SD and were obtained from 4–6 experiments for each group (see text). [a] Values were obtained 30 min after the common bile duct was cannulated, when a steady state bile flow rate was observed. [b] During maximal increase or decrease in bile flow (see text). [c] 60 min after the bile acid infusion (choleresis) or injection (cholestasis) was given. * Significantly different (paired t-test) when compared to the value observed during spontaneous secretion ($p < 0.05$–0.001).

and guinea pigs (n = 5). Bile was collected every 10 min during a 2–3 h experimental period. Blood was withdrawn from the carotid aretery (1.0 ml) during spontaneous secretion, and during and after choleresis or cholestasis. Total phosphorus was measured as described by Bartlett[3], whereas inorganic phosphate was quantitated enzymatically as reported by Hwang and Cha[7]. Choline-containing phospholipids (from now on referred to as phospholipids) were determined enzymatically[4]. The accuracy and reproducibility of these methods were validated in our laboratory.

Results and discussion. Inorganic phosphate, total phosphorus, and phospholipid concentrations in plasma were virtually constant throughout the bile collection period, regardless of whether bile secretion was stimulated of inhibited. Their plasma levels in rats and guinea pigs were, respectively: inorganic phosphate = 0.88 ± 12 (SD) and 0.97 ± 0.15 mmoles/l; total phosphorus = 2.33 ± 0.35 and 1.67 ± 0.27 mmoles/l, phospholipids = 1.68 ± 0.19 and 0.56 ± 0.09 mmoles/l. The biliary concentrations are reported in the table. Two features of these results are of importance. First, inorganic phosphate concentration in bile during spontaneous secretion accounted for approximately 12% of total biliary phosphorus in the rat, but as much as 60% in the guinea pig. Unlike the rat, in fact, the guinea pig's bile contained very low levels of phospholipids. Second, and more importantly, inorganic phosphate concentrations increased significantly in the post-choleretic period, and during and after cholestasis. In either species, the increment in inorganic phosphate levels accounted almost entirely for that seen in total biliary phosphorus.

At present, it is not clear why biliary inorganic phosphate concentration changes following alterations in bile secretion·rate produced by choleretic or cholestatic bile acids. The mechanism by which inorganic phosphate enters bile is not known, so that interpretation of the present results is not possible. Similarly, it remains to be demonstrated whether the changes observed in the rat and guinea pig occur in other laboratory animals and man.

To our knowledge, detailed studies of inorganic phosphate excretion into bile of laboratory animals have not been conducted, and available data are limited to clinical observations. Interestingly, however, Wiegard and Murphy[8] and Sutor and Wilkie[6] have observed a wide variation in inorganic phosphate levels in hepatic biles and duodenal aspirates of patients who underwent cholecystectomy. In some cases, inorganic phosphate accounted for up to 50% of total phosphorus[8]. It is thus possible that in man and other animal species as well, changes in inorganic phosphate concentrations occur, at least in part, secondarily to changes in bile secretory function.

In conclusion, these findings stress the importance of determining inorganic phosphate in bile when total biliary phosphorus, measured in whole bile samples, is taken as an estimate of phospholipid content. Our studies indicate that correction for inorganic phosphate is essential 1) in animal species, e.g. the guinea pig, in which the latter accounts for a major fraction of total biliary phosphorus; and 2) under conditions associated with changes in bile secretion rate.

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Urogastrone-epidermal growth factor is trophic to the intestinal epithelium of parenterally fed rats

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Summary. The weight of the stomach, small intestine and colon and the mucosal crypt cell production rate of these tissues were significantly decreased after 10 days on an isocaloric TPN diet when compared to orally fed controls. Continuous infusion of recombinant beta urogastrone, at a dose below that needed to inhibit gastric acid secretion, largely prevented this decrease in crypt cell production rate and gastrointestinal tissue weights.
Key words. Urogastrone; epidermal growth factor; gastrointestinal tract; trophic; total parenteral nutrition.

Despite the considerable interest shown in the in vitro actions of urogastrone-epidermal growth factor (Uro-EGF) a definitive physiological role for the polypeptide has yet to be confirmed. However the location of the main sites of production of Uro-EGF in the salivary glands and Brunners glands of the duodenum of man[1] and the rat[2] implies that Uro-EGF may have a role in the maintenance of gastrointestinal homeostasis.

β-urogastrone (human epidermal growth factor-hEGF) is a natural human polypeptide which has similar chemical, physical and physiological properties to rat and mouse EGF[3,4]. While the growth promoting actions of Uro-EGF in vitro are well characterized[5], its role in vivo is uncertain: in the fetus and newborn animal, Uro-EGF stimulates the proliferation and differentiation of the epidermis, maturation of the pulmonary epithelium and accelerates the healing of corneal epithelium[5]. Uro-EGF also stimulates the proliferation and maturation of the neonatal intestine[6–8], where it also increases the activity of intestinal ornithine decarboxylase[9], an enzyme associated with the initiation of rapid cell proliferation. The presence of Uro-EGF in a variety of body fluids, including saliva, plasma[5] and milk[10], its production by the salivary and Brunners glands[1,2], the trophic action of saliva on the intestine[11], the demonstration of Uro-EGF receptors in intestinal epithelial cells[12] and the reported cytoprotective effects on the duodenal mucosa[13] suggest that it has a role in the control of gastrointestinal homeostasis other than its ability to inhibit gastric acid secretion[18].

Although Uro-EGF would appear to stimulate intestinal epithelial cell proliferation in the neonate, its role in the adult intestine is not clear. Injection of Uro-EGF in rodents has been reported to increase the incorporation of tritiated thymidine into