is not correlated to their morphology. That is to say, the distribution of electrophoretic mobilities within such a population of lymphocytes or granulocytes does not reflect the fact that large, medium and small lymphocytes are present, neither does it reflect the ratio of eosinophils: basophils : neutrophils in the granulocyte population.

It has been shown[3] that for different cells the relation between the electrophoretic mobility and the sialic acid released is by no means constant. In fact, granulocytes having more sialic acid per μm^2 of surface, have less charge than other cells[3]. These data and the curves reported here suggest the presence of other ionogenic groups, that in the case of platelets and of some lymphocytes become cationic at low pH, such as amino groups.

Riassunto. Viene riportato uno studio sui gruppi carichi alla superficie di alcune cellule del sangue, usando la tecnica della microelettroforesi. Differenze in mobilità elettroforetica, in funzione del pH, per piastrine, eritrociti, linfociti e granulociti sono presentate.

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[10] The authors wish to thank Dr. C. A. BOUVIER and Dr. J. RITSCHARD of the Hôpital Cantonal de Genève for their advice and encouragement.

Effect of Vasopressin on Hepatocytic and Ductal Bile Formation in the Dog

According to the current view[1], the secretion of bile by the hepatocytes is primarily dependent upon bile salt excretion. This 'canalicular bile' may subsequently be modified in bile ductules and ducts by the net addition of water and electrolytes. The latter mechanism is exemplified by the effects of the gastrointestinal hormone secretin[2].

A variety of other hormones have been shown to affect hepatic function and in particular bile formation. Except for hydrocortisone[3], their mechanism of action is as yet ill-defined. Furthermore, disparate results have been reported following administration of the pituitary hormone vasopressin[4,5]. They may reflect differences in methods of bile collection, the interference of anesthetic drugs and the lack of replacing the interrupted enterohepatic circulation with continuous infusions of bile salts.

To resolve these difficulties, the present studies were undertaken in the non-anaesthetized dog. The results clearly demonstrate a choleretic effect of vasopressin in the dose range employed.

Material and methods. The experiments were performed in 8 adult unanaesthetized female dogs (body weight 15–25 kg) at least 1 month previously cholecystectomized and equipped with a THOMAS[6] duodenal cannula. Prior to each study, the animals were fasted for 16 h. The common bile duct was catheterized under direct vision with a ureteral catheter (No. 6, french size) and quantitative collection of the biliary output was obtained by gravity drainage. To compensate for the interrupted enterohepatic circulation of bile salts, a continuous i.v. infusion of pure sodium taurocholate[7] (approximately 12 μEq per min) was administered by way of a polyethylene catheter throughout the study. Suppression of endogenous secretin release was achieved by i.v. injection of the anticholinergic pipenzolate methylbromide[8]. This procedure was shown previously to result in a relative steady state of bile formation[2]. Following a control period of at least 60 min, lysine-8-vasopressin[9] was given as slow single i.v. injections (during 10 min) in a dose of 0.5 U/kg body weight.

In 2 experiments erythritol-^{14}C[10] was administered at a rate of about 0.04 μCi/min after an initial priming dose of approximately 3 μCi[11,12]. An equilibration period of at least 60 min. was followed by the control and experimental periods. In these studies, sodium taurocholate was administered at a rate of 24 μEq/min. Carbon dioxide content in bile was measured with a Natelson microgasometer, and bicarbonate concentrations were calculated assuming a P_{CO_2} of 40 mm Hg. Chloride content was assessed with the Cotlove titrimeter, sodium and potassium concentrations with flame-photometry.

Bile acid concentrations were calculated as the difference between $\Sigma[Na^+ + K^+]$ and $\Sigma[Cl^- + HCO_3^-]$[2]. This procedure was shown to yield results in excellent agreement with direct determination of bile salts using a purified hydroxysteroid dehydrogenase[13]. Radioactivity in plasma and bile samples was measured with a Packard Tri-Carb model 3380 liquid scintillation spectrometer.

Results. An increase in bile flow was noted in all 8 studies within minutes following the start of the vasopressin injection. The increment in flow varied from 10% to 190% and was on the average 55% in excess of control values.

A typical study is depicted in the Figure. During this choleresis, in general, the concentrations of Na^+ and K^+ in bile fell slightly, whereas HCO_3^- and Cl^- concentrations rose. Consequently, the calculated bile acid content decreased in every instance.

Except for the output of bile salts, which remained relatively unchanged during the vasopressin-induced choleresis, the excretion rate of all electrolytes increased significantly in a given experiment. The mean increment amounted to 17.6 μEq/min of Na^-, 0.6 μEq/min of K, 10.1 μEq/min of Cl^- and 7.4 μEq/min of HCO_3^- (Table). The composition of this increment, calculated as the ratio of the increment in output of each constituent and the increment in flow, was characterized by a high bicarbonate (average 66.5 mEq/l) and chloride (average 98.6 mEq/l) concentration, similar to that observed following administration of the hormone secretin[2].

The clearance of erythritol-^{14}C was measured before and after administration of vasopressin in 2 experiments. The average clearance during the control phase ranged

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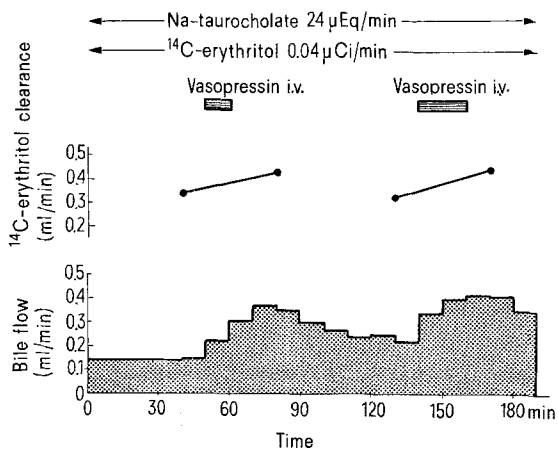
Composition of maximal increment in bile flow during pitressin choleresis

Dog	Maximal increment (μEq/min)					Composition of increment (mEq/l)			
	ΔF ml/min	Na^+	K^+	Cl^-	HCO_3^-	Na^+	K^+	Cl^-	HCO_3^-
Al	0.05	7.13	0.18	3.97	0.52	143	3.6	79	10
Gr	0.04	5.82	0.30	6.18	2.34	146	7.5	155	59
Co	0.07	11.48	0.32	3.11	2.76	164	4.5	44	39
No	0.05	5.45	0.32	5.35	5.55	109	6.4	107	111
No	0.26	39.38	1.21	21.26	16.46	151	4.7	82	63
Ve	0.08	17.04	0.66	10.54	8.86	213	8.3	132	111
Gu	0.16	29.33	1.09	15.72	9.52	183	6.8	98	60
Le	0.16	26.16	1.16	14.34	14.92	164	7.3	90	93
Pi	0.10	16.12	0.30	10.08	5.46	161	3.0	101	55
Averages	0.10	17.6	0.6	10.1	7.4	159.3	5.8	98.6	66.5



Representative study of the vasopressin effect on bile flow in the unanesthetized dog. Note the increase in the erythritol clearance associated with the choleresis.

from 0.33 to 0.39 ml/min and was in accordance with the values in the dog published by others[3,11]. Following vasopressin injection, the clearances increased to 0.43 and 0.47 ml/min. Although these alterations were roughly proportional to those in bile flow, the bile to plasma concentration ratio for erythritol-^{14}C tended to decrease.

Discussion. The biliary clearance of metabolically inert, highly diffusable solutes such as urea, creatinine, mannitol and erythritol has been shown to be linearly correlated with taurocholate excretion in the dog. This relationship forms the basis for assessment of 'canalicular bile flow' by clearance measurements[11,12]. However, the positive intercept for mannitol clearance, when plotted against bile acid excretion[11], the suppression of a fraction of hepatocytic bile by inhibitors of sodium transport[14] and the type of choleresis following hydrocortisone[3] all indicate that mechanisms independent of active bile salt transport may play a role in 'canalicular bile flow'.

Such a mechanism may have been activated by vasopressin. Certainly, the observed increase in the erythritol clearance strongly suggests that vasopressin acts at the hepatocyte. Although this effect was not examined at differing levels of bile acid output, from the relative lack of change in taurocholate excretion during vasopressin choleresis it may be inferred that this hormone appears to act through a mechanism independent of bile acid transfer.

In contrast to the hydrocortisone choleresis, which roughly results in the net addition of plasma water to bile, the electrolyte composition of the vasopressin-induced increment was characterized by a high bicarbonate concentration.

Thus, the calculated HCO_3^- content was considerably higher (average 67 mEq/l) than that following hydrocortisone (average 40 mEq/l). The correspondence of this increment with the bicarbonate and chloride rich fluid 'obligated' by secretin suggests that vasopressin may have a similar effect. Since the secretin-induced choleresis, however, does not affect the clearance of inert solutes[11] – in keeping with the inference that it acts on the ductular and ductal level – the vasopressin choleresis may represent the result of a dual action of this hormone. In this view, vasopressin might have a primary effect in influencing the hepatocellular sodium transport mediated by the Na^+-K^+-activated adenosine triphospatase. A second effect resulting in the net addition of an alkaline fluid might be exerted on the epithelia of the biliary duct system. Whether this is due to a direct action of vasopressin, or whether it could be induced by a secondary release of endogenous secretin, cannot be discerned. Clearly, further work, including an analysis of the potential effects of the well known vasopressin-induced changes in hepatic hemodynamics, will be required to substantiate this hypothesis.

Zusammenfassung. In Studien an 8 cholecystektomierten, mit einer THOMAS-Kanüle versehenen, nicht-anaesthesierten Hunden konnte nach i.v. Injektion von 0,5 E pro kg Körpergewicht Lysin-8-Vasopressin eine Erhöhung des Gallenflusses um durchschnittlich 55% beobachtet werden. Da die biliäre Clearance von ^{14}C-Erythritol – ein Mass des canaliculären Gallenflusses – gleichzeitig anstieg, die Cholerese aber als Zusatz einer HCO_3^- und Cl^- reichen Flüssigkeit imponierte, scheint es wahrscheinlich, dass Vasopressin hepatocytäre und ductuläre Mechanismen der Gallenbildung beeinflusst.

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