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Characteristics Common to Choleretic Increments of Bile Induced by Theophylline, Glucagon and SQ-20009 in the dog[1] (39086)

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The possibility that cyclic-AMP (cAMP) regulates production of bile flow at the canaliculus has been suggested by a number of observations. Theophylline, a compound which increases intracellular cAMP by inhibiting its breakdown by phosphodiesterase, stimulates secretion of the bile salt-independent fraction of canalicular bile flow in the dog (1–5). This fraction of bile in the dog also appears to be stimulated by administration of the dibutyryl derivative of cAMP (1).

In the present study, we report on the characteristics of the increased bile formed in response to administration of additional compounds that also presumably increase the intracellular concentration of cAMP. Glucagon, which stimulates adenylate cyclase (6,7), and SQ-20009, a phosphodiesterase inhibitor (8, 9), were found to enhance canalicular bile production. Moreover, the electrolyte composition of the increment in bile induced by theophylline, glucagon, and SQ-20009 was similar. These observations, in addition to the recent report of Khedis *et al.* (10) that glucagon stimulates this fraction of bile flow, provide support for an important role of cAMP in the sequence of events leading to the formation of the bile salt-independent fraction of canalicular bile in the dog.

Materials and Methods. Animal preparation. Studies were performed on 14 mongrel dogs fasted overnight and anesthetized with sodium pentobarbital administered intravenously in a dose of 30 mg/kg. Preparation included midline laparotomy, ligation of both renal pedicles, ligation of the cystic duct, and cannulation of the common bile duct (PE 190) and both external jugular veins (PE 90). A femoral artery was cannulated with a Cournand needle. Heating pads under the dog were used to maintain a constant body temperature (37°C to 38°C) as monitored by a TeleThermometer (Yellow Springs Company) connected to a rectal probe.

Experimental procedures. Infusions were administered with a Bowman pump into both jugular veins at a constant rate of 0.9 ml/min. A mixture of sodium taurocholate (Calbiochem 2 mg/ml) and ^{14}C-erythritol (Amersham/Searle; 0.15 μCi/ml) was infused continuously into one jugular vein while saline or one of the treatment compounds was administered into the other jugular vein.

The basic design of each experiment was as follows. (1) A recovery period of 60–90 min was allowed after the completion of the preparation of each dog. (2) During a control period of 60–90 min, samples of bile were collected at 10-min intervals, and samples of arterial blood were taken at the midpoint of each bile collection interval. (3) The treatment compound was administered as indicated below and 15 min later, sampling of blood was reinitiated and continued for 60–90 min in the manner described for the control period. Results reported in the tables for the control period represent the mean of all values obtained during that period. Treatment figures reported in the tables represent mean values determined during either the first half or the second half of the treatment period. The same control value was paired with both treatment results.

Treatment compounds. Theophylline (Sigma) was administered intravenously over a 5-min period in an average dose of 19.9 mg/kg (range 19.1–20.8 mg/kg) to five dogs (mean body wt = 19.2 kg; mean liver wt = 493 g).

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Glucagon (Calbiochem) was administered to four dogs (mean body wt = 18.5 kg; mean liver wt = 504 g). In three, an initial loading dose of 108 μg was administered followed by a continuous mean infusion of 0.58 μg/kg/min (range 0.44–0.80 μg/kg/min). In one dog (BB), glucagon was given in a single intravenous dose of 2.2 μg/kg after an infusion of SQ-20009 was stopped and a control period had been reestablished. SQ-20009[3] [1 - ethyl - 4 - (isopropylidenehydrazino) - 1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester, HCl] was administered to five dogs (mean body wt = 20.3 kg; mean liver weight = 572 g). Dog BA received a single intravenous injection of 4.7 mg/kg. The other dogs received an initial loading dose of 2 mg followed by a constant mean infusion of 0.073 mg/kg/min (range 0.040–0.092 mg/kg/min).

Analytical procedures. Total bile salt concentration in bile was determined enzymatically using 3α-hydroxy-steroid dehydrogenase (Worthington Biochemical Corp.) according to a method described previously (5). Sodium and potassium concentrations in bile and plasma were determined by flame photometry (IL Flame Photometer, Model 143, Instrumentation Laboratory, Inc.) and chloride concentrations in bile and plasma were determined by titration with a silver electrode (Buchler–Cotlove Chloridometer, Buchler Instruments, Inc.). The concentration of bicarbonate ion in bile was assayed as described by Shaw and Heath (11). An aliquot of bile (0.2 ml) and of 0.1 *N* HCl (0.3 ml) were mixed and heated to boiling in a sand bath; then the mixture was titrated back to a phenolphthalein endpoint with 0.01 *N* NaOH. The number of milliequivalents of HCl neutralized by the sample of bile was equated to the number of milliequivalents of bicarbonate ion.

Concentrations of ions in the choleretic increments of bile ($[\text{Ion}]_I$) were calculated as follows.

$$[A]_I = \frac{[A]_t(BF)_t - [A]_c(BF)_c}{(BF)_t - (BF)_c}$$

where $[A]$ = concentration of ion, BF = rate of bile flow and subscripts c and t represent time periods, control and treatment, respectively.

For measurement of radioactivity, aliquots of plasma (200 μl) and bile (50 μl) were dissolved in 10 ml of a scintillation cocktail consisting of 0.4% 2,5-phenyloxazole (PPO) and 0.01% 1,4-bis-2-(5-phenyloxazolyl) benzene (POPOP) in toluene. BIO-SOLV BBS-3 (Beckman) was added to the scintillation cocktail (10%, v/v) to solubilize aqueous samples. Radioactivity was measured with a Packard 2420 liquid scintillation spectrometer. Quench was corrected by external standardization.

Erythritol clearance in bile was determined as described by Macarol *et al.* (12).

Statistical analysis. Linear regression analysis by the method of least-squares and testing for significant difference between means by *t*-statistics (paired and unpaired) were determined on a Wang Model 600 programmable calculator (Wang Laboratories, Inc.) using statistical programs supplied by Wang Laboratories (program numbers 1000-2-ST3, 1009-2-ST2 and 1010-2-ST2).

Results. Bile flow, ^{14}C-erythritol clearance and bile salt excretion (Table I). All three compounds significantly increased bile flow. Choleresis, in each instance was accompanied by a proportionate increase in ^{14}C-erythritol clearance in bile (Fig. 1). The average ratio for Δ erythritol clearance/Δ bile flow for each compound was as follows: theophylline, 1.12; glucagon, 1.07; and SQ-20009, 0.85. None of these means was statistically significantly different from 1.0 or from each other. The combination of all of the individual data were described by a line passing through zero with a regression coefficient of 1.05 (Fig. 1).

Bile salt excretion decreased 1.5 μmoles/min on the average in dogs receiving theophylline and did not change significantly in animals given the other compounds. Thus choleresis induced by each of these substances is independent of and cannot be the consequence of an increase in bile salt excretion.

Electrolyte composition. The concentrations of sodium, potassium, chloride, and bi-

[3] SQ-20009 was a gift from The Squibb Institute for Medical Research, Princeton, New Jersey.

TABLE 1. EFFECT OF THEOPHYLLINE, GLUCAGON, AND SQ-20009 ON BILIARY CLEARANCE OF ^{14}C-ERYTHRITOL AND EXCRETION OF BILE SALTS IN THE DOG

Treatment	Number of dogs	Bile flow (ml/min (SD))			^{14}C-erythritol clearance (ml/min (SD))			Bile salt excretion (μmoles/min (SD))		
		Control	Treatment	Δ	Control	Treatment	Δ	Control	Treatment	Δ
Theophylline	5	0.167	0.278[b]	0.111	0.244	0.366[b]	0.122	7.1	5.6[a]	−1.5
		(0.059)	(0.109)	(0.058)	(0.111)	(0.151)	(0.064)	(4.2)	(4.0)	(1.5)
Glucagon	4	0.168	0.251[a]	0.083	0.242	0.330[a]	0.088	6.5	7.6	1.1
		(0.038)	(0.063)	(0.047)	(0.084)	(0.106)	(0.048)	(3.2)	(3.1)	(1.4)
SQ-20009	5	0.151	0.206[b]	0.055	0.201	0.248[b]	0.047	6.4	6.5	0.1
		(0.047)	(0.043)	(0.032)	(0.048)	(0.053)	(0.024)	(3.2)	(2.8)	(1.4)

[a] By paired t-statistics, treatment group significantly different than control group. ($p < 0.01$.)
[b] ($p < 0.001$.)

carbonate ions were determined in bile taken from the dogs during the control and treatment periods. The mean results are presented for each dog receiving theophylline in Table II, glucagon in Table III and SQ-20009 in Table IV. The calculated electrolyte composition of the increment in bile generated after treatment with the respective choleretic agents are reported in their respective tables and are summarized in Table V.

Basically, the electrolyte composition was practically the same after treatment with each compound (Table V). $[K^+]_I$ was somewhat lower after theophylline treatment.

The composition of electrolytes in the induced increments of bile is compared with the composition of the electrolytes in control bile in Table V. The major differences observed are that $[Na^+]_I$ is lower and $[Cl^-]_I$ is higher than the respective ion concentrations in control bile.

Discussion. Theophylline, glucagon, and SQ-20009 induced similar choleretic responses in the dog. The increment in bile flow in each instance was accompanied by a proportionate increase in erythritol clearance, suggesting the canaliculus as the anatomical site of increased bile formation (13–15). Moreover, bile salt excretion either fell slightly or did not change. Thus, these agents stimulated formation of the bile salt-independent fraction of canalicular bile flow. These observations agree with other findings reported for theophylline (1–5) and glucagon (10, 16).

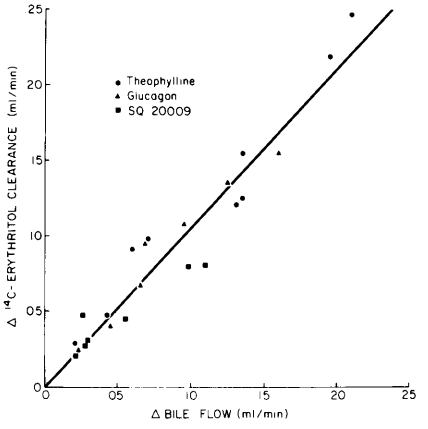

FIG. 1. Effect of theophylline, glucagon and SQ-20009 on bile flow and clearance of ^{14}C-erythritol in dogs. Each point represents the mean of data obtained from at least three 10-min collection periods.

The concentrations of electrolytes in incremental bile were similar for all three compounds used. They were also similar to values we have calculated for sodium (130 μEq/ml) from data presented by Erlinger and Dumont after administration of theophylline (4); for sodium (133), potassium (5.2), chloride (111) and bicarbonate (40), from data presented by Khedis *et al.* (10), during infusion of glucagon; and for chloride and bicarbonate concentrations in the electrolyte fraction of bile observed during glucagon choleresis by Jones *et al.* (16).

Bile generated by the compounds utilized in the present study was isosmotic. In four dogs given either theophylline or SQ-20009, osmolarity of bile determined by freezing

TABLE II. EFFECT OF THEOPHYLLINE ON ELECTROLYTE COMPOSITION OF BILE ON THE DOG

Dog	Δ bile flow[a] (ml/min)	[Na] μEq/ml Control	Treat	Δ	[K⁺] μEq/ml Control	Treat	Δ	[Cl⁻] μEq/ml Control	Treat.	Δ	[HCO_3^-] μEq/ml Control	Treat.	Δ
EA1[b]	0.021	164	159	123	6.6	6.1	2.5	61.0	70.7	140	36.4	38.0	49.6
EA2[b]	0.042	164	154	118	6.6	5.8	2.9	61.0	75.0	126	36.4	38.9	47.9
M2	0.061	177	158	138	5.5	4.4	3.3	48.9	53.4	58.0	68.8	79.8	91.2
M1	0.071	177	162	149	5.5	4.1	2.9	48.9	56.2	62.7	68.8	71.8	74.5
N1	0.131	185	168	139	7.4	6.1	3.9	63.3	77.7	102	40.1	34.3	24.3
N2	0.135	185	163	126	7.4	5.9	3.4	63.3	80.4	110	40.1	30.4	14.2
EK2	0.136	168	154	133	6.7	4.8	2.0	56.6	75.0	102	44.6	47.7	52.3
EK1	0.143	168	157	141	6.7	4.6	1.6	56.6	76.0	103	44.6	46.4	48.9
EH2	0.194	158	149	138	6.2	5.0	3.6	68.0	85.0	105	35.2	35.4	35.6
EH1	0.210	158	151	143	6.2	5.1	3.9	68.0	84.0	101	35.2	38.0	41.0
Mean	0.114	170	158	135[c]	6.5	5.2	3.0[c]	59.6	73.3	101[c]	45.0	46.1	48.0
SD	0.063	10.1	5.8	9.7	0.66	0.74	0.78	6.8	10.7	24.9	13.0	16.6	22.4

[a] Theophylline-induced increment in bile flow.

[b] Number refers to respective half of treatment period in this and other tables; 1 = 1st half; 2 = 2nd half.

[c] By paired *t*-statistics, concentration in theophylline-induced increment significantly different from concentration in control group ($p < 0.001$).

TABLE III. EFFECT OF GLUCAGON ON ELECTROLYTE COMPOSITION OF BILE IN THE DOG

Dog	Δ bile flow[a] (ml/min)	[Na^+] μEq/ml Control	Treat.	Δ	[K^+] μEq/ml Control	Treat.	Δ	[Cl^-] μEq/ml Control	Treat.	Δ	[HCO_3^-] μEq/ml Control	Treat.	Δ
BB[b]	0.023	154	151	130	3.8	4.2	6.2	72.0	74.4	91.5	—	—	—
EG1	0.046	159	151	115	7.3	7.1	6.2	80.0	80.0	80.0	27.2	33.2	59.8
QF1	0.066	168	167	165	5.2	5.1	5.0	84.7	90.7	101	37.0	39.0	42.5
EG2	0.068	159	152	125	7.3	6.9	5.4	80.0	85.0	104	27.2	27.8	30.1
QF2	0.095	168	166	163	5.2	5.1	5.0	84.7	94.7	107	37.0	36.1	35.0
QA1	0.124	149	152	157	5.6	6.0	6.6	66.4	80.5	102	35.1	33.4	30.8
QA2	0.159	149	155	170	5.6	6.2	7.2	66.4	84.3	109	35.1	31.4	29.1
Mean	0.083	158	156	146	5.7	5.8	5.9	76.3	84.2	99.2[c]	33.1	33.5	37.9
SD	0.047	8.0	7.1	22.4	1.20	1.05	0.84	8.0	6.8	10.2	4.6	3.8	11.8

[a] Glucagon-induced increment in bile flow.

[b] SQ-20009 given to dog in a treatment period prior to glucagon dosage.

[c] By paired *t*-statistics, concentration in glucagon-induced increment significantly different from concentration in control group ($p < 0.01$).

point depression did not change significantly during choleresis induced by these agents (control period mean osmolarity 277 ± SD 21 mOsm; during choleresis, mean osmolarity 286 ± SD 15 mOsm); and the calculated osmolarity of the incremental bile in all experiments averaged 292 Osm/liter.

Because of the diverse chemical nature of the compounds, and the impossibility that they could have induced cholersis as a result of osmotic activity of the compound itself excreted into bile (complete biliary excretion of the respective compounds would not account for observed increment in bile (5)), it seems likely that they stimulated bile flow through some common pathway, presumably via cAMP. All of the compounds have the potential to increase intracellular concentration of cAMP, glucagon via stimulation of adenylate cyclase, theophylline and SQ-20009

TABLE IV. EFFECT OF SQ-20009 ON ELECTROLYTE COMPOSITON OF BILE IN THE DOG

Dog	Δ Bile flow[a] (ml/min)	[Na^+] μEq/ml			[K^+] μEq/ml			[Cl^-] μEq/ml			[HCO_3^-] μEq/ml		
		Control	Treat.	Δ	Control	Treat.	Δ	Control	Treat.	Δ	Control	Treat.	Δ
QH	0.022	172	168	122	5.8	6.2	9.6	77.7	82.0	136	38.9	39.3	45.5
BA	0.026	161	159	148	5.2	5.1	4.5	53.8	58.2	82.9	—	—	—
BB2	0.028	163	152	99	4.3	3.8	1.4	58.6	73.0	142	—	—	—
BB1	0.031	163	159	142	4.3	3.9	2.2	58.6	70.0	119	—	—	—
QH	0.055	163	167	180	6.1	5.9	5.1	82.0	83.0	86.9	44.1	45.0	48.5
BC2	0.061	140	145	155	5.0	6.0	8.0	50.7	65.7	95.4	—	—	—
BC1	0.063	140	126	99	5.0	4.8	4.4	50.7	57.3	70.1	—	—	—
QC1	0.098	148	154	161	5.1	5.1	5.1	59.0	78.5	103	39.5	49.1	61.0
QC2	0.111	148	155	163	5.1	4.8	4.5	59.0	78.8	101	39.5	46.8	54.8
Mean	0.055	155	154	141	5.1	5.1	4.9	61.1	71.8	104[b]	40.5	45.0	52.4
SD	0.032	11.5	12.7	28.5	0.59	0.86	2.56	11.2	9.7	24.1	2.4	4.2	6.9

[a] SQ-20009-induced increment in bile flow.

[b] By paired *t*-statistics, concentration in SQ-20009-induced increment significantly different from concentration in control group ($p < 0.001$).

TABLE V. COMPARISON OF ELECTROLYTE COMPOSITION IN INCREMENTS OF BILE GENERATED IN RESPONSE TO THEOPHYLLINE, GLUCAGON AND SQ-20009 TO ELECTROLYTE COMPOSITION IN CONTROL BILE

	Number of dogs	[Na^+]	[K^+]	[Cl^-]	HCO_3^-]
			(μEq/ml)		
Choleretic increment					
Theophylline	5	135	3.0	101	48.0
Glucagon	4	146	5.9	99.2	37.9
SQ-20009	5	141	4.9	104	52.4
Composite mean	14	140[b]	4.5[a]	102[b]	45.8
(± SD)		(20.5)	(2.0)	(20.9)	(17.7)
Control bile	14	162	5.8	64.6	40.5
(± SD)		(12.0)	(1.0)	(11.2)	(10.7)

[a] Concentration significantly different from concentration in control bile. ($p < 0.01$.)

[b] ($p < 0.001$.)

by inhibition of phosphodiesterase activity. Moreover, Morris, in a preliminary communication, has reported that administration of dibutyryl cAMP induces choleresis in the dog (1), an observation confirmed in two dogs in unpublished observations in our laboratory.

Hydrocortisone also has been shown to stimulate the bile salt-independent fraction of canalicular bile flow in the dog (12). This may also be linked to cAMP since a number of observations indicate that hydrocortisone and other corticosteroids may inhibit phosphodiesterase activity in several tissues including liver (17–19).

The mechanism by which increased cAMP leads to choleresis is not yet established but probably involves stimulation of electrolyte transport at the canaliculus. Inhibition of water and electrolyte reabsorption in the bile ducts does not seem to be an important factor. During the control period, erythritol clearance exceeded bile flow. Since erythritol enters bile by passive processes primarily at the canaliculus (13–15), an erythritol clearance/bile flow ratio greater than one indicates reabsorption of water to a greater extent than erythritol at some site distal to erythritol entry into bile. In the present studies, a broad range of increments in bile

flow induced by theophylline, glucagon, and SQ-20009 was accompanied by proportionate increments in erythritol clearance (See Table I and Fig. 1). It is unlikely that this would be the consequence of inhibition of ductular reabsorption of water.

Summary. Theophylline, glucagon, and SQ-20009 induce a choleresis in the dog characterized by a proportionate increase in erythritol clearance and bile flow, no increase in bile salt excretion, and by an isosmotic solution of similar electrolyte composition. The increment in bile appears to originate at the canaliculus in response to increased cyclic-AMP.

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