[Chem. Pharm. Bull. 34(1) 349—359 (1986)]

Effects of Various Cathartics and Deoxycholic Acid on the Disposition of Endogenous Bile Acids in the Bile, Portal Blood and Feces of Rats

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(Received May 28, 1985)

The effects of various cathartics (magnesium sulfate, mannitol, dioctyl sodium sulfosuccinate (DSS), castor oil and pilocarpine) and deoxycholic acid (DCA) on the disposition of endogenous bile acids in the bile, portal blood and feces of rats were investigated. The biliary bile acids of free type increased significantly after the oral administration of cathartics except for DSS. The amounts of glyco- and tauro-conjugated bile acids showed different patterns after the oral administration of various cathartics. However, the proportion of DCA significantly increased in the case of diarrhea induced by cathartics, and the proportion of cholic acid (CA) conversely decreased compared with the control. Thus, it was suggested that unconjugated bile acids eliminated into the intestinal lumen and DCA formed from CA participate in the occurrence of diarrhea after oral administration of various cathartics. Moreover, the ratio of glycine- to taurine-conjugated bile acid of rat bile increased when diarrhea was induced by various cathartics. Thus result suggested that the reabsorption of tauro-conjugated bile acids is decreased, or that the reabsorption of glyco-conjugated bile is increased in diarrhea.

On the other hand, DCA caused diarrhea in rats. The occurrence of diarrhea was concentrated within 20 to 24 h after the oral administration of DCA. Since biliary unconjugated and glyco-conjugated bile acids were increased at 24 h after DCA treatment, whereas glyco-conjugated bile acids were decreased in the portal blood, it is conceivable that deconjugation is accelerated by changes in the activity of intestinal bacteria and in the liver after DCA treatment. Active transport of tauro-conjugated bile acids from the end of the ileum may have been impaired, because the absorption of tauro-conjugated bile acids was inhibited from 1 h after the administration of DCA.

Keywords——cathartic; deoxycholic acid; cholic acid; bile; portal blood; feces; unconjugated bile acid; glyco-conjugated bile acid; tauro-conjugated bile acid; diarrhea

Bile salts have important roles as physiological surfactants in the intestinal absorption of lipid.[1,2] It is well-known that, besides the acceleration of bile secretion, bile acids inhibit the transport of water and electrolytes in the colon,[3-7] and also inhibit the over-growth of bacteria in the intestine.[8] However, the effects of individual bile acids differ, and information about the activity of several bile acids is not complete.

On the other hand, bile acids undergo biochemical transformations such as hydroxylation, dehydroxylation or epimerization by the action of intestinal bacteria or in the liver.[9,10] Therefore, when a change in the environment of the gastrointestinal lumen occurs, or in the case of hepatobiliary disease, the effect of endogenous bile acids may be greatly modified by changes in metabolism or reabsorption during enterohepatic circulation. In fact, inhibition of reabsorption of bile acids was observed in a functional disorder of the ileum[11,12] and accleration of deconjugation of bile acids was observed in over-propagation of gut flora during diarrhea.[13,14]

In the present study, diarrhea was chosen as a model disease in which the environment of

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the intestinal lumen is changed. It is gnerally believed that certain types of diarrhea are related to a stimulation of intestinal motility and mesenteric blood vessel permeability, and to an inhibition of water reabsorption. We previously reported that these actions were mediated by endogenous substances, serotonin, histamine, polyamine[15] and cholecystokinin.[16] In this work, we examined the disposition of bile acids in the bile, portal blood and feces and studied the physiological significance of bile acids in diarrhea. The diarrheal model was produced by administering cathartics of various types: magnesium sulfate and mannitol as saline cathartics: dioctyl sodium sulfosuccinate (DSS) as a wetting cathartic; castor oil as an irritant cathartic and pilocarpine corresponding to a cholinergic stimulant.

It is also known that deoxycholic acid (DCA) causes diarrhea, due to the stimulation of intestinal motility, or the inhibition of the transport of Na and water in the intestinal mucosa.[17-19] This paper also reports the changes of biliary, portal blood and fecal bile acids in diarrhea induced after the oral administration of exogenous DCA and discusses the physiological significance of endogenous bile acids in the case of diarrhea induced by DCA.

Experimental

Animals——Male Wistar rats weighing 180 to 230 g were used in this investigation. Standard food (F-2, Funabashi Laboratory) was withheld for 20—24 h prior to all the experiments; there was free access to drinking water.

Induction of the Diarrheal Model——Non-diarrheal rats were selected before the experiment. Magnesium sulfate (300 mg/kg), mannitol (4000 mg/kg), DSS (1500 mg/kg), castor oil (5 ml/kg) and pilocarpine (10 mg/kg) were orally given through a stomach tube. All rats developing diarrhea at 2 h after the administration of cathartics were used a diarrheal rats for the following experiments.

Cathartic Effect of DCA——Non-diarrheal rats were selected before the experiment. DCA was orally given through a stomach tube at a dose of 25 to 1500 mg/kg. After the administration of DCA, the rats were observed form 0 to 4 h according to the method described by Tsurumi *et al.*[20]

A dose with a cathartic efficiency of 100%, 100 mg/kg, was selected to produce the model of diarrhea.

Collection of Samples——Collection of the Bile: Rats were kept in an air-conditioned room (25 ± 1 °C, 50—60% humidity). Under anesthesia with ether, the distal common bile duct was cannulated with polyethylene tubing (P-E 10) following a midline abdominal laparotomy. Bile samples were collected from 0 to 1 h, 1 to 2 h and 2 to 8 h after the oral administration of various cathartics. In the case of DCA, the bile was pooled from 0 to 6 h, 0 to 12 h, 0 to 20 h, 0 to 24 h or 0 to 28 h after the oral administration of DCA.

Collection of the Portal Blood: The abdomen of diarrheal rat induced with various cathartics was opened under anesthesia with ether, and a heparinized syringe was inserted into the portal vein. The blood was allowed to drain slowly. In the case of DCA, the portal blood samples were collected from the portal vein at 1, 3, 6, 12, 20, 24 and 28 h after the oral administration of DCA.

Collection of Feces: Rats were housed individually in metabolism cages. The feces were collected from 0 to 8 h after oral administration of various cathartics, and from 0 to 12 h and 12 to 24 h after oral administration of DCA.

Extraction and Analysis of Bile Acids——The bile, portal blood and feces samples were each refluxed for 2 h with ethanol. After filtration, the ethanol extracts were quantitatively transferred to a Sep-Pak C 18 cartridge (Waters Assoc.) and eluted with the same solvent. Unconjugated, glyco- and trauro-conjugated bile acids were separated form total bile acids extracted with ethanol according to the method described by Goto *et al.*[21] The conjugated bile acids were hydrolyzed with cholylglycine hydrolase (Clostridium Welchii acetone powder, Sigma Chemical Co.). The methyl ester-trifluoroacetate derivatives of individual free bile acids obtained by hydrolysis and 5β-cholanic acid as an internal standard were subjected to gas-liquid chromatography.

A Hitachi gas chromatograph (model 164) equipped with a flame-ionization detector was used. The coiled glass column (1.0 × 3 mm i.d.) was packed with 2% silicon DC QF-1 on 60—80 mesh Uniport HP (Packard). The column temperature was kept at 220 °C. The nitrogen (carrier gas) flow-rate was 40 ml/min and the hydrogen and air flow rates were 0.9 and 1.4 kg/cm^2, respectively.

In this experiment, free type, glyco- and tauro-conjugated lithocholic acid (LCA), DCA, ursodeoxycholic acid (UDCA), chenodeoxycholic acid (CDCA) and cholic acid (CA) were measured. Hyodeoxycholic acid was determined as a measure of UDCA because the retention time of HDCA is the same as that of UDCA.

Determination of Na and K in the Serum——Electrolytes (Na and K) levels were determined by means of a flame photometer (Corning Ltd., type 435).

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Results

Cathartic Effect of Deoxycholic Acid

DCA at doses from 25 to 100 mg/kg (*p.o.*) caused diarrhea in a dose-dependent manner. In contrast, large doses of DCA (over 300 mg/kg, *p.o.*) inhibited the occurrence of diarrhea. The occurrence of diarrhea was concentrated within 20 to 24 h after the administration of DCA (Table I). This is delayed as compared with the action of other cathartics.[15] Mortality due to cardiac toxicity was noted at a dose of 300 mg/kg, *p.o.*, or more.

Alteration of Biliary Bile Acids after Administration of Various Cathartics and DCA

Tables II and III show the bile acids contents of bile collected from 0 to 1 h and 1 to 2 h after the oral administration of various cathartics. Biliary bile acids contents were expressed as $\mu g \cdot ml^{-1}$, because no significant variation in the bile flow was observed at 0—8 h after the oral administration of various cathartics to rats. After magnesium sulfate, mannitol, castor oil or pilocarpine treatment, there was an increase in the proportion of free bile acids. The TDCA content of rat bile at 0—1 h after administration of magnesium sulfate or castor oil, the TCA content of rat bile at 0—1 h after the administration of various cathartics except for mannitol, and the GDCA or GCA content of rat bile at 0—1 h after the administration of mannitol or castor oil were increased significantly compared with those of control groups. In the bile collected from 1 to 2 h, the TDCA and TCA contents were increased after magnesium sulfate treatment. The amounts of biliary bile acids of diarrheal rats induced by various cathartics are shown in Table IV. Increase of free bile acids contents was observed in the case of diarrhea induced by magnesium sulfate, mannitol, castor oil and pilocarpine. As regards conjugated bile acids in the bile, GCA, GDCA and TDCA contents in magnesium sulfate- or mannitol-induced diarrhea, and GDCA, GUDCA, GCA, TDCA, TCDCA, TUDCA and TCA contents in pilocarpine-induced diarrhea were significantly increased. However, TUDCA and TCA contents in castor oil-induced diarrhea, and GDCA, GUDCA, GCA, TCDCA, TUDCA and TCA contents in DSS-induced diarrhea were significantly decreased.

From these data, the composition ratio of biliary bile acids (total contents of unconjugated and conjugated individual bile acid/total contents of bile acids) was calculated. There was no significant difference in the composition ratio between the initial stage of diarrhea and the control stage. However, in the case of diarrhea induced by various cathartics, the proportion of DCA was significantly increased, whereas that of CA was decreased compared with the control.

The ratio of glycine- to taurine-conjugated bile acids (G/T ratio) of bile in diarrheal rats was calculated, and was significantly increased in the case of diarrhea induced by various cathartics except for DSS (Table V).

TABLE I. Cathartic Effect of Deoxycholic Acid in Rats

Dose (mg/kg, *p.o.*)	Time (h)							
	0—1	1—2	2—3	3—4	4—20	20—24	24—28	0—28
1500	1/3[a)]	2/3	Death					3/3
500	0/5	0/5	0/5	Death				0/3
300	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
100	0/5	0/5	0/5	0/5	0/5	5/5	5/5	5/5
50	0/5	0/5	0/5	0/5	1/5	0/5	3/5	4/5
25	0/5	0/5	0/5	0/5	0/5	1/5	2/5	3/5

a) Two of five rats died immediately after oral administration of DCA.

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TABLE II. Biliary Bile Acid Contents at 0—1 h after Oral Administration of Various Cathartics to Rats

		LCA (μg/ml)	DCA (μg/ml)	CDCA (μg/ml)	UDCA (μg/ml)	CA (μg/ml)
Control	F	Trace	Trace	Trace	Trace	Trace
	G	Trace	7.28±1.18	Trace	12.0±3.9	24.9±2.3
	T	45.3±7.9	2320±164	474±89	1327±304	11584±2088
Magnesium sulfate	F	1.91±0.38	1.15±0.23	1.72±0.51	3.11±0.43	7.09±1.87
	G	Trace	10.2±0.9	6.11±2.01	15.9±2.4	37.8±6.8
	T	26.1±2.4	3755±420[c]	1062±212	2638±309[c]	23103±2551[c]
Mannitol	F	Trace	1.19±0.24	1.13±0.22	1.64±0.69	11.6±1.3
	G	Trace	47.3±10.1[c]	11.6±6.6	12.0±4.0	142±21[a]
	T	48.0±7.6	2450±596	585±86	625±206	9161±1238
Castor oil	F	Trace	0.93±0.20	Trace	3.30±0.90	9.30±2.19
	G	Trace	20.7±4.0[b]	Trace	18.6±3.1	131±29[c]
	T	37.9±2.5	2358±240[c]	962±166	2320±633	30964±3208[a]
DSS	F	Trace	Trace	Trace	Trace	Trace
	G	Trace	14.1±2.9	Trace	14.5±2.6	56.6±8.0[b]
	T	27.8±6.8	2572±124	794±132	1467±90	31656±6240[c]
Pilocarpine	F	Trace	1.70±0.85	Trace	1.79±0.18	19.7±3.0
	G	Trace	14.0±2.9	3.13±0.76	14.4±4.5	78.5±12.6[b]
	T	57.8±11.9	2154±501	920±199	877±92	31244±6124[c]

Each result represents the mean±S.E. of 6 rats. *a*) Differences are significant at $p<0.01$. *b*) Differences are significant at $p<0.02$. *c*) Differences are significant at $p<0.05$. LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; F, unconjugated bile acid; G, glycine-conjugated bile acid; T, taurine-conjugated bile acid; DSS, dioctyl sodium sulfosuccinate.

TABLE III. Biliary Bile Acid Contents at 1—2 h after Oral Administration of Various Cathartics to Rats

		LCA (μg/ml)	DCA (μg/ml)	CDCA (μg/ml)	UDCA (μg/ml)	CA (μg/ml)
Control	F	Trace	Trace	Trace	Trace	Trace
	G	Trace	6.26±0.98	Trace	6.02±1.44	19.5±3.3
	T	33.9±6.4	1306±159	494±86	851±91	9275±2806
Magnesium sulfate	F	2.29±0.63	1.27±0.51	1.25±0.31	2.67±0.38	4.98±1.09
	G	Trace	10.5±2.6	Trace	12.2±3.1	41.9±8.1
	T	30.1±7.8	3039±586[c]	941±200	2164±412	14506±826[c]
Mannitol	F	Trace	1.67±0.52	1.03±0.36	2.50±0.70	21.7±9.1
	G	Trace	20.3±3.9[a]	2.80±0.28	7.96±3.11	50.4±7.1[c]
	T	43.6±14.3	3012±48	485±82	528±196	7317±1620
Castor oil	F	Trace	4.15±1.55	Trace	3.08±0.65	24.6±8.0
	G	Trace	16.3±1.3[a]	Trace	14.0±1.8[c]	92.6±17.6[b]
	T	30.9±6.6	1689±380	821±195	1216±251	17905±3512
DSS	F	Trace	Trace	Trace	Trace	Trace
	G	Trace	7.22±1.41	Trace	8.49±1.34	37.2±12.7
	T	32.3±2.7	1647±152	703±75	1265±245	16895±3650
Pilocarpine	F	Trace	17.3±8.7	3.06±0.42	2.11±0.69	22.4±7.3
	G	Trace	16.3±1.4[b]	12.2±5.7	13.5±4.1	124±19[a]
	T	59.5±5.5	2041±390	1519±197	1003±198[a]	35460±2866[a]

Each result represents the mean±S.E. of 6 rats. *a*) Differences are significant at $p<0.01$. *b*) Differences are significant at $p<0.02$. *c*) Differences are significant at $p<0.05$. LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, unsodeoxycholic acid; CA, cholic acid; F, unconjugated bile acid; G, glycine-conjugated bile acid; T, taurine-conjugated bile acid; DSS, dioctyl sodium sulfosuccinate.

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TABLE IV. Biliary Bile Acid Contents at 2—8 h after Oral Administration of Various Cathartics to Rats

		LCA (μg/ml)	DCA (μg/ml)	CDCA (μg/ml)	UDCA (μg/ml)	CA (μg/ml)
Control	F	Trace	Trace	Trace	Trace	Trace
	G	Trace	5.90±0.76	Trace	7.43±0.93	21.3±2.4
	T	28.7±2.1	1075±184	491±43	959±84	8766±967
Magnesium sulfate	F	1.13±0.27	1.72±0.75	3.09±0.72	3.61±0.86	28.6±8.9
	G	Trace	22.2±5.9[c]	Trace	15.5±3.7	28.1±4.2
	T	33.9±5.1	3558±704[b]	586±75	1596±367	8525±537
Mannitol	F	Trace	1.19±0.38	2.48±0.75	2.41±0.60	24.1±7.1
	G	Trace	23.5±1.8[a]	Trace	7.71±1.83	24.8±3.0
	T	20.4±4.0	2446±256[a]	424±85	730±189	4019±360[b]
Castor oil	F	1.12±0.25	Trace	1.89±0.73	1.35±0.22	4.45±0.98
	G	Trace	6.31±0.80	3.24±0.61	5.10±0.98	11.4±1.4
	T	24.8±3.6	1050±122	423±88	619±89[d]	2049±224[a]
DSS	F	Trace	Trace	Trace	Trace	Trace
	G	Trace	3.19±0.78[b]	1.02±0.26	1.60±0.38[b]	3.71±0.63[a]
	T	27.8±5.2	372±79	173±30[a]	249±50[a]	1246±266[a]
Pilocarpine	F	0.96±0.08	1.46±0.17	3.66±0.82	1.50±0.22	10.4±2.3
	G	0.43±0.23	14.5±3.1[a]	Trace	22.7±4.0[a]	76.7±11.2[a]
	T	36.8±8.3	1982±470[d]	2054±559[a]	2423±97[a]	19300±2948[a]

Each result represents the mean±S.E. of 6 rats. *a*) Differences are significant at $p<0.001$. *b*) Differences are significant at $p<0.01$. *c*) Differences are significant at $p<0.02$. *d*) Differences are significant at $p<0.05$. LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; F, unconjugated bile acid; G, glycine-conjugated bile acid; T, taurine-conjugated bile acid; DSS, dioctyl sodium sulfosuccinate.

TABLE V. Ratio of Glycine- to Taurine-Conjugated Bile Acids of Rat Bile in Diarrhea Induced by Various Cathartics and Deoxycholic Acid

	G/T ratio
Control	0.0030±0.00037
Magnesium sulfate	0.0058±0.00100[b]
Mannitol	0.0069±0.00047[d]
Castor oil	0.0049±0.00034[c]
DSS	0.0042±0.00046
Pilocarpine	0.0047±0.00070[a]
Deoxycholic acid	0.0138±0.00370[d]

Each result represents the mean±S.E. of 6 rats. *a*) Differences are significant at $p<0.05$. *b*) Differences are significant at $p<0.02$. *c*) Differences are significant at $p<0.01$. *d*) Differences are significant at $p<0.0001$.

On the other hand, changes in the disposition of biliary bile acids are indicated in Figs. 1(a, b), 2(a, b) and 3(a, b). GCA and GDCA contents kept increasing till 28 h after oral administration of DCA, and the contents of free bile acids and tauro-conjugated bile acids were increased at 24 h after DCA treatment. The beginning of the increase of free bile acids contents is delayed compared with that in the case of cathartics. Moreover, the G/T ratio in the case of diarrhea induced by DCA was significantly increased, as in the case of the cathartics except for DSS (Table V).

Alterations of Portal Blood Bile Acids after Administration of Various Cathartics and DCA

As shown in Table VI, TCA was decreased significantly in diarrhea induced by the

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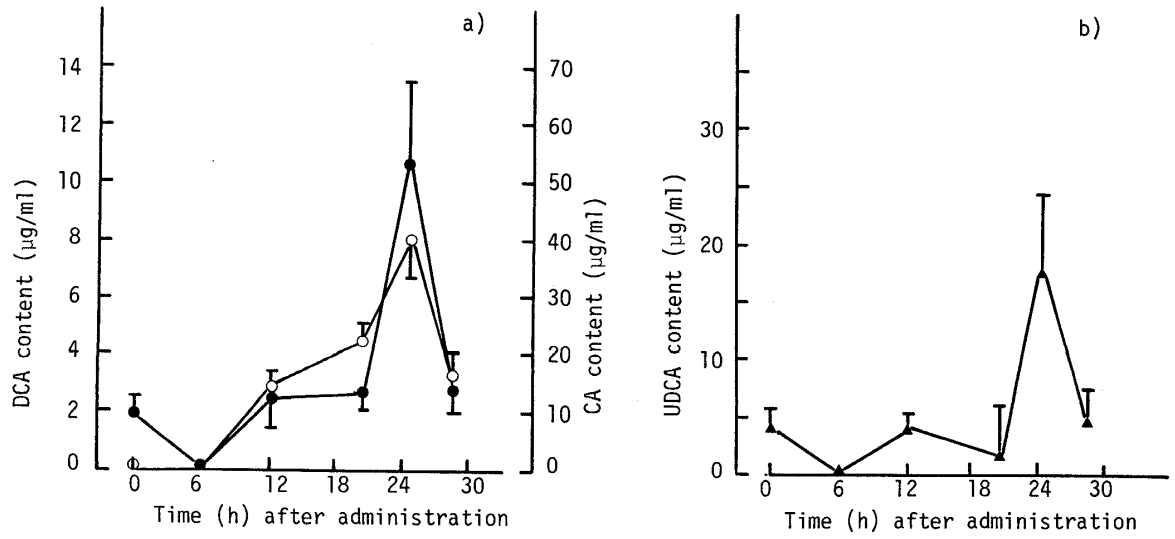

Fig. 1. Contents of Biliary Free Bile Acids after Oral Administration of Deoxycholic Acid to Rats

Each result represents the mean ± S.E. of 6 rats.
a) —○—, deoxycholic acid; —●—, cholic acid. b) —△—, ursodeoxycholic acid.

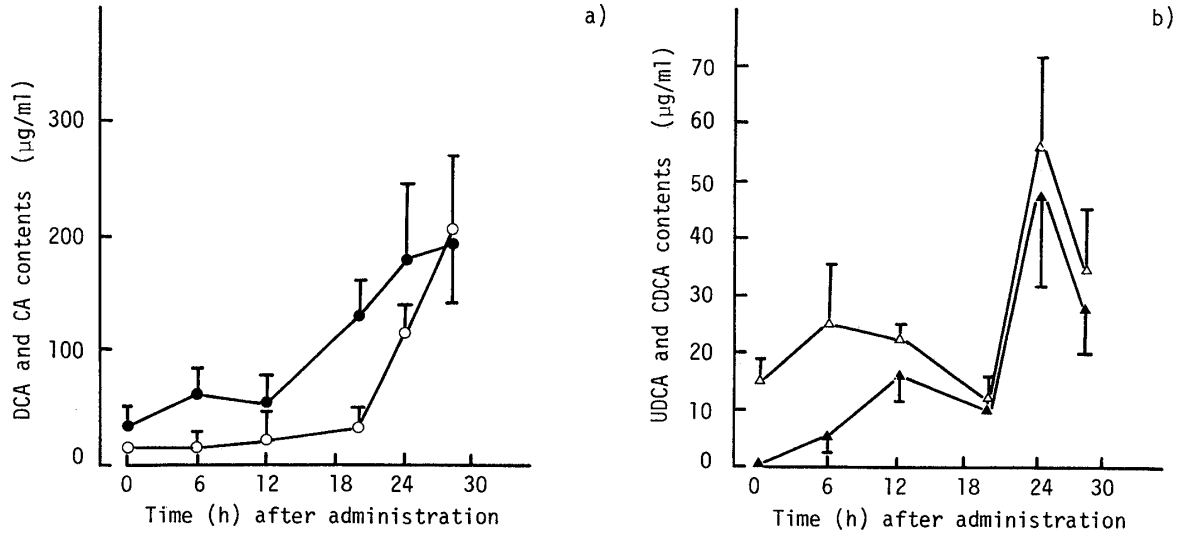

Fig. 2. Contents of Biliary Glycine-Conjugated Bile Acids after Oral Administration of Deoxycholic Acid to Rats

Each result represents the mean ± S.E. of 6 rats.
a) —○—, deoxycholic acid; —●—, cholic acid. b) —△—, ursodeoxycholic acid; —▲—, chenodeoxycholic acid.

various cathartics, and TDCA was increased in the portal blood of diarrheal rats induced with magnesium sulfate, mannitol and DSS. However, there was no significant difference in free bile acids contents between the various cathartics-treated and non-treated rats. Moreover, from calculation of the composition ratio of portal blood bile acids, it was clear that the proportion of DCA was increased, and the proportion of CA was decreased.

On the other hand, the effects of DCA on the portal blood bile acids are indicated in Figs. 4(a, b), 5(a, b) and 6(a, b). The amounts of bile acids conjugated with glycine after DCA treatment were significantly lower than in the control. Each of the tauro-conjugated bile acids showed a different pattern of absorption. However, it is interesting that TDCA attained a plateau at 6 h and TCA decreased from 12 h after DCA treatment. Moreover, free bile acids

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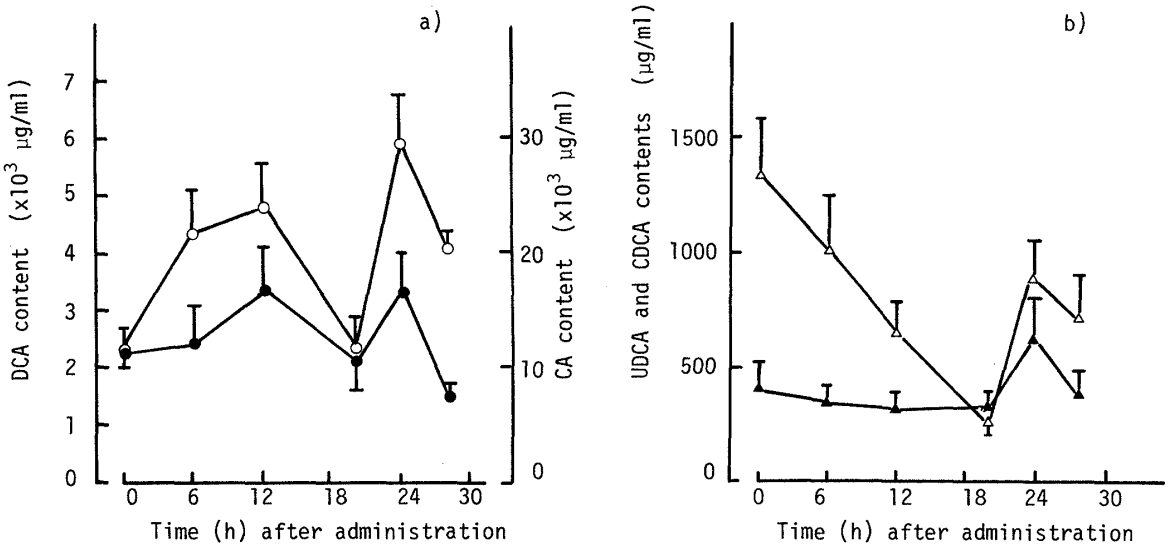

Fig. 3. Contents of Biliary Taurine-Conjugated Bile Acids after Oral Administration of Deoxycholic Acid to Rats

Each result represents the mean ± S.E. of 6 rats.
a) —○—, deoxycholic acid; —●—, cholic acid. b) —△—, ursodeoxycholic acid; —▲—, chenodeoxycholic acid.

TABLE VI. Bile Acid Contents of the Portal Blood in Cathartics-Induced Diarrhea

		LCA (μg/ml)	DCA (μg/ml)	CDCA (μg/ml)	UDCA (μg/ml)	CA (μg/ml)
Control	F	Trace	3.19±0.33	1.56±0.49	7.21±0.54	2.39±0.65
	G	Trace	Trace	Trace	Trace	Trace
	T	Trace	9.69±1.73	5.92±1.00	15.4±2.6	128±25
Magnesium sulfate	F	Trace	13.5±3.4[c]	1.79±0.44	6.02±0.93	2.32±0.45
	G	Trace	Trace	Trace	Trace	Trace
	T	Trace	34.0±8.9[c]	11.0±2.7	20.2±8.1	39.8±10.6[c]
Mannitol	F	Trace	6.46±1.11	1.02±0.17	5.10±1.68	2.94±0.63
	G	Trace	3.33±0.94	Trace	2.32±0.68	1.74±0.44
	T	1.18±0.11	47.9±11.9[c]	13.2±0.4[a]	32.0±6.7	50.6±11.2[c]
Castor oil	F	Trace	3.54±0.80	0.44±0.01	5.68±1.30	1.55±0.22
	G	Trace	Trace	Trace	Trace	Trace
	T	Trace	7.82±1.15	2.15±0.11[c]	5.17±0.98[c]	17.5±3.6[c]
DSS	F	Trace	8.20±2.00[c]	3.84±0.56	15.5±2.5[c]	3.32±0.90
	G	Trace	3.17±0.81	0.70±0.09	1.60±0.36	0.62±0.16
	T	Trace	17.8±5.1	2.42±0.85	7.64±2.40	10.5±4.0[b]
Pilocarpine	F	Trace	1.02±0.23[a]	0.93±0.27	3.03±0.27	Trace
	G	Trace	Trace	Trace	Trace	Trace
	T	Trace	2.97±0.57[a]	1.91±0.48	1.67±0.29[a]	34.1±7.3[a]

Each result represents the mean ± S.E. of 6 rats. *a*) Differences are significant at $p<0.01$. *b*) Differences are significant at $p<0.02$. *c*) Differences are significant at $p<0.05$. LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; F, unconjugated bile acid; G, glycine-conjugated bile acid; T, taurine-conjugated bile acid; DSS, dioctyl sodium sulfosuccinate.

contents were increased in the portal blood from 24 h after DCA administration, as in the bile, but the increase (percent) was lower than that in the bile.

Alterations of Fecal Bile Acids after Administration of the Various Cathartics and DCA

As shown in Table VII, free bile acids contents were decreased significantly in diarrheal

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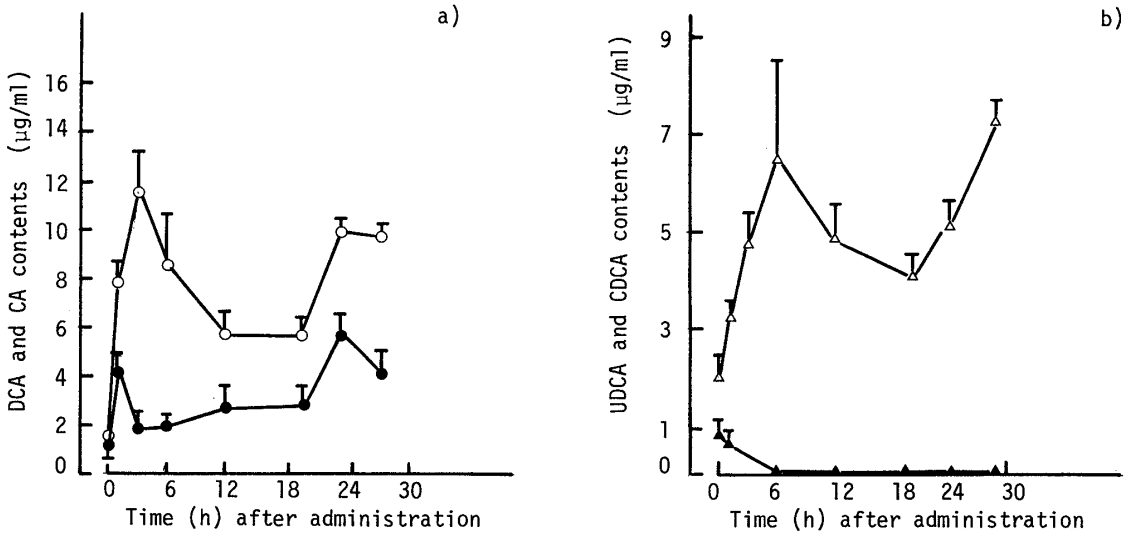

Fig. 4. Contents of Portal Blood Free Bile Acids after Oral Administration of Deoxycholic Acid to Rats

Each result represents the mean ± S.E. of 6 rats.
a) —○—, deoxycholic acid; —●—, cholic acid. b) —△—, ursodeoxycholic acid; —▲—, chenodeoxycholic acid.

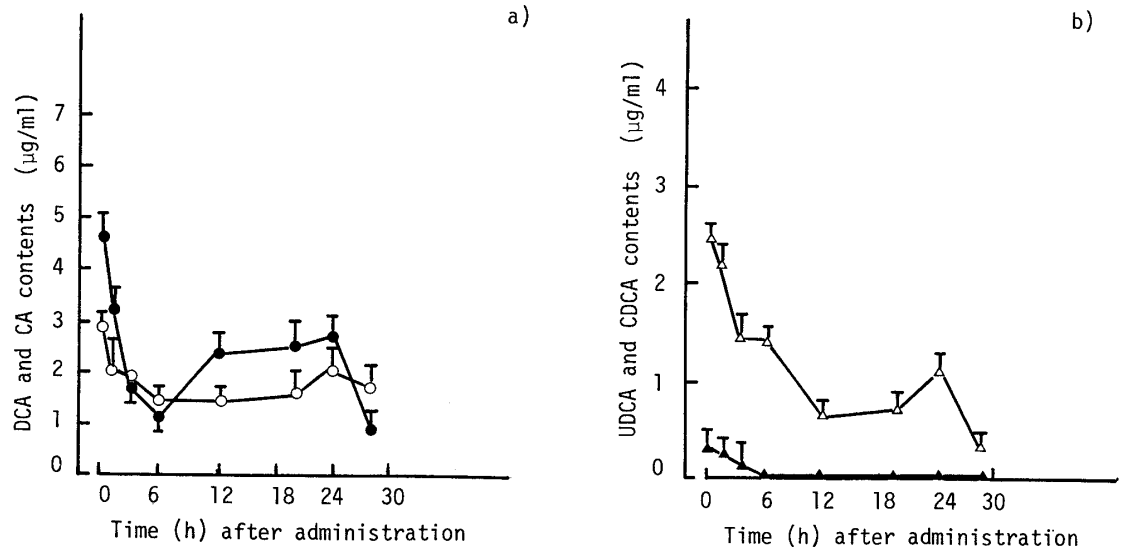

Fig. 5. Contents of Portal Blood Glycine-Conjugated Bile Acids after Oral Administration of Deoxycholic Acid to Rats

Each result represents the mean ± S.E. of 6 rats.
a) —○—, deoxycholic acid; —●—, cholic acid. b) —△—, ursodeoxycholic acid; —▲—, chenodeoxycholic acid.

rats induced with magnesium sulfate, mannitol, castor oil and pilocarpine, but the amounts of bile acids conjugated with taurine were significantly increased in the case of diarrhea induced with magnesium sulfate, mannitol, DSS and pilocarpine. Moreover, from the composition ratio of fecal bile acids, the proportion of DCA was decreased, and the proportion of CA was increased.

On the other hand, the fecal bile acids contents after DCA treatment were increased except for total LCA content (Fig. 7).

Electrolytes (Na and K) Level in the Serum of Diarrheal Rats Induced with Various Cathartics

The Na level in the serum after treatment with various cathartics was significantly

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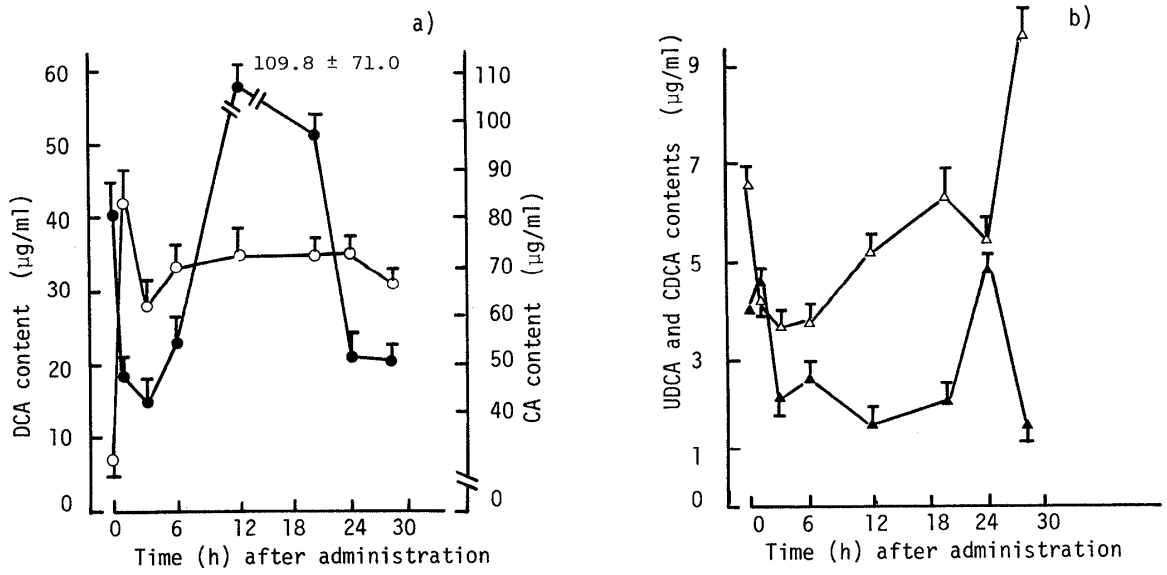

Fig. 6. Contents of Portal Blood Taurine-Conjugated Bile Acids after Oral Administration of Deoxycholic Acid to Rats

Each result represents the mean ± S.E. of 6 rats.
a) —○—, deoxycholic acid; —●—, cholic acid. b) —△—, ursodeoxycholic acid; —▲—, chenodeoxycholic acid.

TABLE VII. Fecal Bile Acid Contents in Cathartics-Induced Diarrhea

		LCA (μg/8 h)	DCA (μg/8 h)	CDCA (μg/8 h)	UDCA (μg/8 h)	CA (μg/8 h)
Control	F	36.0 ± 6.4	145 ± 48	32.1 ± 8.0	528 ± 122	217 ± 75
	G	Trace	4.89 ± 0.73	Trace	4.03 ± 1.23	Trace
	T	Trace	2.25 ± 0.17	2.31 ± 0.64	2.92 ± 0.49	6.92 ± 2.21
Magnesium sulfate	F	Trace	44.2 ± 6.1[d]	7.22 ± 1.84[c]	81.3 ± 23.3[c]	144 ± 19
	G	Trace	4.15 ± 1.52	Trace	7.33 ± 2.21	Trace
	T	Trace	9.98 ± 2.17[b]	3.96 ± 0.73	7.80 ± 1.44[b]	38.9 ± 14.3[d]
Mannitol	F	Trace	17.6 ± 4.7	Trace	27.0 ± 8.8[b]	31.6 ± 6.3
	G	Trace	2.51 ± 0.56	Trace	1.41 ± 0.31	Trace
	T	Trace	9.02 ± 2.52[d]	4.14 ± 1.15	2.81 ± 0.65	8.55 ± 1.14
Castor oil	F	Trace	55.0 ± 12.1	2.32 ± 0.41[c]	30.3 ± 7.8[b]	41.8 ± 9.7
	G	Trace	3.87 ± 0.59	Trace	3.17 ± 0.60	4.45 ± 1.19
	T	Trace	76.0 ± 9.6[a]	27.7 ± 2.2[a]	34.6 ± 5.0[a]	330 ± 32[a]
DSS	F	22.4 ± 9.3	526 ± 102[b]	15.6 ± 6.2	467 ± 99	940 ± 97[a]
	G	Trace	27.8 ± 6.4[b]	Trace	14.1 ± 1.9[b]	60.1 ± 13.9
	T	Trace	648 ± 174[b]	86.9 ± 22.9[c]	198 ± 54[b]	1472 ± 337[a]
Pilocarpine	F	Trace	71.1 ± 6.1	3.18 ± 0.71[b]	79.9 ± 15.2[b]	192 ± 33[b]
	G	Trace	1.50 ± 0.18	Trace	2.25 ± 0.41	5.94 ± 0.75
	T	Trace	159 ± 46[c]	55.1 ± 9.0[b]	262 ± 50[a]	1943 ± 498[c]

Each result represents the mean ± S.E. of 6 rats. *a*) Differences are significant at $p<0.001$. *b*) Differences are significant at $p<0.01$. *c*) Differences are significant at $p<0.02$. *d*) Differences are significant at $p<0.05$. LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; F, unconjugated bile acid; G, glycine-conjugated bile acid; T, taurine-conjugated bile acid; DSS, dioctyl sodium sulfosuccinate.

decreased in comparison with the control level (Table VIII). Therefore, enteropooling (the accumulation of fluid in the small intestine) may have been induced by the various cathartics.

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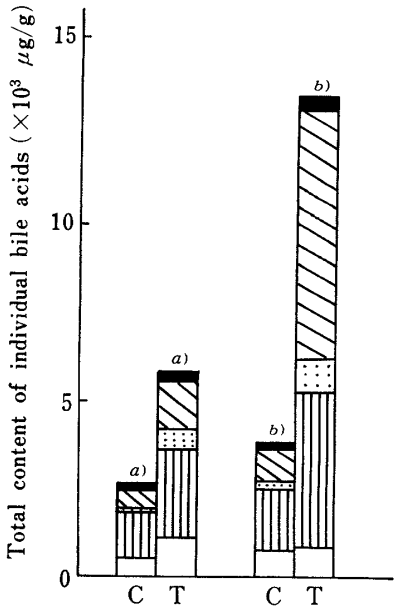

Fig. 7. Total Contents of Individual Bile Acids in the Feces of Rats after Oral Administration of Deoxycholic Acid

a) 0—12 h after oral administration of deoxycholic acid. *b*) 12—24 h after oral administration of deoxycholic acid.
C: control; T: treatment with deoxycholic acid.
■, lithocholic acid; ▨, deoxycholic acid; ⁞⁞⁞, chenodeoxycholic acid; ▥, ursodeoxycholic acid; □, cholic acid.

TABLE VIII. Alteration of Serum Electrolytes (Na and K) in Diarrhea Induced by Various Cathartics

	Na (meq/l)	K (meq/ml)
Control	145.7±0.3	7.4±0.2
Magnesium sulfate	133.7±1.2[b)]	6.7±0.2[a)]
Mannitol	133.7±0.7[b)]	6.8±0.2
Castor oil	140.0±0.3[a)]	7.5±0.1
DSS	139.3±1.7[a)]	7.8±0.2[a)]
Pilocarpine	138.3±0.3[b)]	5.9±0.4[a)]

Each result represents the mean±S.E. of 6 rats. *a*) Differences are significant at $p<0.05$. *b*) Differences are significant at $p<0.001$.

Discussion

The purpose of this study was to determine the disposition of biliary, portal blood and fecal bile acids in diarrheal rats, and to investigate the physiological significance of endogenous bile acids. The biliary bile acids of free type increased significantly in the initial stage and in diarrhea after the oral administration of various cathartics except for DSS. Since there was no significant change in the free bile acid contents of portal blood in diarrhea, it is likely that unconjugated bile acids eliminated into the intestinal lumen are not reabsorbed from the ileum in diarrheal rats. Therefore, we supposed that the biliary excretion of endogenous unconjugated bile acids after cathartics treatment might induce the occurrence of diarrhea.

The amount of bile acids conjugated with glycine and taurine varied depending on the cathartics administered. However, the proportion of DCA was significantly increased in the case of diarrhea induced by cathartics and DCA, and the proportion of CA was conversely decreased compared with the control. This result suggested that 7α-dehydroxylation of endogenous CA to DCA is accelerated by the action of intestinal bacteria or the liver. Previously, DCA was shown to inhibit the transport of water and Na in the intestinal mucosa.[17-19] In this experiment, inhibition of intestinal absorption of Na was confirmed.

Next, the G/T ratio of biliary bile acids was calculated in order to determine the

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conjugation and absorption of bile acid during enterohepatic circulation. The G/T ratio was increased in the case of injury of the ileum. As regards the mechanism, Hislop *et al.*[22] showed that the G/T ratio rises because tauro-conjugated bile acids do not efficiently return to the liver in ileal disease, since bile acids conjugated with taurine are absorbed only from the ileum by active transport, in contrast with glyco-conjugated bile acids, which are absorbed from all the absorption sites of the small intestine by passive transport. We observed an increase of the G/T ratio in biliary bile acids when diarrhea was induced by castor oil and pilocarpine. This might be attributed to lower reabsorption of tauro-conjugated bile acids, because the intestinal transit time of tauro-conjugated bile acids in the absorption site was increased in rats with diarrhea. Moreover, the G/T ratio of biliary bile acids increased in the case of diarrhea induced by magnesium sulfate and mannitol. These results suggested that the reabsorption of glyco-conjugated bile acids increased significantly in diarrhea, since no alteration of fecal tauro-conjugated bile acids was observed.

The above results suggested that a secondary bile acid, DCA, participates in the occurrence of diarrhea after the oral administration of various cathartics. Therefore, we investigated the occurrence of diarrhea after the oral administration of exogenous DCA. DCA caused diarrhea in rats. The occurrence of diarrhea was concentrated within 20 to 24 h after the oral administration of DCA. A cathartic efficiency of 100% was obtained at a dose of 100 mg/kg, *p.o.*

Since biliary unconjugated and glyco-conjugated bile acids were increased at 24 h after DCA treatment, but glyco-conjugated bile acids were decreased in the portal blood, deconjugation may be accelerated as a result of changes of the activity of intestinal bacteria and in the liver after DCA treatment. Thus, diarrhea may be induced by the increase of free bile acids in the intestinal lumen. Moreover, the active transport of tauro-conjugated bile acids from the end of the ileum may have been impaired, becuase the absorption of tauro-conjugated bile acids was inhibited from 12 h after the administration of DCA.

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