Changes in biliary lipid and biliary bile acid composition in patients after administration of ursodeoxycholic acid

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Abstract Twenty-three patients with gallstones were treated with two dosage levels of ursodeoxycholic acid, 600 mg/day and 150 mg/day. Two to three months after the treatment, the molar percentage of cholesterol in bile significantly decreased (from 7.4 to 4.5 mole% in the 600 mg group and from 7.6 to 4.0 mole% in the 150 mg group), so that bile became unsaturated in most patients in both treatment groups. However, there was no significant difference between the two groups. Biliary ursodeoxycholate increased in proportion to dose, and the sum of ursodeoxycholic acid plus chenodeoxycholic acid in biliary bile acids was over 70%. There was no significant increase in the proportion of lithocholate in bile. The major fecal bile acid of patients receiving ursodeoxycholic acid was lithocholic acid. Serum bile acid concentration rose slightly after 3 months of ursodeoxycholic acid treatment, and the major circulating bile acid became ursodeoxycholic acid. Ursodeoxycholic acid is well absorbed from intestine, undergoes little biotransformation during hepatic passage, and is 7-dehydroxylated by colonic bacteria. The litholytic activity of ursodeoxycholic acid was demonstrated in two patients receiving 450 mg and 150 mg, respectively, of the bile acid per day.

Supplementary key words lithogenic index · serum bile acids · fecal bile acids

Following the report by Danzinger, et al. (1) that chenodeoxycholic acid (CDCA) dissolves cholesterol gallstones in man, the metabolism of CDCA has been studied in detail by many investigators (2, 3). In 1975, we reported (4) that the administration of ursodeoxycholic acid (UDCA), the 7-β epimer of CDCA, reduced biliary cholesterol concentrations, and this result suggested the possibility that UDCA could dissolve gallstones (5). However, little work has been done to study the metabolism of UDCA in man.

This report describes the changes of biliary lipid and bile acid composition in bile, feces, and serum after administration of UDCA, and presents two case histories dealing with the dissolution of gallstones.

MATERIALS AND METHODS

Ursodeoxycholic acid

UDCA was synthesized by Tokyo Tanabe Pharmaceutical Co. (Tokyo, Japan), and was better than 99% pure by gas–liquid chromatography and thin-layer chromatography.

Patients

All patients with radiolucent gallstones in radiologically visualizing gallbladders were treated at the Department of Medicine, Hokkaido University Hospital. The control subjects were persons without hepatobiliary diseases selected from the medical staff and nurses of our clinic.

Analysis of duodenal bile

Twenty-three patients with gallstones were allocated to two groups. One group (n = 11) was given 150 mg of UDCA daily, and the other group (n = 12) was given 600 mg daily. After an overnight fast, bile-rich fluid was obtained through a duodenal tube before and 2–3 months after UDCA treatment. The gallbladder bile was collected after stimulation by infusion of 25% magnesium sulfate solution. One ml of bile was added to 20 ml of chloroform–methanol 2:1 and the mixture was agitated in an ultrasonic bath for 15 min. The sample was then centrifuged and the whole extract was further analyzed for biliary lipid and biliary bile acid composition.

Cholesterol

An aliquot of the extract was evaporated to dryness and the residue was dissolved in 2 ml of 70% (v/v)

Abbreviations: CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid.

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ethanol. The cholesterol was extracted with hexane, and the hexane phase was evaporated to dryness. The sample was analyzed by the enzymatic method of Allain, et al. (6) (cholesterol oxidase and cholesterol hydrolase were kindly supplied by Kyowa Hakko., Ltd, Tokyo, Japan).

Phospholipid

An aliquot of the extract was evaporated to dryness, and phospholipid phosphorus was determined by the method of Hoeflmayr and Fried (7).

Bile acid

An aliquot of the extract was evaporated to dryness, and total bile salts were enzymatically measured by using 3α-hydroxy steroid dehydrogenase (Nyegaad Co., Oslo, Norway) (8). Bile acid composition in bile was determined by gas–liquid chromatography as described previously (9). Briefly, the procedure was as follows. An aliquot of the extract was evaporated, and the residue was subjected to solvolysis, hydrolysis, extraction, and methylation. The methyl esters of the bile acids were applied to a column containing 15 g of aluminum oxide (grade V) and the column was washed with 100 ml of benzene–hexane 3:7 (v/v). Monohydroxy bile acids were eluted with 100 ml of benzene, and di- and trihydroxy bile acids were eluted with 100 ml of methanol. The eluate was evaporated to dryness and the methyl esters were converted to the trifluoroacetate derivatives, which were then analyzed by gas–liquid chromatography on QF-1 columns.

The individual composition of biliary lipid (cholesterol, phospholipid, and bile salt) was expressed in mole%, and cholesterol saturation of bile was computed by the method of Thomas and Hofmann (10).

Analysis of serum bile acid

After an overnight fast, blood samples were obtained in the morning from nine patients with normal liver function before and after 3 months of treatment with UDCA (450 mg/day). Serum bile acids were extracted with Amberlite XAD-2 (Rohm and Haas, Philadelphia, PA), and the solvolysis, hydrolysis, and methylation procedures were as described previously (11). The methyl esters of serum bile acids were applied to a column containing 5 g of neutral aluminum oxide (grade V), and the column was washed with 50 ml of benzene–hexane 3:7 (v/v). Monohydroxy bile acids were eluted with 50 ml of benzene, and di- and trihydroxy bile acids with 50 ml of methanol. Each eluate was analyzed by gas–liquid chromatography as described for biliary bile acids.

Analysis of fecal bile acid

Fecal samples were obtained from four patients during 450 mg/day UDCA treatment and from three normal subjects. Fecal bile acid was analyzed by the method of Grundy, Ahrens, and Miettinen (12).

RESULTS

Changes of biliary lipid and bile acid composition in bile

As shown in **Table 1,** biliary cholesterol during pretreatment was 7.4 ± 2.0 mole% in the 600 mg UDCA group and 7.6 ± 3.7 mole% in the 150 mg UDCA group. After UDCA treatment, the molar percentage of cholesterol significantly decreased to 4.5 ± 1.5 mole% in the former group and to 4.0 ± 1.2 mole% in the latter group, so that the lithogenic index decreased from 1.20 ± 0.37 to 0.69 ± 0.30 and from 1.23 ± 0.50 to 0.65 ± 0.21, respectively. However, there was no significant difference between these two groups.

The effect of the two doses of UDCA on biliary bile acid composition is indicated in **Table 2.** After 2 months of treatment with a dose of 150 mg/day, there was a substantial increase in the proportion of UDCA. At a dose of 600 mg/day, UDCA predominated so that almost half of the biliary bile acids consisted of UDCA. The sum of UDCA plus CDCA was over 70%. At the same time, the proportion of biliary cholic acid and CDCA was decreased.

There was no significant increase in the proportion of lithocholate in bile after UDCA treatment (both in the 150 mg group and the 600 mg group).

Changes in fecal bile acids

Before treatment, the fecal bile acids showed the usual pattern consisting mainly of deoxycholic acid (50.5%) and lithocholic acid (32.0%) (**Fig. 1**). After UDCA treatment, no UDCA was found in feces, and the major fecal bile acid was lithocholic acid (60.3%); yet, appreciable amounts of deoxycholic acid persisted (21.9%). This result indicates that the administered UDCA must have been 7β-dehydroxylated to lithocholic acid in the human large intestine.

Changes of serum bile acid

Serum bile acids rose slightly after 3 months of UDCA treatment (**Table 3**). The range was 0.3–4.9 μg/ml (2.38 ± 1.68). All liver function tests remained normal. The most striking change was that the major circulating bile acid became UDCA; thus, the pattern of serum bile acids now mirrored that of biliary bile acids.

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TABLE 1. Biliary lipid composition before and during treatment with ursodeoxycholic acid

Patients	Dosage	Pretreatment				During Treatment			
		PL[a]	CHO[a]	BS[a]	Lithogenic Index	PL	CHO	BS	Lithogenic Index
	mg/day		*mole %*				*mole %*		
1) S.N.	600	22.0	10.6	67.5	1.39	19.1	2.9	78.0	0.43
2) A.S.	600	18.3	5.2	76.6	0.80	22.7	2.9	74.4	0.37
3) I.J.	600	18.7	8.7	73.1	1.32	19.2	6.5	74.3	0.96
4) K.T.	600	13.9	4.1	82.1	0.80	12.5	6.7	80.9	1.40
5) S.K.	600	15.5	7.3	77.3	1.30	19.3	7.0	73.7	1.03
6) K.S.	600	20.0	6.7	73.2	0.96	18.7	3.6	77.7	0.55
7) K.N.	600	12.5	7.9	79.7	1.58	18.0	4.4	77.7	0.64
8) O.F.	600	22.8	9.9	67.3	1.27	23.6	4.4	72.0	0.55
9) H.T.	600	22.1	5.5	72.5	0.72	17.7	4.5	77.8	0.71
10) N.S.	600	12.7	9.4	78.0	1.96	14.7	2.4	82.9	0.44
11) M.M.	600	15.0	7.0	78.0	1.30	22.6	4.9	72.5	0.64
12) Y.W.	600	20.2	6.7	73.0	0.94	20.0	3.8	76.2	0.54
Mean ± SD[b]		17.8 ± 3.8	7.4 ± 2.0	74.9 ± 4.6	1.20 ± 0.37	19.0 ± 3.2	4.5[c] ± 1.5	76.5 ± 3.3	0.69[d] ± 0.30
13) Y.M.	150	17.1	5.0	78.0	0.82	13.4	3.4	83.3	0.68
14) I.F.	150	17.4	11.4	71.2	1.84	31.1	3.6	65.3	0.47
15) K.K.	150	9.6	7.5	82.9	1.83	18.2	4.0	77.8	0.63
16) J.K.	150	11.9	5.8	82.3	1.26	17.4	3.3	79.2	0.53
17) S.K.	150	23.9	3.3	72.8	0.41	17.9	5.9	76.2	0.94
18) N.M.	150	19.4	11.2	69.4	1.62	17.5	5.6	76.9	0.90
19) Y.Y.	150	13.6	4.7	81.8	0.94	16.0	5.8	78.2	1.02
20) N.T.	150	28.4	15.0	56.7	1.81	25.4	3.6	71.0	0.43
21) S.W.	150	19.0	8.8	72.3	1.31	12.9	2.5	84.7	0.51
22) M.I.	150	12.5	3.5	84.0	0.73	15.0	2.6	82.5	0.48
23) K.J.	150	20.6	7.3	72.1	1.01	19.0	4.0	77.0	0.60
Mean ± SD[b]		17.6 ± 5.6	7.6 ± 3.7	74.9 ± 8.1	1.23 ± 0.50	18.5 ± 5.3	4.0[c] ± 1.2	77.5 ± 5.5	0.65[d] ± 0.21

[a] PL, phospholipid; CHO, cholesterol; BS, bile salt.

[b] There was no statistical difference in biliary lipid composition before UDCA treatment between the groups receiving either 600 or 150 mg of UDCA.

[c] Statistical evaluation of decrease in mole % of biliary cholesterol after UDCA treatment: UDCA 600 mg group, $P < 0.01$; and UDCA 150 mg group, $P < 0.001$.

[d] Statistical evaluation of decrease in lithogenic index after UDCA treatment: UDCA 600 mg group, $P < 0.01$; UDCA 150 mg group, $P < 0.01$. However, there were no significant differences in biliary lipid composition after UDCA treatment between 600 mg group and 150 mg group.

Case reports

Recently, we observed that UDCA was clearly effective in dissolving cholesterol gallstones in two patients.

Case 1. In August 1973, the patient, a 50-year-old woman, had right hypochondrial pain. Oral cholecystography revealed a functioning, slightly deformed gallbladder with more than 10 radiolucent calculi of about 3–5 mm in diameter (**Fig. 2*A***). She began taking UDCA, 0.45 g daily. In November 1973, after 3 months of the treatment, it was observed that the number of calculi was markedly diminished, and only four calculi remained in the gallbladder. In March 1974, after 7 months of the treatment, no gallstones were found on cholecystography (Fig. 2*B*). Further X-ray studies were carried out, and the disappearance of the stones was confirmed. During the treatment, liver function tests and serum cholesterol remained normal and she had no anorexia, diarrhea, or abdominal pain.

Case 2. A 54-year-old woman had an attack of right hypochrondrial pain in 1975. Oral cholecystography revealed a functioning gallbladder with radiolucent multiple calculi (more than 20 stones) less than 5 mm in diameter (**Fig. 3*A***). She began to take UDCA, 0.15g daily. After 3 months of treatment, the number of calculi was markedly diminished. After 5 months, no gallstones were observed by oral cholecystography (Fig. 3*B*). There were no abnormalities of liver function or abdominal pain during the treatment.

DISCUSSION

The results of the present study show that bile became significantly unsaturated in most patients after

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TABLE 2. Bile acid composition in bile before and during treatment with ursodeoxycholic acid

Patients	Dosage	Pretreatment					During Treatment				
		LCA[a]	DCA	CDCA	UDCA	CA	LCA	DCA	CDCA	UDCA	CA
	mg/day			%					%		
1) S.N.	600	tr.	19.1	40.4		40.4	0.1	tr.	34.0	56.7	9.3
2) A.S.	600	2.6	29.8	27.8		39.8		3.0	20.4	68.4	8.2
3) I.J.	600	0.8	19.2	52.7		27.3		38.9	18.9	39.9	2.2
4) K.T.	600	tr.	2.8	51.2		46.0	0.9	11.3	41.6	43.4	2.8
5) S.K.	600	0.6	20.1	55.1		24.2	6.5	9.3	27.3	48.7	8.2
6) K.S.	600	0.9	32.3	31.4		35.3	1.1	9.8	14.7	48.7	25.8
7) K.N.	600	0.5	46.7	39.9		12.9	4.6	28.4	13.6	49.8	3.7
8) O.F.	600	4.5	24.3	43.9		27.3	1.5	6.5	23.0	65.1	3.9
9) H.T.	600	0.6	20.1	46.7		32.7	2.4	6.8	32.6	52.1	6.3
10) N.S.	600	1.6	23.6	40.8		34.0	1.4	10.4	19.9	59.3	9.0
11) M.M.	600	1.0	25.0	37.5		36.5	2.7	19.2	15.0	51.5	11.5
12) Y.W.	600	2.4	42.6	29.1		26.0	0.5	8.7	27.9	54.3	8.6
Mean ± SD		1.3 ± 1.3	25.5 ± 11.5	41.4 ± 9.0		31.9 ± 8.9	1.8 ± 2.0	12.7 ± 11.1	24.1 ± 8.7	53.2 ± 8.3	8.3 ± 6.3
13) Y.M.	150	1.6	21.0	48.0		29.4	4.2	18.4	51.1	22.1	4.2
14) I.F.	150	2.0	4.9	50.6		42.5	0.2	5.0	38.2	24.3	32.4
15) K.K.	150	3.6	47.2	33.6		15.7		26.3	37.7	20.9	15.1
16) J.K.	150	0.6	10.4	58.0		31.0	1.0	5.8	53.9	22.5	16.8
17) S.K.	150	1.9	38.4	49.3	tr.	10.4	5.6	30.4	29.4	26.7	8.0
18) N.M.	150		6.0	68.6		25.4	0.3	10.7	46.4	24.5	18.1
19) Y.Y.	150	tr.	17.8	50.5	tr.	31.6	0.5	5.1	41.8	26.5	26.1
20) N.T.	150		4.4	86.1		9.6	tr.	tr.	44.8	49.3	5.8
21) S.W.	150	tr.	tr.	52.8		47.2	tr.		61.7	34.0	4.3
22) M.I.	150	0.8	26.9	57.0		15.3	0.9	9.8	46.1	32.7	10.4
23) K.J.	150	1.3	30.0	37.7		30.9	2.5	19.0	27.1	36.3	15.1
Mean ± SD		1.1 ± 1.1	18.8 ± 15.4	53.8 ± 14.3	tr.	26.3 ± 12.4	1.4 ± 1.9	11.9 ± 10.3	43.5 ± 10.2	29.1 ± 8.4	14.2 ± 9.0

[a] Abbreviations: LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; and CA, cholic acid.

the administration of UDCA, the 7β-epimer of CDCA. These findings are similar to those reported by Salen, et al. (13). However, in contrast to CDCA, the cholesterol saturation was not related to UDCA dosage; the lithogenic index was not lower in patients receiving 600 mg/day than in patients receiving 150 mg/day (14). Therefore, expansion of the bile acid pool might be achieved by a dose of UDCA as low as 150 mg/day. In other words, maximal suppression of HMG-CoA reductase in liver could be obtained after the administration of a dose as low as 150 mg/day, and no additional response would occur at a larger UDCA dose. In 1972, Hatanaka, et al. (15) showed that UDCA could inhibit ^{14}C incorporation from [^{14}C]acetate into the nonsaponifiable fraction of cell-free extract of yeast, and further studies should be dealing with the effect of UDCA on the enzymatic activity of HMG-CoA reductase and cholesterol 7α-hydroxylase in the liver.

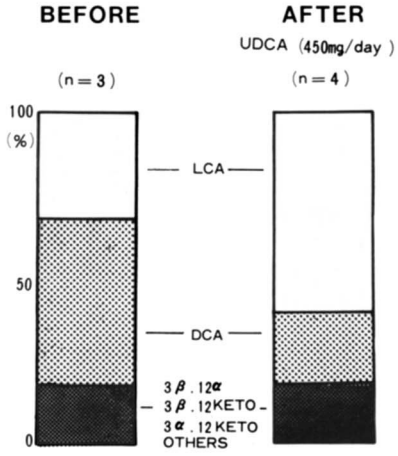

Fig. 1. Fecal bile acid composition after administration of ursodeoxycholic acid. LCA, lithocholic acid; DCA, deoxycholic acid.

Biliary UDCA increased in proportion to the dose administered, and the sum of UDCA plus CDCA was over 70% after UDCA treatment. This finding suggests that UDCA is well absorbed from the intestine but undergoes relatively little transformation during passage through the liver.

Previously, CDCA was shown to be metabolized to UDCA only when the enterohepatic circulation is intact, suggesting a requirement for bacterial transformation (16). Similarly, Salen, et al. (3) reported the

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TABLE 3. Serum bile acid level after three months' treatment with ursodeoxycholic acid

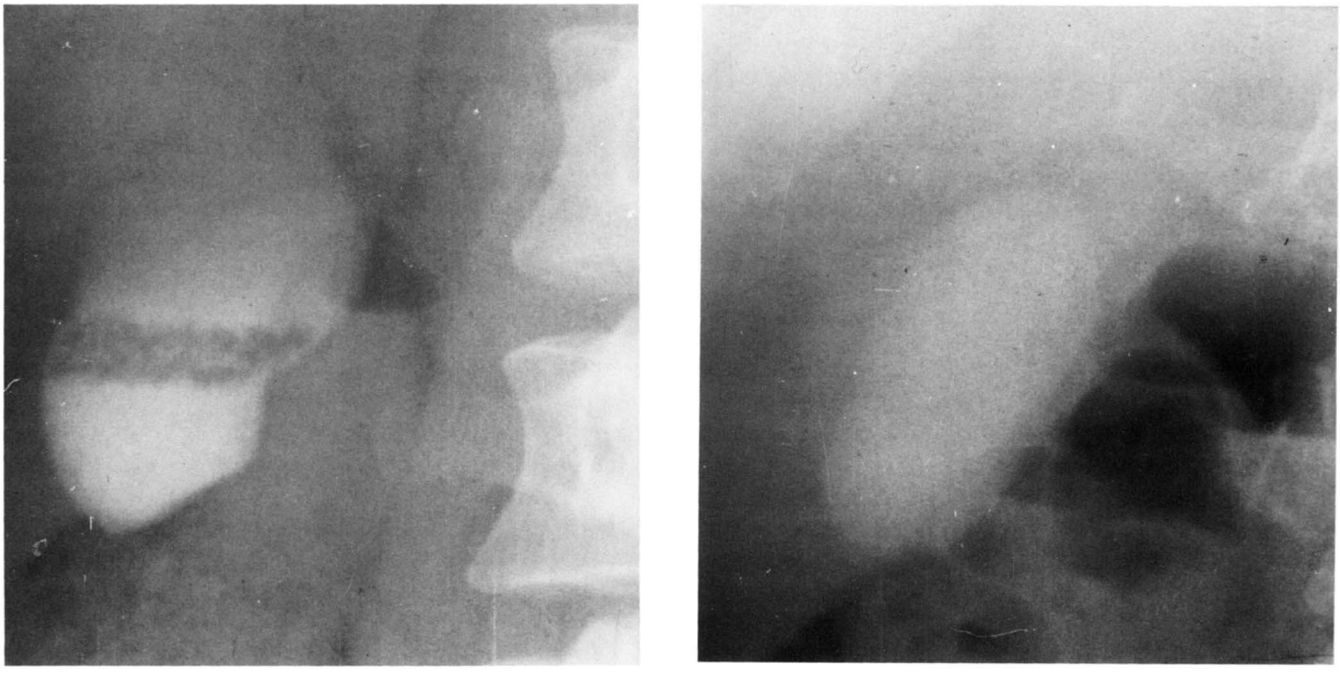
Patients	Nonsulfated					Sulfated					Total
	LCA[a]	DCA	CDCA	UDCA	CA	LCA	DCA	CDCA	UDCA	CA	
			μg/ml					μg/ml			μg/ml
1.		tr.	0.35	0.35	0.35			tr.	tr.		1.05
2.		0.30	0.27	0.47	tr.			tr.			1.04
3.			0.13	0.13				tr.			0.26
4.		0.20	2.41	2.01	0.26						4.88
5.	tr.	0.21	0.36	1.32		0.08		0.08	0.44		2.49
6.		0.12	0.74	2.49	tr.			0.22	tr.		3.57
7.	0.07	0.25	0.27	1.91	0.17	tr.		0.27			2.94
8.	0.03	0.36	0.89	2.61	tr.	0.03		0.19	0.26		4.37
9.		0.17	0.15	0.37				0.12			0.81
Normal (n = 8)			0.63 ± 0.27					0.06 ± 0.05			0.69 ± 0.30

[a] Abbreviations: LCA, lithocholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; UDCA, ursodeoxycholic acid; and CA, cholic acid.

UDCA dosage: 450 mg/day.

formation of UDCA in patients during CDCA therapy, and indicated that there might be a precursor–product relationship between CDCA and UDCA in man.

Despite considerable evidence for the safety of CDCA in man, widespread therapeutic trials of CDCA have been undertaken reluctantly because CDCA is converted to lithocholic acid in the human intestine and it is consistently hepatotoxic in rabbits and many nonhuman primates (17, 18). The major fecal bile acid of patients receiving UDCA was lithocholic acid, indicating that bacterial 7β-dehydroxylation of UDCA occurs in the human intestine. However, there was no significant increase in the proportion of lithocholic acid in bile. UDCA has been widely used as a cholagogue in Japan with no known side effects except mild diarrhea. Therefore, UDCA could be a safe drug to use in expanded clinical studies concerning gallstone dissolution by the oral administration of bile acid. Per-



(a) (b)

Fig. 2. *A*. Case 1, August 1973, before treatment. Upright position. *B*. Case 1, March 1974, after 7 months of UDCA treatment (0.45 g/day). Upright position.

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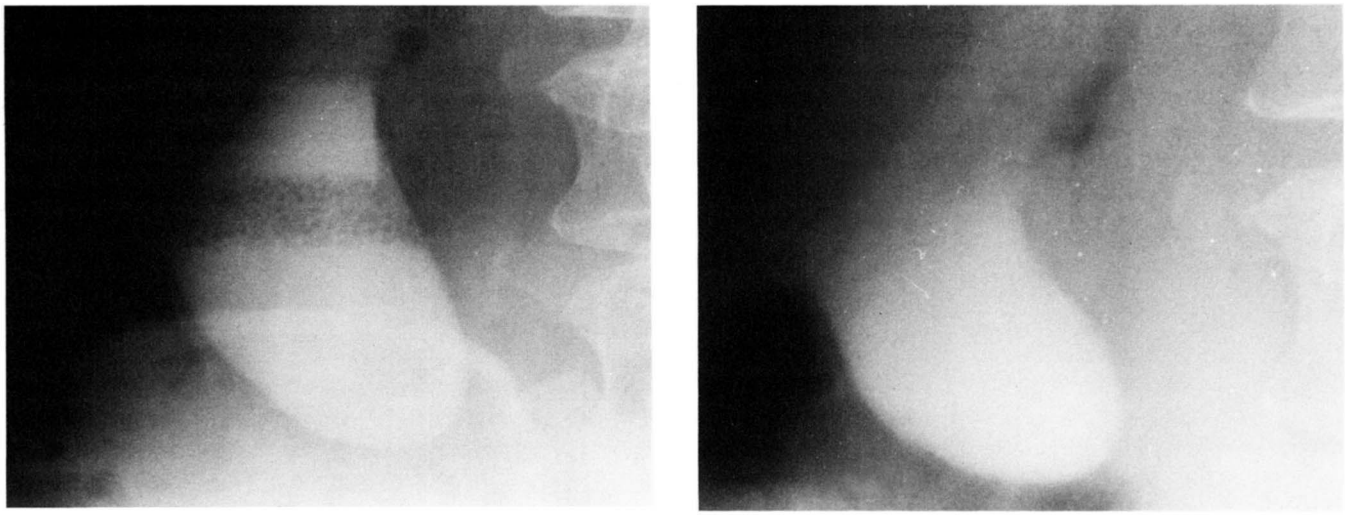

(a) (b)

Fig. 3. *A*. Case 2 before treatment. Upright position. *B*. Case 2 after 5 months of UDCA treatment (0.15 g/day). Upright position.

haps UDCA could be safer than CDCA in the medical treatment of gallstones.

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