APPENDIX I

Review of Journal Articles:

Roda A, Minutello A., Angellotti M.A. et al. Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partiion coeffcient and reverse phase HPLC. J. Lipid Res. 1990; 31:1433-1443.

Method:

The octanol/water partition coefficients were determined using ^{14}C labeled bile acids and unlabeled bile acids. The partition coefficient was determined at concentrations below the water solubility of the ionized species and below the water solubility of the protonated species. The aqueous buffer was pre-saturated with 1-octanol before the partitioning was determined. Samples were left to equilibrate for 2 weeks at 25°C.

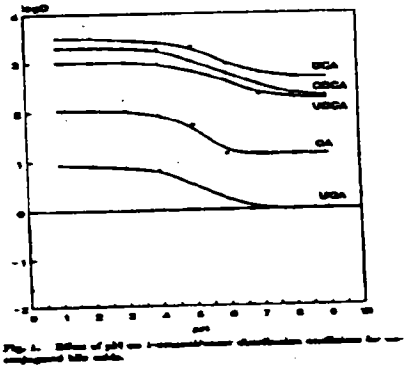
Results:

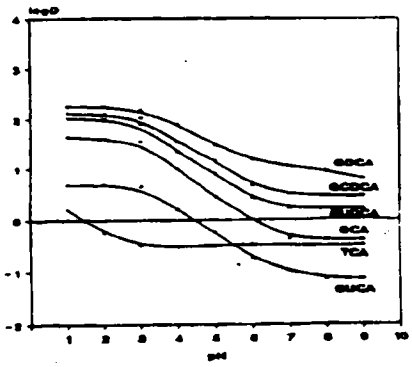
The distribution coefficient determination took into account both ionized and protonated species.

$$D = P'_{HA}[H^+] + P'_A K_a/([H^+] + K_a)$$

where P'_{HA} and P'_A are the intrinsic partition coefficients of HA and A^- respectively
K_a the dissociation constant of the bile acid.

Results:





Salvioli G., Lugli R., Pradelli J.M. and Gigliotti G. Bile acid binding in plasma : the importance of lipoproteins. FEBS Letters 1985; 187 (2): 272-276.

Method:

Blood samples were collected form healthy fasting subjects. Lipoproteins and the lipoprotein-free fractions were separated by sequential ultracentrifugation. Equilibrium dialysis was carried out at 37°C with an incubation time of 36 hours against Tris buffer . The bile acid concentration was at 100μM below CMC. Plasma concentrations for UDCA would be in the 0 to 16 μM range. Also, the distribution between lipoprotein classes was determined. The % bound to plasma proteins was determined, but the proteins were not identified per se.

Results:

This is a published article that uses human serum to determine binding. Most of the other published literature uses rat or bovine serum albumin, which may give erroneous conclusions. Lipoproteins may be a reserve transport system for BA in serum, especially for hydrophilic BA that are less bound to proteins. From previous publications, albumin was identified as the major bile acid carrier.

Composition of pooled plasma and lipoprotein fractions used in dialysis experiments

	Protein*	Phospho-lipids	Cho-lesterol	Trigly-cerides
Plasma	7.0	182	229	154
VLDL	0.035	15	39	110
LDL	0.12	42	150	48
HDL	0.30	107	40	16
$d > 1.210$ g/ml	3.5	18	–	–

* g/dl

Values expressed as mg/dl

Table 2

Binding of different bile acids to plasma and lipoprotein fractions

	VLDL	LDL	HDL	$d > 1.210$	Plasma
DCA	15.4 ± 2.2	20.6 ± 3.4	49.5 ± 5.9	95.3 ± 8.5	97.6 ± 9.6
CDCA	16.2 ± 1.7	21.7 ± 3.7	50.2 ± 5.1	94.6 ± 9.1	96.2 ± 8.3
HDCA	9.6 ± 1.4	11.7 ± 1.2	28.4 ± 2.8	74.3 ± 7.6	81.2 ± 9.3
UDCA	10.8 ± 1.0	16.1 ± 1.7	27.6 ± 3.1	71.7 ± 6.2	76.4 ± 7.7
CA	7.3 ± 0.3	9.5 ± 1.2	19.7 ± 1.2	48.3 ± 4.5	50.2 ± 4.9
UCA	7.1 ± 0.4	9.8 ± 0.8	16.6 ± 2.1	28.2 ± 3.0	29.6 ± 3.1

Values are expressed as percentage of initial bile salt concentration (0.1 mM) bound to the studied fraction. Mean values ± SD of 5 experiments

Table 3

Distribution of bile salts between the lipoprotein fractions

	VLDL	LDL	HDL	$d > 1.210$
DCA	–	1.8 ± 0.4	6.1 ± 1.7	92.0 ± 3.1
CDCA	–	2.2 ± 0.3	6.7 ± 1.7	91.1 ± 5.2
HDCA	0.9 ± 0.3	7.6 ± 2.2	12.1 ± 1.8	79.1 ± 3.8
UDCA	0.6 ± 0.2	4.7 ± 0.9	10.6 ± 1.4	84.2 ± 4.2
CA	1.4 ± 0.8	10.2 ± 1.8	18.3 ± 2.1	70.1 ± 5.4
UCA	2.1 ± 0.6	18.2 ± 2.3	29.2 ± 2.4	50.5 ± 6.8

Values (mean of 5 experiments ± SD) indicate the bile acid content of each fraction (as percentage of the initial amount)

The concentration used for UDCA (0.1mM) is higher than would be seen clinically, where concentrations can be at the 30μM level. In healthy subjects not treated with UDCA, the UDCA plasma level is in the range 0.15 to 0.27 μM. More protein binding information is needed for a range of concentrations of UDCA and its conjugates in patient serum.

Stiehl A, Raedsch R, and Rudolph G. Ileal excretion of bile acids:comparison with biliary bile composition and effect of ursodeoxycholic acid treatment. Gastroenterol. 1988; 94: 1201-6.

Method:

Five male and three female ileostomy patients were studied. The age range was 46 to 67 years. These patients had no evidence of liver disease. All had resection of the sigmoid colon and/or of the descending colon, but no ileal resection. Their ileostomies were within 15 cm of the ileocaecal valve. Ileal excretion was measured at least 4 weeks after surgery. Patients were taken off drugs known to interfere with bile acid metabolism.

Day 1: Duodenal intubation was used to collect concentrated bile after administration of cholecystokinin.

Day 2: Ileostomy bags were sampled every 2 hours.

Day 3: UDCA as 2 capsules =500 mg, was given before breakfast and ileal samples were collected.

Nonsulfated, nonglucuronidated BA were determined enzymatically. Total BA were also determined. Fecal BA were determined from 8 healthy subjects acting as control group.

Results:

Table 1. Bile Acids in Bile and Terminal Ileum of Patients With Ileostomy

	Litho	Deoxy	Chenо	Ursо	Cholic	Total
Bile						
Nonsulfated, nonglucuronidated bile acids	–	0.30 ± 0.14	42.53 ± 4.57	0.00 ± 0.00	56.12 ± 4.93	98.94 ± 0.30
Sulfates	–	0.01 ± 0.01	0.42 ± 0.14	–	0.14 ± 0.07	0.57 ± 0.22
Glucuronides	–		0.30 ± 0.22	–	–	0.30 ± 0.22
Terminal ileum						
Nonsulfated, nonglucuronidated bile acids	–	0.44 ± 0.22	30.00 ± 2.22*	1.06 ± 0.37	68.51 ± 2.56*	98.63 ± 0.50
Sulfates	–	0.02 ± 0.01	1.38 ± 0.43*	–	0.20 ± 0.07*	1.40 ± 0.40*
Glucuronides	–	–	0.80 ± 0.05*	–	–	0.80 ± 0.01*

Chenо, chenodeoxycholic acid; cholic, cholic acid; deoxy, deoxycholic acid; litho, lithocholic acid; ursо, ursodeoxycholic acid. Values are means ± SEM percent of total. * Significantly different from bile with $p < 0.05$.

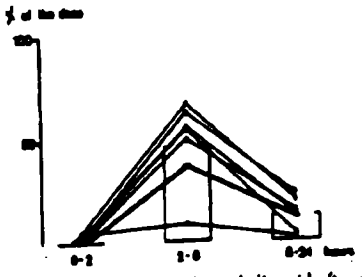


Figure 1. Ileal excretion of ursodeoxycholic acid after oral administration of 500 mg of this bile acid. Data are given as a percentage of the administered dose.

Most of the ileal excretion occurs in the 2-8 hour collection period.

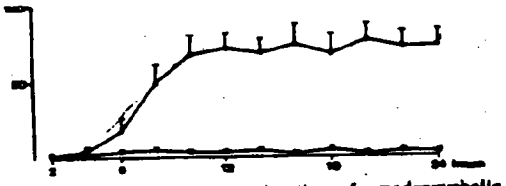


Figure 2. Glycine and taurine conjugation of ursodeoxycholic acid (urso) in ileal fluid after oral administration of 500 mg of this bile acid at time 0. Glycine-conjugated ursodeoxycholic acid ●—●, taurine-conjugated ursodeoxycholic acid ○—○.

UDCA is mostly glycine-conjugated with much smaller amounts of taurine-conjugated product. Conjugates of UDCA appear in the ileum 2 hours post administration of UDCA.

Parquet M., Metman E.H., Raizman A. et al. Bioavailability, gastrointestinal transit, solubilization and fecal excretion of ursodeoxycholic acid in man. Eur. J. Clin. Investig. 1985; 15: 171-178.

Method:

12 healthy subjects (six males and 6 females) aged 21 to 24 years were divided into two study groups.

Group 1: Four males and three females whose gastrointestinal tract was catheter-free.

Group 2: Two males and three females had a gastrointestinal catheter placed.

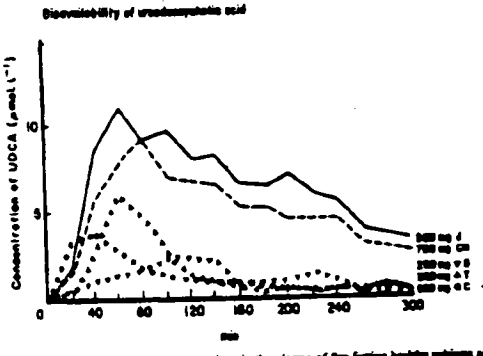
Group 1 had ^{3}H-UDCA administered as 500 mg a capsule with 50 mL water. 10 mL blood samples were taken at 20 minute intervals up to 4 hours. Fecal and urine samples were collected for 24 hours for 3 days.

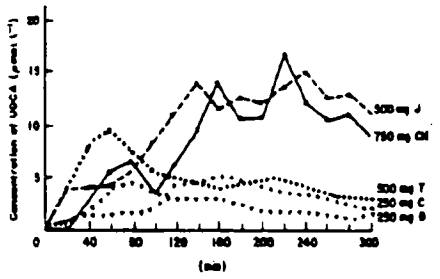
Group 2 on Day 1, five subjects ingested 250, 500 or 750 mg ^{14}C-UDCA and 200 mL of water containing ^{3}H- PEG 4000. Jejunal contents were sampled at 20 minute intervals for 5 hours (distal jejunum, ileum and colon were excluded). Blood was sampled every 20 minutes up to 5 hours.

On Day 2, the subjects ingested 250, 500 or 750 mg ^{3}H-UDCA and 200 mL of water containing ^{14}C - PEG 4000. Jejunal contents were sampled 20 minutes before the UDCA was administered and at 20 minute intervals for 5 hours, however an aliquot was taken and the remainder reinjected into the intestinal lumen. Blood was sampled every 20 minutes up to 5 hours.

Total UDCA was determined (free and conjugated) in blood. ^{14}C and ^{3}H radioactive UDCA was measured in the jejunal samples with separation of free from conjugated using t.l.c. Fecal and samples had total UDCA measured.

Results:





From these graphs, it can be seen that the UDCA plasma time profiles vary according to where the UDCA is being absorbed. It must be kept in mind that total UDCA was being measured in the plasma.

Table 2. Distribution of soluble and solid forms of ursodeoxycholic acid in jejunal content

Subjects		Dose (mg)	% of soluble UDCA	% of insoluble UDCA	% of total UDCA recovered	UDCA absorbed in mg	% of total polyethylene glycol recovered		% of UDCA soluble
C ♂	Ileum excluded	250	27	50	77	57·5	100	Ileum reinjected	27
DE ♀		250	53	25	78	55	100		35
T ♂		500	22	26	48	250	83		25
J ♀		500	44	21	65	175	83		
CH ♀		750	33	41	74	195	84		30

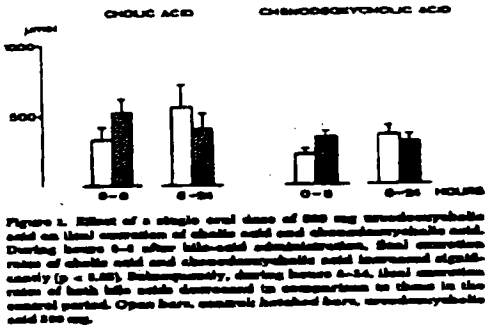
There is slow solubilization of UDCA in the jejunum. This was under fasting conditions. After food, solubilization is enhanced and the intestinal absorption would probably change. The table is incorrect in the amount of UDCA absorbed. The amount absorbed has not been corrected for the PEG recovered. The assumption of course is that the disappearance from the jejunum means that this is the amount that is absorbed across the gastrointestinal wall and into the bloodstream.

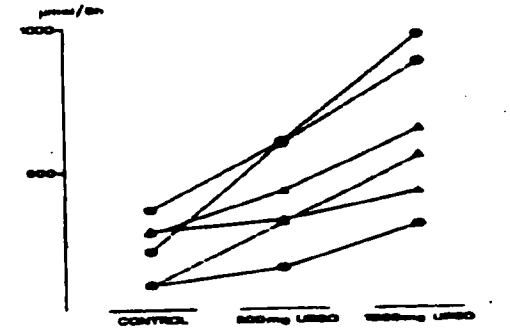
Stiehl A, Raedsch R, and Rudolph G. Acute effects of ursodeoxycholic and chenodeoxycholic acid on the small intestinal absorption of bile acids. Gastroenterol. 1990; 98: 424-428.

Method:

Six male and two female ileostomy patients aged 43 to 55 and whose weight ranged from 55 to 78 Kg, took part in this study. These patients had no evidence of liver disease. All had resection of the sigmoid colon and/or of the descending colon, but no ileal resection. Their ileostomies were within 15 cm of the ileocaecal valve. Patients took one dose of 500 mg CDCA (from Chenofalk, Germany) and after one week they took 500 mg of UDCA (Ursofalk, Germany) in a randomized crossover design. One week later 3 patients took one dose of 1000 mg of UDCA. Ileal fluid samples were collected at 2 h intervals. There was a prior control period in which no UDCA/CDCA was taken.

Results:





Conclusions:

UDCA interferes with the absorption of common bile acids in the small intestine to decrease their

absorption.

Roda A., Roda E., Marchi E. et al. Improved intestinal absorption of an enteric-coated sodium ursodeoxycholate formulation. Pharm. Res. 1994; 11(5): 642-647.

Method:

Six healthy subjects received the following treatments in a randomized order and separated by a ten-day washout period:

Trt 1- 450 mg UDCA in a gelatin capsule

Trt 2- 475 mg sodium salt UDCA in a gelatin capsule

Trt 3- 475 mg of enteric-coated sodium salt UDCA

Trt 4- 515 mg of glycoursodeoxycholic acid in a gelatin capsule

Trt 5- 540 mg of enteric-coated sodium salt of glycoursodeoxycholic acid

An assay specific for UDCA was used.

Results:

All treatments were taken after a standard meal.

The first peak occurred after 1 hour and a second peak was observed in the plasma after four hours post-administration of UDCA (trt 1). The intersubject variability was high for Cmax. Trt 2 gave comparative plasma-time profiles to trt 1. Trt 3 showed almost no UDCA levels for 2 hours and then reached a Cmax at about 3 to 4 hours. Cmax was on average about four times higher after trt3 than either trt 1 or 2 and was highly variable. The mean AUC was higher after trt 3 than the other treatments.

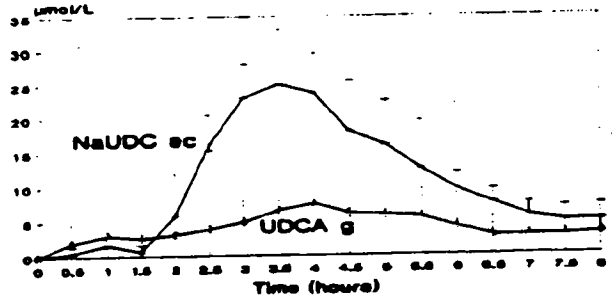


Fig. 3. Time profile of mean serum ursodeoxycholic acid concentrations after a single-dose administration of UDCA and enteric-coated sodium ursodeoxycholate. Each point is the mean value ± SD of six experiments.

Table II. Peak Serum UDCA Concentration (C_{max}; μM), Time to Peak Serum Concentration (T_{max}; min), and Area Under the Curve to 8 hr (μM · hr) in the Same Subject Receiving (UDCA g) 450 mg of UDCA in a Gelatin Capsule, (NaUDC g) 475 mg of NaUDC in a Gelatine Capsule, and (NaUDC ec) 475 mg of Enteric-Coated NaUDC

Formulation	1	2	3	4	5	6	Mean ± SD
UDCA g							
C_{max}	7.5	8.4	4.2	12.2	6.5	7.9	6.6 ± 3.9
T_{max}	4.0	3.2	2.8	4.1	2.8	3.0	3.8 ± 0.6
AUC	30.2	27.6	20.5	18.9	26.6	30.2	25.6 ± 4.8
NaUDC g							
C_{max}	8.7	12.5	7.2	6.5	10.6	6.8	8.7 ± 2.4
T_{max}	2.6	2.4	2.4	2.8	1.7	2.5	2.4 ± 0.4
AUC	31.4	29.5	20.8	17.6	20.7	31.6	25.2 ± 6.2
NaUDC ec							
C_{max}	30.5	28.9	24.4	18.7	24.7	26.8	25.5 ± 9.1
T_{max}	3.0	2.5	4.1	4.1	2.9	3.8	3.4 ± 0.7
AUC	53.2	46.6	36.7	32.7	48.2	51.6	44.8 ± 8.2

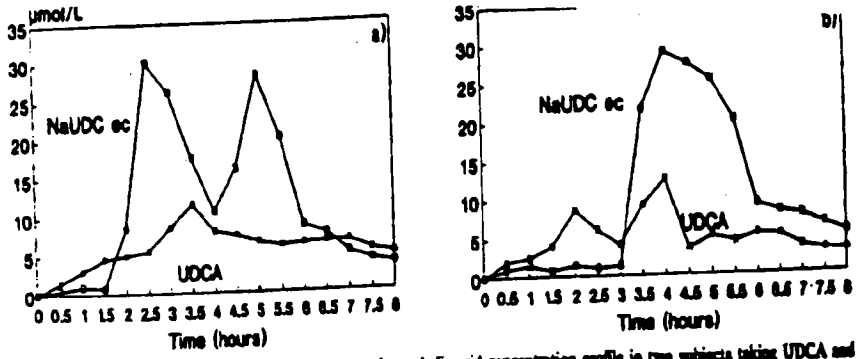


Fig. 4. Intersubject variability of the time-serum ursodeoxycholic acid concentration profile in two subjects taking UDCA and NaUDC, enteric coated.

Fedorowski T., Salen G., Colallilo A., et al. Metabolism of ursodeoxycholic acid in man. Gastenterol.1977; 73 (5) : 1131-1137.

Method:

Seven subjects took part in this study. Most subjects had normal liver function test results, however one subject had elevated triglycerides and another was diagnosed as having cholelithiasis. All subjects were given the same diet. The subjects were given 1G of UDCA orally per day for two weeks and then given a dose of IV [24-^{14}C]UDCA. A two-month washout was followed by 1G CDCA orally per day for two weeks and then a dose of IV [24-^{14}C]UDCA. This was a sequential design and not all subjects took both treatments. Intestinal bile samples were taken after stimulation of the gallbladder with CCK. Free and amidated bile acids were measured. Stool samples were collected daily during week 2 and 3 of the study.

TABLE 2. *Biliary lipid composition before and during treatment with ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA)*

Subject	Cholesterol			Bile acids			Phospholipids			Cholesterol saturation		
	Control	UDCA*	CDCA*	Control	UDCA	CDCA	Control	UDCA	CDCA	Control	UDCA	CDCA
	%			%			%			%		
A. S.	8.8	5.4	5.2	72.1	83.5	91.8	19.9	11.1	3.0	18.8	17.5	18.2
J. S.	5.6	5.4	5.5	79.9	90.6	87.7	14.5	4.1	6.8	16.9	17.5	17.2
O. W.	5.9	3.2	4.3	84.3	91.1	89.4	9.8	5.7	6.3	15.9	30.3	22.3
J. Sw.	8.8	5.0		84.4	90.7		6.8	4.3		18.4	19.0	
P. J.	9.5	4.5		79.4	89.7		11.1	4.8		9.5	21.0	
H. J.	8.6		4.8	77.8		87.2	13.6		8.1	18.6		19.9
Mean	7.9	4.7	5.0	79.7	89.1	89.0	12.5	6.0	6.1	11.5	21.0	19.6
± SD	±1.7	±0.5	±0.5	±4.6	±3.2	±2.1	±4.3	±2.9	±2.2	±2.4	±3.8	±3.8

* UDCA, 1 g per day.
* CDCA, 1 g per day.

TABLE 3. [illegible]

Study parameters, UDCA kinetics	Subjects							Mean ± SD
	A. S.	J. S.	O. W.	P. J.	J. Sw.	H. J.	E. E.	
UDCA treatment								
UDCA pool (μmol/kg)	96.4	28.5	16.4	57.6	21.7			44.1 ± 33.3
UDCA FTR (day⁻¹) [K]	1.09	0.59	0.76	1.34	0.93			0.91 ± 0.32
UDCA input								
μmol/kg/day	71.3[a]	39.8	17.8	35.4	20.7			26.4 ± 10.8
mg/day [production rate]	2446.2[a]	1491.4	458.0	924.2	724.8			609.6 ± 438.3
CDCA treatment								
UDCA pool (μmol/kg)	4.6	3.3	0.7			8.5	3.1	4.1 ± 2.9
UDCA FTR (day⁻¹)	1.10	0.96	0.57			1.92	0.52	1.01 ± 0.58
UDCA synthesis								
μmol/kg/day	3.4	2.5	0.9			3.7	5.0	3.1 ± 1.5
mg/day	117.1	94.3	23.9			111.0	114.2	92.4 ± 39.1

[a] UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; FTR, fractional turnover rate.

[b] A. S. was not included in the so because substantial amounts of UDCA were present in the bile before treatment and thus the turnover value reflects both endogenous synthesis and absorption of UDCA.

34 100

This assumes that there is a single pool and steady-state conditions exist. Total bile acid is being measured ie. free bile acid has not been isolated from its metabolites. This study showed that there was some precursor relationship between CDCA and UDCA. The method is based on the Lindstedt isotope dilution method in which back extrapolation of the radioactive decay curve to the value at time zero gives the pool size. One measurement is being taken per day during which there will be 6 to 12 enterohepatic cycles. If bile acid is being given orally, the input into the pool reflects synthesis of bile acid and absorption. This assumes that the rate of absorption is constant. This particular journal article is using the fact that the dose of UDCA being given is much higher than the endogenous bile acid synthesis of UDCA. The UDCA production(absorption) rate has been incorrectly calculated, since this should be the product of the fractional turnover rate and the pool size. Also, if the free bile acid is not being measured then errors will be incurred by following total bile acids that includes metabolites. This technique has not been validated as far as reflecting production/absorption of UDCA.

Roda E., Mazzella G., Bazzoli F., et al. Effect of ursodeoxycholic acid administration on biliary lipid secretion in primary biliary cirrhosis. Dig. Dis. Sci. 1989; 14(12) Dec. Suppl.:52S-58S.

Method:

Seven female PBC patients aged 34 to 58 years with histological confirmation of the stage of their disease, took part in this study. A dose of 600 mg/d of UDCA was given in two divided doses after lunch and dinner. After four weeks treatment, the cholesterol index, biliary lipid and bile acid were measured as well as bile acid pool size, serum bile acid levels and liver function tests.

Results:

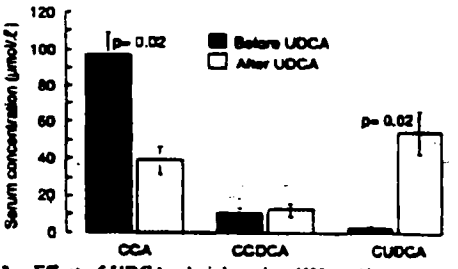


Fig 2. Effect of UDCA administration (600 mg/day) on serum bile acid levels in seven subjects with primary biliary cirrhosis. (CCA = conjugated cholic acid; CCDCA = conjugated chenodeoxycholic acid; CUDCA = conjugated ursodeoxycholic acid.)

TABLE 3. BILIARY BILE ACID PATTERN IN FASTING GALLBLADDER BILE EVALUATED BY HPLC

	Percent Molar*				
Patient	*LCA*	*DCA*	*CDCA*	*CA*	*UDCA*
Before UDCA					
1	—	23	38	39	—
2	1	11	34	49	5
3	2	24	32	42	—
4	4	5	23	62	6
5	1	10	33	56	—
6	7	17	29	47	—
7	5	28	31	36	—
Mean	2.9	16.8	31.4	47.3	1.8
± SE	0.1	3.2	1.7	3.5	1.0
After UDCA					
1	1	11	22	28	38
2	2	5	17	42	34
3	—	6	26	38	30
4	2	-	15	44	39
5	8	4	17	40	31
6	—	9	26	30	35
7	6	11	26	26	31
Mean	2.8	6.6†	21.3†	35.4†	34.0
± SE	0.9	1.5	1.8	2.6	1.3

*HPLC = high performance liquid chromatography; LCA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; CA = cholic acid; UDCA = ursodeoxycholic acid.

†$P < 0.02$ vs baseline.

TABLE 5. INDIVIDUAL BILE ACID SECRETION RATES

Patient	Output (μmol/hr)*				
	LCA	DCA	CDCA	CA	UDCA
Before UDCA					
1	—	426	703	721	—
2	19	211	653	941	96
3	38	456	608	798	—
4	77	97	444	1197	116
5	16	158	521	885	—
6	112	272	464	752	—
7	60	336	372	432	—
Mean ± SE	46 ± 15	279 ± 51	538 ± 46	818 ± 88	30 ± 20
After UDCA					
1	47	517	1034	1316	1786
2	38	95	323	789	646
3	—	162	347	1026	810
4	39	—	290	849	733
5	176	88	374	880	682
6	—	146	421	486	567
7	97	178	421	421	502
Mean ± SE	57 ± 23	169 ± 62	459 ± 98	825 ± 116	821 ± 166†

*LCA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; CA = cholic acid; UDCA = ursodeoxycholic acid.
†$P < 0.01$.

Patient	Total bile acid pool (mmol)	Individual bile acid pool sizes (mmol)*				
		LCA	DCA	CDCA	CA	UDCA
Before UDCA						
1	4.41	—	1.01	1.67	1.72	—
2	5.32	0.05	0.58	1.80	2.60	0.3
3	4.32	0.09	1.04	1.38	1.81	—
4	4.02	0.16	0.20	0.92	2.49	0.3
5	6.24	0.06	0.62	2.06	3.49	—
6	2.51	0.17	0.42	0.73	1.18	—
7	4.02	0.20	1.12	1.24	1.45	—
Mean ± SE	4.38 ± 0.44	0.1 ± 0.03	0.71 ± 0.13	1.40 ± 0.18	2.10 ± 0.30	0.07 ± 0.05
After UDCA						
1	5.21	0.05	0.57	1.15	1.46	1.98
2	5.82	0.12	0.29	1.08	2.40	1.98
3	5.22	—	0.26	1.40	1.98	1.51
4	4.52	0.09	—	0.68	1.59	1.76
5	4.20	0.34	0.17	0.71	1.08	1.30
6	4.82	—	0.43	1.25	1.45	1.69
7	5.01	0.30	0.60	1.30	1.30	1.55
Mean ± SE	4.96 ± 0.19	0.09 ± 0.04	0.33 ± 0.08	1.11 ± 0.09	1.73 ± 0.13	1.68 ± 0.09†

*LCA = lithocholic acid; DCA = deoxycholic acid; CDCA = chenodeoxycholic acid; CA = cholic acid; UDCA = ursodeoxycholic acid.
†$P < 0.02$.

These tables show the enrichment of the bile with UDCA. The theory behind the use of UDCA in the treatment of PBC is to replace the more cytotoxic bile acids with hydrophilic, noncytotoxic UDCA.

Batta A.K., Salen G., Mirchandani R. et al. Effect of long-term treatment with ursodiol on clinical and biochemical features, and biliary bile acid metabolism in patients with primary biliary cirrhosis. Am. J. Gastroenterol. 1993; 88(5):691-700.

Fourteen patients with PBC took part in this study. There were 12 females and two males with mean age of 53 years. After discontinuing all medications for three months, the patients then took three capsules of 300 mg UDCA (Actigall) or 10-12 mg/Kg/day for six to 12 months. It was not stated whether this was taken as a divided dose or not. Bile, fasting serum and urine samples were taken.

Results:

TABLE 1

*Effect of 2 Yr of Ursodiol Treatment on the Biochemical Parameters in Patients with Primary Biliary Cirrhosis**

Treatment	Alk. Phos.	GGT	ALT	AST	TB
Pretreatment†	577 ± 394	443 ± 253	109 ± 59	127 ± 83	2.0 ± 2.1
Placebo‡	−11 ± 22%§	−6 ± 37%	−7 ± 38%	−5 ± 31%	21 ± 33%
	(NS)‖	(NS)	(NS)	(NS)	(NS)
Ursodiol¶ (6 mo)	−39 ± 18%	−63 ± 48%	−42 ± 20%	−47 ± 18%	−13 ± 37%
	(<0.001)	(<0.002)	(<0.007)	(<0.002)	(NS)
Ursodiol** (12 mo)	−58 ± 23%	−56 ± 27%	−49 ± 23%	−39 ± 15%	−6 ± 18%
	(<0.001)	(<0.002)	(<0.006)	(<0.003)	(NS)
Ursodiol** (18 mo)	−50 ± 21%	−50 ± 21%	−40 ± 17%	−44 ± 22%	2 ± 27%
	(<0.001)	(<0.002)	(<0.005)	(<0.002)	(NS)
Ursodiol** (24 mo)	−44 ± 24%	−52 ± 39%	−34 ± 12%	−41 ± 21%	11 ± 47%
	(<0.02)	(<0.03)	(<0.03)	(<0.001)	(NS)

* Alk. Phos., alkaline phosphatase (upper limit of control, 140 IU/L); GGT, γ-glutamyltransferase (upper limit of control, 38 IU/L for males; 29 IU/L for females); ALT, alanine aminotransferase (upper limit of control, 45 IU/L); AST, aspartate aminotransferase (upper limit of control, 40 IU/L); TB, total bilirubin (upper limit of control, 1.2 mg/dl).

† Fasting pretreatment serum was obtained from 14 patients.

‡ Fasting serum was obtained every month from 11 patients on placebo treatment. The values shown are means from the last 3 months.

§ The values shown are percent changes from the pretreatment enzyme levels, obtained by single sample *t* test.

‖ The significance values were calculated by paired Student's *t* test.

¶ Fasting serum was obtained from 11 patients receiving 900 mg/day ursodiol. The values shown are means from last 3 months.

** Fasting serum was obtained from eight patients receiving 900 mg/day ursodiol. The values shown are means from last 3 months.

TABLE 4

*Effect of Ursodiol Treatment on Serum Bile Acids in Patients with Primary Biliary Cirrhosis**

Treatment	Bile Acids (μM)				
	Unconjugated			Others¶	Total
	Endogenous†	Hydroxylated‡	Ursodiol		
Patients					
Pretreatment‖	46.1 ± 15.4	2.4 ± 0.4	1.4 ± 0.4	2.9 ± 0.2	52.8 ± 12.6
Placebo¶	53.3 ± 16.2	2.4 ± 0.5	1.7 ± 0.3	3.5 ± 0.2	60.6 ± 14.3
Ursodiol** (6 mo)	20.0 ± 4.2††	3.5 ± 0.6	19.8 ± 6.7††	3.4 ± 2.8	48.7 ± 9.4
Ursodiol** (12 mo)	23.0 ± 4.1††	3.0 ± 0.4	26.7 ± 8.2††	6.2 ± 2.7	58.9 ± 11.2
Ursodiol** (24 mo)	22.7 ± 3.8††	3.3 ± 0.4	30.9 ± 7.6††	3.2 ± 2.2	60.3 ± 9.8
Controls‡‡					
Pretreatment	3.4 ± 0.5		0.2 ± 0.1		3.6 ± 0.2
Ursodiol (2 wk)	0.6 ± 0.3	0.2 ± 0.1	7.2 ± 0.8	0.3 ± 0.1	8.3 ± 0.6

* Fasting serum bile acids were quantified by capillary gas-liquid chromatography.

† Chenodeoxycholic acid, cholic acid, deoxycholic acid, lithocholic acid.

‡ 1β,3α,12α-Trihydroxy-5β-cholanoic acid, 1β,3α,7β-trihydroxy-5β-cholanoic acid, 1β,3α,7α,12α-tetrahydroxy-5β-cholanoic acid.

§ Iso-ursodeoxycholic acid, ursocholic acid, hyocholic acid, ω-muricholic acid.

‖ Fasting serum was obtained immediately pretreatment. Values reported are mean ± SD (n = 14).

¶ Fasting serum was obtained every month from patients receiving placebo. Average of last two determinations for each patient was used to obtain mean ± SD (n = 11).

** Fasting serum was obtained every month from patients receiving ursodiol. Average of [illegible] effect, n = 11), 11 and 12 (12-month effect, n = 8), or 23 and 24 (24-month effect, n = 8) for each patient was used to obtain mean ± SD.

†† $p < 0.001$.

‡‡ Fasting serum was obtained from four healthy subjects. ursodiol (900 mg/day) was fed for 10 days, and serum was collected before and on the last day of bile acid feeding.

Treatment	Bile Acids (μM/G creatinine)				
	Uncommon			Others§	Total
	Endogenous†	Hydroxylated‡	Ursodiol		
Patients					
Pretreatment‖	37.3 ± 16.7	14.2 ± 10.2	2.9 ± 0.5	7.8 ± 2.8	62.2 ± 22.2
Placebo¶	35.7 ± 14.7	12.4 ± 8.9	2.7 ± 0.6	8.1 ± 2.4	58.9 ± 18.6
Ursodiol** (6 mo)	23.5 ± 8.7††	13.7 ± 5.4	77.9 ± 19.7‡‡	23.9 ± 7.8	139.0 ± 28.9‡‡
Ursodiol** (12 mo)	24.9 ± 7.8††	15.0 ± 4.8	120.1 ± 33.6‡‡	38.6 ± 12.7	198.6 ± 42.7‡‡
Ursodiol** (24 mo)	18.5 ± 4.5††	11.5 ± 4.7	96.9 ± 23.9‡‡	35.0 ± 8.7	161.9 ± 34.7‡‡
Controls§§					
Pretreatment	1.7 ± 0.3		0.3 ± 0.1	0.5 ± 0.1	2.5 ± 0.4
Ursodiol (2 wk)	2.5 ± 0.5	2.1 ± 0.3	33.0 ± 6.7	11.1 ± 2.2	48.7 ± 7.3

* Urinary bile acids were quantified by capillary gas-liquid chromatography. Values are reported as percentage of total bile acids.
† Chenodeoxycholic acid, cholic acid, deoxycholic acid, lithocholic acid.

In terms of the composition of bile acid, it can be seen that % CDA out of the total bile acid pool remains the same irrespective of treatment with UDCA. The % CA shows differences between studies in that there may be a decrease or its % contribution remains the same. DCA is present in healthy subjects in comparison to CA and there is a decrease in its % contribution with UDCA treatment. The consistent change can be seen in the % UDCA irrespective of the disease state ie there are marginal levels pre-treatment and then substantial increase post-treatment. In terms of the presence of conjugated vs. unconjugated bile acid, about 6% unconjugated bile acids can be found in the bile. CA predominates. In healthy subjects (control), this % is much lower being about 0.5%. UDCA treatment results in UCDA becoming the predominant bile acid and a decrease in other free bile acids. It can be seen that UDCA excretion increases in the urine with treatment.

TABLE 4

*Effect of Ursodiol on Biliary Bile Acids in Primary Biliary Cirrhosis**

Bile acid	Patients			Controls‖	
	Pre†	Placebo‡	Ursodiol§	Pre	Ursodiol
CDCA¶	33 ± 8**	41 ± 2	29 ± 8	24 ± 5	18 ± 3
CA	62 ± 8	57 ± 2	33 ± 9††	49 ± 8	15 ± 2‡‡
UDCA	0.3 ± 0.2	0.1 ± 0.1	31 ± 12§§	1 ± 1	55 ± 7§§
LCA	0.7 ± 0.3	0.5 ± 0.2	1 ± 1	1 ± 0.2	2 ± 0.4
DCA	3 ± 3	0.4 ± 0.2	5 ± 3	25 ± 6	9 ± 0.3
Others***	1 ± 0.2	1 ± 0.4	1 ± 1		1 ± 0.2

* Biliary bile acids were quantified by capillary gas-liquid chromatography. Values are reported as percentage of total bile acids

TABLE 5

*Unconjugated Bile Acids as Percent of Total Biliary Bile Acids in Patients with Primary Biliary Cirrhosis**

Patient	Treatment	Free Bile Acids as % of Total Bile Acids				
		CDCA†	CA	DCA	UDCA	Total
EZ	Pretreatment‡	0.4§	5.1	0.5	—	6.0
	Placebo‖	1.8	2.4	1.8	—	6.0
RG	Pretreatment	0.2	0.4	—	—	0.6
	Placebo	0.1	0.1	—	—	0.2
DD	Pretreatment	7.4	9.5	0.4	—	17.3
	Placebo	4.1	12.5	0.3	—	16.9
SD	Pretreatment	1.3	2.4	0.4	—	4.1
	UDCA¶	0.2	0.7	0.6	4.9	6.4
ME	Pretreatment	0.1	1.2	—	—	1.3
	UDCA	0.3	0.4	0.1	0.5	1.3
EA	Pretreatment	2.6	5.4	0.5	—	8.5
	UDCA	2.2	2.6	0.9	2.7	8.4
Control**	Pretreatment	—	0.5	—	—	0.5
	UDCA	—	—	—	0.6	0.6

* Biliary bile acids were quantified by capillary gas-liquid chro-

Marigold J.H., Bull H.J., Gilmore I.T. et al. Direct measurement of hepatic extraction of chenodeoxycholic acid and ursodeoxycholic acid in man. Clin. Sci. 1982; 63: 197-203.

Method:

Ten subjects were enrolled into the study, who had neither liver nor gallbladder disease. These subjects were undergoing cardiac catheterization. Six of the subjects got ^{14}C-CDCA as an iv bolus and four subjects got ^{14}C-UDCA as an iv bolus. Another 22 patients with liver disease (divided into mild or severe according to serum albumin) were studied: 10 of these had ^{14}C-CDCA as an iv bolus and 10 got ^{14}C-UDCA as an iv bolus. Some of the patients also had ICG injected as a bolus (0.25 mg/Kg) immediately after the radiolabeled bile acid. Blood samples were taken at 5 , 7.5 and 9 minutes post-injection from the hepatic vein. Blood samples were also taken from a peripheral vein or the aorta in the cardiac catheterization patients. Total bile acid was measured. Hepatic extraction was determined from the following calculation:

$$E = \frac{\text{(peripheral vein or aorta) - (hepatic vein)}}{\text{(peripheral vein or aorta)}}$$

Results:

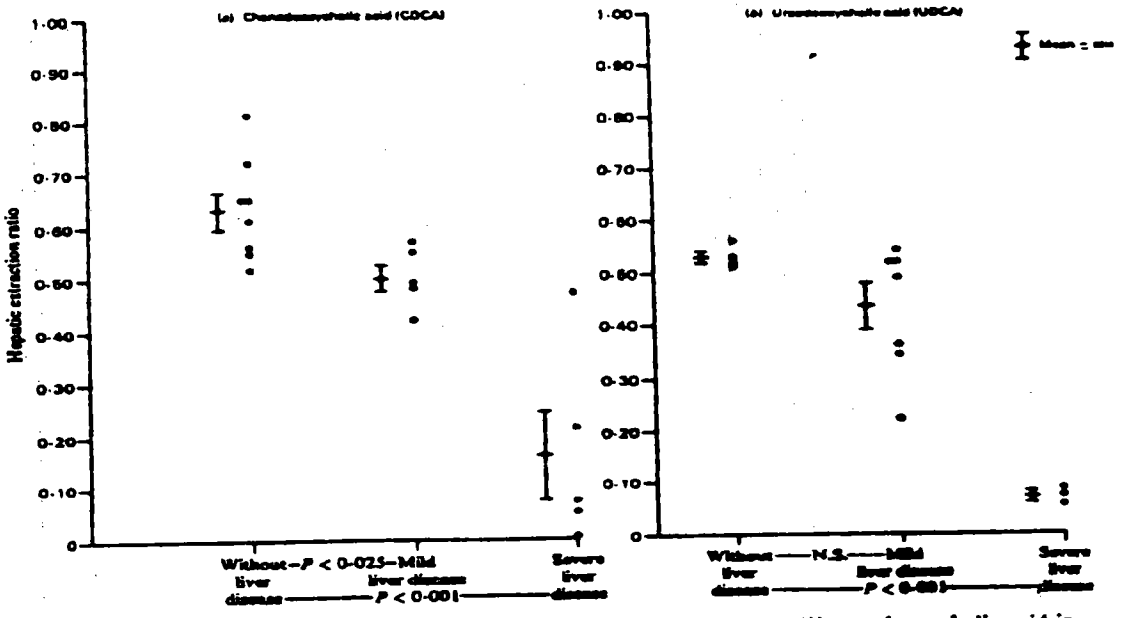


FIG. 1. Hepatic extraction ratios for (*a*) chenodeoxycholic acid and (*b*) ursodeoxycholic acid in patients with and without liver disease. N.S., Not significant.

The extraction ratios for the two bile acids seem to correlate with ICG extraction ratio ($r=0.94$, $N=8$, $p<0.001$ for UDCA and ICG). The extraction ratio for UDCA was 0.53 ±

0.01 (SEM) in patients without liver disease and was 0.43 ± 0.05 (SEM) in mild and 0.07 ± 0.01 (SEM) in severe liver disease. A nonspecific assay was used for measuring UDCA in serum.

Beuers U, Spengler U, Zwiebel F.M. et al. Effect of ursodeoxycholic acid on the kinetics of the major bile acids in health and in chronic cholestatic liver disease. Hepatology 1992; 15: 603-608.
Method:
Five patients (three with PBC and two with PSC) were studied along with four healthy men. Stable isotopes were used. Between 10 am and noon, 50 mg of 24-^{13}C-CDCA and 50 mg of 2,2,4,4,-$^{2}H_4$-DCA dissolved in 200 mL 0.25% sodium bicarbonate were given orally. Postprandial blood samples were collected before the radiolabeled compounds were given and once a day 2 hours post cibum at 24 hour intervals for the next four days. After four days, subjects were given 13-15 mg/Kg/day UDCA. There is no description whether UDCA was given with food or in divided doses. After four weeks of UDCA treatment and between 10 am and noon, 50 mg of 24-^{13}C-CDCA and 50 mg of 2,2,4,4,-$^{2}H_4$-DCA dissolved in 200 mL 0.25% sodium bicarbonate were given orally. Postprandial blood samples were collected before the radiolabeled compounds were given and once a day 2 hours post cibum at 24 hour intervals for the next four days. Fasting bile acids were also determined the two days that the radiolabeled compounds were administered.
Note that the radiolabeled compounds were given orally: this may lead to less than simple interpretation of pool size, fractional turnover and synthesis/input rates compared with iv administration.

Results:

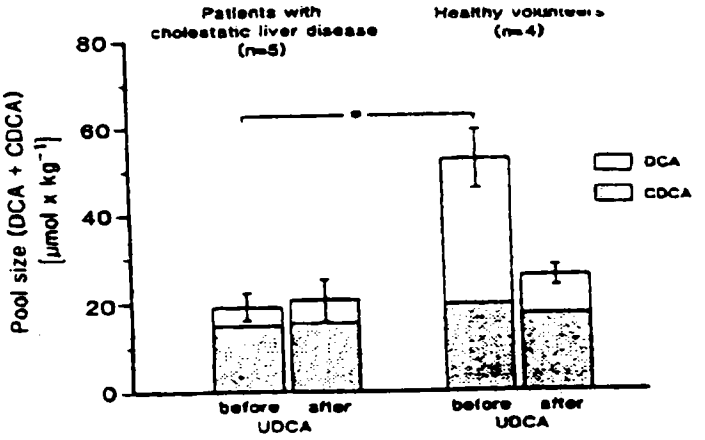


FIG. 2. Pool size of hydrophobic dihydroxy bile acids (CDCA + DCA) DCA before and 1 mo after the start of UDCA treatment in patients with chronic cholestatic liver disease (n = 5) and healthy subjects (n = 4). *p < 0.02.

In healthy subjects UDCA treatment results in a decrease in the DCA pool size. UDCA does not change the CDCA pool size. The results were consistent across healthy subjects, but in patients, there were conflicting results. These are very small numbers that are being looked at. From the clinical trials, however, there is enrichment of the bile acid composition with UDCA and a lowering of the %CA and %CDCA with UDCA treatment.

Meischer G., Paumgartner G. and Preisig R. Portal spill-over of bile acids: a study of mechanisms using ursodeoxycholic acid. Europ. J. Cli. Investigat. 1983; 13: 439-445.

Method:

Ten subjects (5 females and five males) acted as control group. 14 patients , six with chronic liver disease and eight with cirrhosis of the liver were the comparison group. UDCA was administered orally at a dose of 1.5 mg/Kg in a solution of sodium bicarbonate. Note that this a much lower dose than given in the treatment of PBC. Unconjugated UDCA was measured for 2 hours. The subjects were also given an iv solution of UDCA and blood samples were taken 3,5,7,10,20,30,60,90 and 120 minutes after injection. This was a crossover designed study. Elimination and distribution rate constants were erroneously calculated by determining the constants between fixed time points. Systemic availability was calculated from the ratio of the AUC extrapolated to infinity for the oral compared with the iv. Since UDCA was administered as an oral solution, the time to maximum absorption was shorter than seen with studies where a capsule or tablet was given.

Results:

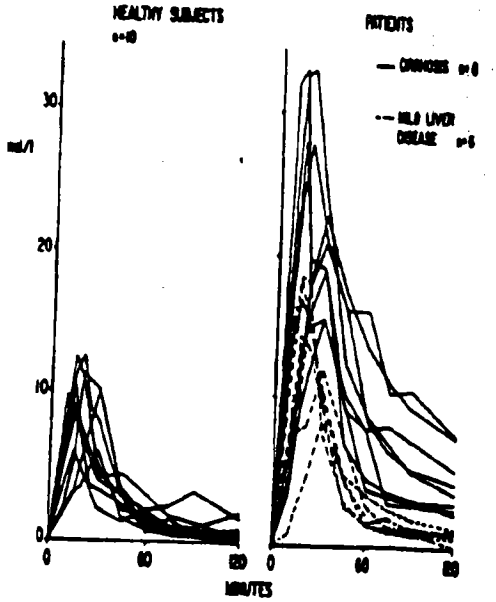


Figure 1. Serum concentration time curves of unconjugated ursodeoxycholic acid in ten healthy subjects and fourteen patients with liver disease following oral administration of 1·5 mg/kg body weight.

Only two of the healthy subjects and four of the patients with liver disease had data available for both orally and iv administered UDCA. From the plasma UDCA-time profiles, it can be seen that the peak plasma concentration of UDCA increases in liver disease particularly in patients with cirrhosis. The bioavailability of UDCA increased to 136% in a patient with a portacaval shunt. The availability in the two healthy subjects was in the order of 50%. Some errors in AUC determinations are introduced by not sampling for a sufficient time particularly in patients where the elimination rate was prolonged compared to healthy subjects.

Bioavailability Study:
Protocol
Title: Bioavailability of Ursodeoxycholic acid
Investigator: [redacted]
Clinical Study Date: October 1991
Study Centers: [redacted]

OBJECTIVE:

To determine the bioequivalence of 4 preparations of ursodeoxycholic acid (UDCA).

METHODS:
Study Design:
Single-dose 5-way (six sequence) crossover study. 5 mL blood samples were taken pre-dose and at one-hourly intervals up to 6 hours. T- tube bile collections were also taken for eleven of the subjects. Three subjects took part in an extension of the study protocol involving the administration of Drug A at doses of 250, 500 and 750 mg. Blood samples alone were collected.

Analytical Method:
[redacted]

Subjects:
Treatment and Administration:
Drug A: 2 [redacted] Tablets = 500 mg total dose
Drug B: 2 Actigall capsules = 600 mg dose
Drug C: 2 [redacted] tablets = 500 mg total dose
Drug D: 3 [redacted] capsules = 600 mg total dose

24 Healthy subjects took part in the study. Different numbers of subjects repeated treatments. See Table 1. Each subject took each of the four different drugs/treatments with a one week washout period between each treatment.

Formulation: Ursofalk Canada is essentially URSO manufactured at [redacted] (Lot No. 1-[redacted]01, 1-[redacted]02, 1[redacted]03) and Ursofalk USA is essentially URSO manufactured at [redacted] (Lot No. L-0824-91).

Biological sampling:
5 mL of blood was taken every hour for 6 hours. Bile acid composition was also measured by collecting bile acid through a T-tube.

Pharmacokinetic Analysis:
AUC's were adjusted to 500 mg dose on the assumption that the pharmacokinetics of UDCA are linear. This has not been confirmed in studies. AUC's for total bile acid and ursodeoxycholic acid alone were determined from 0 to 6 hours. The tabulated AUC's were provided, but no individual plasma data. No report was given of the Cmax nor Tmax. Tabulated results are included in the Appendix.

Statistical Analysis:
An independent t test for all AUC's including repeats and a paired t-test was undertaken for 24 subjects who had taken all treatments. The sponsors did not describe how the 24 subjects were selected to form the repeats. The comparison of treatment A to treatment C is relevant to this submission. This showed no significant difference in the means from the independent t test for all values of AUC for both total bile acid and ursodeoxycholic acid ($p=0.269$ and 0.127 respectfully, two-tailed probability). There was no significant difference in the means from the paired t test for values of AUC of 24 subjects for both total bile acid and ursodeoxycholic acid ($p=0.391$ and 0.212 respectfully, two-tailed probability). Tabulated results are included in the Appendix. These results are incorrect since the assay was not validated and its accuracy in measuring low levels of UDCA in the presence of other bile acids is dubious.

Comment:

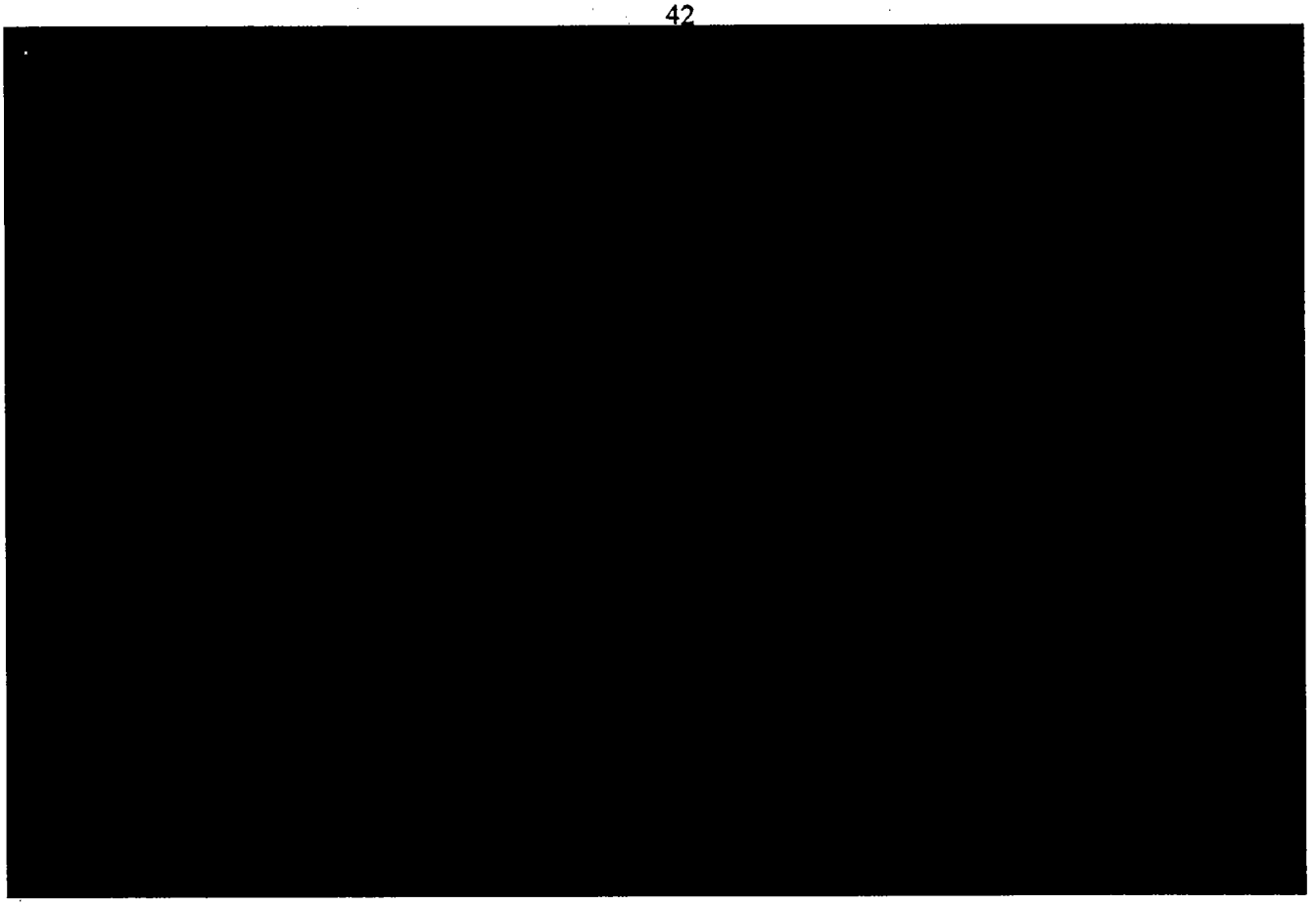
The sponsors submitted NDA 19-809 in March 1990 to the Agency in which ursodeoxycholic acid was to be indicated for the dissolution of radiolucent gallstones. There were five drug products compared. The formulations used in the clinical studies included [redacted] as the same formulation used in the clinical studies in this submission, but not the same Lot numbers and not [redacted]. The study looked at Cmax and AUC(0 to 6 hours) for 24 subjects. None of the products were bioequivalent based on unconjugated ursodeoxycholic acid in plasma and non-transformed data. Re-analysis of the data using log-transformation by this reviewer revealed that the formulations would not be bioequivalent under the current criteria (see Appendix). However, the study used essentially a truncated AUC.

FORMULATIONS AND DISSOLUTION DATA

[redacted]

Dissolution:

[redacted]



APPEARS THIS WAY ON ORIGINAL

APPENDIX II

81 Pages
Trade Secret/
Confidential
Commercial