CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 020675

MEDICAL REVIEW(S)

SEP 19 1996

MEDICAL OFFICER'S REVIEW

NDA 20-675

URSO™ (ursodiol) Tablets, 250 mg

Treatment of Patients With All Stages of

Primary Biliary Cirrhosis

Submitted by

Axcan Pharma US Inc.

Reviewer:
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HFD-180

DIVISION OF GASTROINTESTINAL AND COAGULATION DRUG PRODUCTS
MEDICAL OFFICER'S REVIEW

NDA:	20-675
Date Submitted:	March 26, 1996
Sponsor:	Axcan Pharma US Inc.
Drug:	URSO™ (Ursodiol) [Ursodeoxycholic acid]
Formulation:	Film coated tablets
Route of Administration:	Oral
Pharmacologic Category:	[Anticholelithogenic], Anticholestatic, Hepatoprotective, Immunomodulator, Bile Acid Substituent
Proposed Indication:	Treatment of all Stages of Primary Biliary Cirrhosis

Of the material submitted by the sponsor, the following is considered pertinent to the present MOR:

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MOR OF NDA 20-675

URSO™ (Ursodiol) Tablets

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(Selected Topics)

I. GENERAL INFORMATION

A. Drug Substance

1. Names

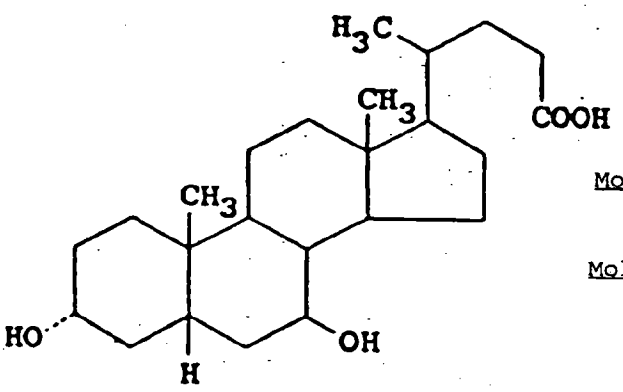
- Chemical Name: (3α,5β,7β)-3,7-Dihydroxycholan-24-oic acid; 17β-(1-methyl-3-carboxypropyl)etiocholane-3α,7β-diol; 3α,7β-dioxycholanic acid

- Generic Name: Ursodeoxycholic acid; ursodiol

- Trade (Proprietary) Name: URSO

- CAS Registry Number: 128-13-2

2. Physical and Chemical Characteristics



Melting Point
203°

$[\alpha]_D^{20}$ + 57°

Molecular Formula
C_{24} H_{40} O_4

Molecular Weight
392.56

URSODEOXYCHOLIC ACID (UDCA)

- UDCA is epimer with chenodeoxycholic acid (CDCA) with respect to the hydroxyl group at C_7.

- Appearance and color : Colorless or almost colorless crystalline powder

- Solubility : Freely soluble in ethanol, glacial acetic acid; slightly soluble in chloroform and sparingly soluble in ether. Practically insoluble in water.

3. Manufacturer: [redacted]

B. Drug Product

1. Dosage Form and Composition
URSO (ursodiol) Tablets, 250 mg are white, elliptical, bioconvex film-coated tablets engraved with "785"

Quantitative Composition of Tablet

INGREDIENT	[redacted] mg/tablet
Ursodeoxycholic acid	[redacted]
Microcrystalline cellulose [redacted]	
Povidone [redacted]	
Polyethylene glycol 3350 [redacted]	
Sodium starch glycolate [redacted]	
Magnesium stearate [redacted]	
[redacted]	[redacted]
Hydroxypropyl methylcellulose [redacted]	[redacted]
[redacted]	
Dibutyl sebacate [redacted]	
Polyethylene glycol 8000 [redacted]	
Carnauba wax [redacted]	

2. Manufacturer

URSO (ursodiol) tablets will be manufactured, packaged and labeled by the following contract manufacturer:

[redacted]

Release and stability testing of the final drug product will be performed at the following contract laboratory:

[redacted]

5. Stability

Based on stability analysis data, the sponsor proposes an expiry dating of 36 months.

II. INTRODUCTORY NOTE

The compound which is the subject of the present NDA submission is chemically described as (3α,5β,7β)-3,7-Dihydroxycholan-24-oic acid. The generic name of the free acid is ursodeoxycholic acid (abbreviated UDCA) or ursodiol (a name created by Alan Hofmann, in analogy to chenodiol).

The drug product proposed by the sponsor of NDA 20-675, URSO™ (ursodiol) tablets, 250 mg, has not been approved in the U.S. for any indication.

A drug product from another pharmaceutical company (Ciba-Geigy, Co., Summit, NJ), known by the proprietary name of ACTIGALL®, was approved in 1988 for the dissolution of radiolucent, noncalcified stones.[1] This Ciba-Geigy product is supplied in 300 mg [redacted] capsules, to be given at a recommended dose of 8 to 10 mg/Kg/day in 2 to 3 divided doses. On February 19, 1991, this product was granted orphan status for "the management of the clinical signs and symptoms with PBC". In 1994, Ciba-Geigy submitted NDA 19-594/S-015 requesting approval of ACTIGALL® for the treatment of PBC. This NDA was the subject of two reviews by the MO (R. Prizont, July 8, 1994 and November 22, 1994) in addition to being reviewed at the July 28, 1994 meeting of the Gastrointestinal Drugs Advisory Committee. In action letter dated April 18, 1995, Ciba-Geigy was told that the information presented was inadequate and that the supplemental

[1] ≤20 mm in greatest diameter for patients in whom elective cholecystectomy would be undertaken except for the presence of increased surgical risk due to systemic disease, advance age, idiosyncratic reaction to general anesthesia, or for those patients who refuse surgery.

application was not approvable.[2] To establish the effectiveness of ACTIGALL® in the treatment of PBC, the FDA requested of Ciba-Geigy either a new adequate and well-controlled study or a full report of the Poupon et al. study published in the NEJM 330:1342-1347 (1994) and a bioequivalence study of ACTIGALL® and the ursodiol preparation used in that study. It so happens that the ursodiol preparation used in the Poupon et al. study was precisely URSO™, the ursodiol from Axcan Pharma. This formulation of ursodiol, granted orphan drug status for the treatment of PBC (June 20, 1991), is the subject of the present NDA.

Throughout the present NDA review, the Axcan Pharma product is identified as **UDCA**. Important clinical efficacy data are mentioned or quoted only when the source of ursodiol (either Ciba-Geigy or Axcan Pharma) can be properly identified. But it is important to note that, in innumerable pre-clinical and clinical studies, the test-drug has been identified as UDCA (only); the actual source of the drug is not provided.

III. SCIENTIFIC RATIONALE/DEFINITIONS

A. Generalities

Ursodeoxycholic acid (UDCA) is an **enterohepatic drug** and thus differs from typical drugs that are **systemic** drugs. The differences between these two types of drugs have been extensively reviewed by several authors, including A. Hoffman [Scand. J. Gastroenterol. 29(Suppl. 204):1-15 (1994)]. Enterohepatic drugs (EH drugs) are targeted to the organs of the enterohepatic circulation (EHC) - the liver, biliary tract, and intestine - whereas systemic drugs are targeted to the systemic circulation or to organs by way of the systemic circulation. For EH-drugs, biliary and intestinal levels (and eventually intrahepatic levels) are important in efficacy, whereas for systemic drugs, blood levels (and eventually target organ levels) are important in efficacy. EH-drugs are eliminated virtually entirely, by the fecal route often after extensive bacterial biotransformation; a fraction of the bacterial metabolic products may be absorbed from the intestine and circulate with the parent drug. Systemic drugs are eliminated in urine and usually to a smaller extent in bile. Since EH-drugs have the liver and biliary tract as their first target organs, first pass hepatic extraction is usually high; in contrast, because the efficacy of systemic drugs is generally considered to be dependent on blood levels, first pass hepatic extraction is kept as low as possible during the development stage when congeners are selected for clinical testing.

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[2] Reasons cited in the action letter included that ACTIGALL® had what seemed to be a potentially favorable biochemical effect but its effect on clinical endpoints was not established for any group of patients. Results in Study 906 (the Coombs study) cast some doubt on the meaningfulness of these acute effects. The study showed no effect at all in any subgroup, on fibrosis and, more important, on the pre-specified components of treatment failure (identified in the Statistical Methodology section of the protocol as the primary analysis): death without transplant, transplant, bleeding varices/survival/encephalopathy, development of varices, or development of cirrhosis. The results suggested at the most a trend in favor of ACTIGALL® regarding piecemeal necrosis and portal inflammation. There was no overall efficacy of ACTIGALL® in either stabilizing histology or preventing progression of histologic stage.

Finally, since EH-drugs are eliminated solely in bile, they are tightly bound to plasma proteins (usually albumin), whereas systemic drugs may or may not have extensive binding to plasma proteins [A.F. Hofmann, J. Controlled Release 2:2-11 (1985) and Pharmacology of chenodeoxycholic and ursodeoxychotic acid in man. In G. Paumgartner et al., editors. Bile Acids and Cholesterol in Health and Disease. Boston.MTP Press, pp.301-336 (1983)].

UDCA is a naturally-occurring bile acid (BA) in many mammals, including man. It was first isolated from polar bear bile near the turn of the century[3] and was named after this species. The chemical structure of UDCA was definitely established ca. 30 years later when T. Iwazaki[4] showed that it was an epimer of chenodeoxycholic acid (CDCA), the major BA present in human bile. After that, the [redacted] developed a method for converting cholic acid (CA), the most commonly marketed BA, to UDCA[5]. UDCA, when taken orally in "large doses" (500 to 700 mg/day; 8 to 10 mg/Kg-day), desaturates bile and dissolves cholesterol gallstones. Its efficacy for cholesterol gallstone dissolution is at least equal to that of CDCA which had been introduced for this purpose [CDCA is another EH-drug]. But UDCA differed from CDCA in not inducing diarrhea, in being devoid of hepatotoxicity and in causing no increase in the level of serum cholesterol. Because of its apparent superiority to CDCA, UDCA gradually replaced CDCA for the medical treatment of cholesterol gallstones and, at present, it is the most widely used agent for this purpose.[6]

B. Rationale Based on Reported Clinical Data

The rationale to properly test the safety and efficacy of CDCA in the treatment of cholestatic liver disease, including primary liver cirrhosis (PBC), is based on information that can be summarized as follows. [Note: additional information on the physicochemical, pharmacological, immunological, PKs, metabolism and PD actions of UDCA is given in the subsequent section of this review.]

Having observed no apparent hepatotoxicity during UDCA administration, U. Leuschner et al. [Dig. Dis. Sci. 30:642-649 (1985)] explored the use of UDCA in patients with chronic active hepatitis who also had gallstones. Not only did the gallstones dissolve, but laboratory test actually improved. This group of German investigators had performed toxicity studies of CDCA and UDCA

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[3][. Hammarsten, Hoppe-Seyler's Z. Physiol. Chem. 32:435-466 (1901). II. Abschnitt. 36:525-555 (1902)].

[4][Z. Physiol. Chem. 244:181-193 (1936)].

[5]Total synthesis of CDCA was accomplished by Kametani et al. [J. Amer. Chem. Soc. 103:2891-2892 (1981)]. The structure of UDCA crystals has been elucidated by Lindey and Carey [J. Crstall. Spec. Res. 17:231-249 (1987). NMR spectra have been published [A. Baillet-Guffroy et al., J. Pharm. Sci. 73:847-849 (1984)].

[6]In the U.S., Ciba-Geigy produces a form of UDCA [(Actigall™) 300 mg capsules] that is available to patients (approved by FDA in 1987).

in rats. Convincing morphological evidence that CDCA was much more hepatotoxic than UDCA was acquired.[7] Also, T. Krol et al.[8] had performed animal studies which suggested that pre-treatment of the hamster with UDCA decreased hepatocellular damage caused by bile duct ligation. An Abstract from Canada [M.M. Fisher and M.T. Paradine, Gastroenterology 90:A1725 (1986)] reported that patients with PBC showed improvement in biochemical parameters of cholestatic liver disease during UDCA administration. A double-blind, PL-controlled, multicenter trial, aimed at defining the efficacy and safety of UDCA in Japan was initiated ca. 1983. But recruitment was slow because of the rarity of this disease in that country. Ca. one year later Poupon and his colleagues in France initiated a PL-controlled study of UDCA at a dose of 12 mg/Kg-day in patients with PBC. The results were published in 1987 [R. Poupon et al., Lancet, 1:834-836 (1987)]. The changes were remarkable:

- pruritus decreased
- serum AP, an accepted sign of cholestasis, decreased
- γ-GT level, a test of biliary ductular cell injury, improved
- serum amino transferase and BA levels, tests that indicate hepatocyte injury, also improved.

The first publication by Poupon et al. aroused enormous interest for a number of reasons. The sample size (n=15) seemed adequate, the investigation appeared to be carefully performed, the changes in laboratory tests were considerable and statistically significant. The paper, published in Lancet, had been reviewed by Dame Sheila Sherlock, and she quoted it widely. During initial uncontrolled observations, Poupon et al. [Communicated at the 11/87 Chicago meeting] claimed to have observed histological regression in several patients. Also, by this time, UDCA was being increasingly used for gallstone dissolution. Shockwave lithotripsy, which featured adjunctive use of UDCA, was being evaluated as a potentially powerful new approach for the treatment of symptomatic gallstone disease.

Since the time of their first publication (1987), Poupon et al. as well as other presentations have confirmed and extended the initial results. Poupon et al. proposed the novel therapeutic approach on the basis of evidence that hepatic lesions in PBC can result from intracellular accumulation of potentially toxic BAs. They postulated that L-T administration of UDCA [a

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[7][U. Leuschner. Liver tissue injury due to chenodeoxycholic acid: metabolic pathways and toxicity. In: G. Paumgartner, A. Stiehl and W. Gerok, eds. Biological Effects of Bile Acids. MTP Press Limited, Lancaster, pp 191-203 (1979); U. Leuschner, M. Schneider and L. Korte. The influence of chenodeoxycholic acid and ursodeoxycholic acid on the hepatic structure of the rat. Z. Gastroenterol. 17:244-255 (1979)].

[8][T. Krol et al., Tauroursodeoxycholate reduces ductular proliferation and portal inflammation in bile duct-ligated hamsters. Hepatology 72:132 (1983)]

hydrophilic BA devoid of cytotoxicity in humans and in vitro[9]], by modifying the composition of the endogenous BA pool, might be beneficial to patients with PBC. This hypothesis was supported by the results on an uncontrolled prospective pilot trial [Locus cited, Lancet (1987)] and an interim analysis [R.E. Poupon et al., J. Hepatol. 11:16-21 (1990)] of a 2-year, randomized, multicenter, double-blind trial (comparing the efficacy of UDCA (n=73) to PL (n=73) [NEJM 324:1548-1554 (1991)].

In the Poupon et al. trial, Treatment Failure (Tx F) was defined as a doubling of BIL levels to more than 70 μmol per liter or the occurrence of a severe complication (ascites or variceal bleeding) or an adverse reaction. The results, taken from the Abstract of this NEJM publication were summarized as follows.

> Treatment failed in 6 patients in the UDCA group, compared with 13 in the PL group (p<0.01 by Cox regression model). A single patient in each group withdrew because of minor AEs. After two years of treatment, the proportion of patients with clinically overt disease decreased only in the UDCA group (p<0.02). The patients treated with UDCA had significant improvements in serum levels of BIL, AP, ALT, AST, γ-GT, cholesterol, and IgM (all p<0.001); the antimitochondrial-antibody (AMA) titer (p<0.01); and the Mayo risk score[10] (p<0.001). Follow-up analysis of 95 liver-biopsy specimens showed a significant improvement in the mean histologic score (p<0.002) and in all the characteristic histologic features except fibrosis only in the group given UDCA:

Progression of Disease in the UDCA and PL Groups
During the Two-Year Study, According to
Histologic Analysis of Liver-Biopsy Specimens[a]

Group	Improvement		No Change	Worsening	
	≤-5	-4 to -2	-1 to 1	2 to 4	≥5
Ursodiol	10	18	12	6	4
Placebo	5	8	10	13	9

Liver-biopsy specimens obtained at entry and after two years were paired and reassessed at the end of the trial. Histologic stage was determined according to "accepted criteria" [J. Ludwig et al., Virchows Arch. (Pathol. Anat) 379:103-112 (1978)]. The stage was determined in terms of the most advanced lesions in each specimen. The main histologic features were graded as follows:

fibrosis, 0 to 3
piecemeal necrosis, 0 to 2
parenchymal lobular necrosis, 0 to 2
cholestasis, 0 to 3
portal and periportal inflammation, 0 to 3
ductular proliferation, 0 to 2
inflammation, 0 to 3

The degree of bile-duct paucity was calculated as the number of interlobular bile ducts divided by the number of portal tracts. These values were used to calculate a liver-histology score for each liver-biopsy specimen. Histologic progression was analyzed according to changes in the grade and was deemed to be improving, unchanged, or worsening.

a) The specimens were graded according to changes in histologic scores: ≤-2 indicated improvement, -1 to 1 no change, and ≥2 worsening. Overall, the changes were significant (p<0.002).

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[9] [A.F. Hofmann. Bile acid hepatotoxicity and the rationale of UDCA therapy in chronic cholestatic liver disease: some hypotheses. In: Paumgartner G., Stiehl A., Barbara L., Roda E., eds. Strategies for the treatment of hepatobiliary diseases. Dordrecht, the Netherlands: Kluwer Academic, pp 13-33 (1990)].

[10] The Mayo Risk Score is a cross-validated index predictive of survival in PBC. It consists of six variables: age, total serum BIL, serum albumin, prothrombin time, presence or absence of edema and whether or not the patient was on diuretic therapy for edema [P.M. Grambsch et al. Hepatology 10:846-850 (1989)].

In 1994, Poupon et al reported on the benefit of L-T treatment with UDCA. After two years of follow-up, all patients completing the study (reported in 1991) received ursodiol in an open trial [NEJM 330:1342-1347 (1994)] and were monitored for 2 more years. The end points in the assessment of efficacy were: progression of disease, as defined by the presence of hyperbilirubinemia, variceal bleeding, ascites or encephalopathy; liver transplantation or a referral for that procedure; and liver transplantation (or a referral) or death. The results, taken from the Abstract of that publication were summarized as follows:

> Disease progressed significantly less frequently in the UDCA group than in the PL group ($p<0.002$; relative risk, 0.28; 95 percent confidence interval, 0.12 to 0.63). The probability of liver transplantation or a referral for that procedure and the probability of transplantation or death were significantly lower in the group assigned to UDCA than in the group assigned to PL (for transplantation alone, $p=0.003$; relative risk, 0.21; 95 percent confidence interval, 0.07 to 0.66; for transplantation or death $p=0.005$; relative risk, 0.32; 95 percent confidence interval, 0.14 to 0.74). High BIL levels and to a lesser extent, signs of cirrhosis at entry into the trial were predictive of disease progression, liver transplantation or a referral, and transplantation or death.

From their data published in 1994, Poupon et al. concluded that L-T UDCA treatment slows the progression of PBC and reduces the need for liver transplantation.

From the above discussed and other publications there seemed to be good reasons to believe that UDCA might be a safe and effective treatment for PBC. Many issues remain unsettled (see reviewer's comments to the Mayo Clinic data) but all of these are sound reasons to further delineate the safety and efficacy of UDCA in the treatment of PBC.

C. Disease to be Treated: Primary Biliary Cirrhosis (PBC) - Summary

PBC[11] is a disease of unknown cause in which intra-hepatic bile ducts are progressively destroyed. It is of interest to note that, in the early stages, nodular regeneration is inconspicuous and cirrhosis is not present. The disease is associated with a profound immunological disturbance[12] which has been related to the bile duct destruction.[13] The disease might represent a failure of immuno-regulation, with loss of tolerance to tissues bearing a rich display of histocompatibility antigens. In many respects PBC is analogous to

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[11]Termed by E.H. Ahrens, Jr. and co-workers [Medicine (Baltimore) 29:299 (1950)]. The term "chronic non-suppurative destructive cholangitis", proposed by E. Rubin et al. [Amer. J. Pathol. 46:387 (1965)] is a better one although too cumbersome to replace the popular "primary biliary cirrhosis".

[12]Cytotoxic T-cells have been seen infiltrating the bile duct epithelium [G. Yamada et al., Hepatology 6:385 (1986)] as have class 2 restricted T-4 lymphocytes [G. Colucci et al., Immunol. Immunopath. 41:35 (1986)]. The final event is an attack by cytotoxic T-cells on biliary epithelium. Suppressor T-cells are reduced in number and function [G. Ballardini et al., Lancet ii:1009 (1984)]. Upregulated display of HLA class 1 antigens and de novo expression of HLA class 2 antigens are compatible with immuno-mediated duct destruction [G. Ballardini et al. (locus cited) (1984)].

[13][R.A. Fox et al. Lancet i:959 (1969); M.E. Gerschin and I.R. Mackay, Gastroenterology 100:822 (1991)]

the graft-versus-host (GVH) syndrome. The condition can be viewed as a dry gland syndrome[14]. PBC may be regarded as a spontaneous form of chronic liver rejection. Circulating antibodies (A_b) against mitochondria are found in virtually 100% of patients with PBC [L.E. Munoz et al., Gut 22:136 (1981); J.G. Walker, Lancet i:827 (1965)] but they are non-organ and non-species specific.[15] At present, AMA_b are tested routinely by indirect immunofluorescence on rat kidney substrate.

Death rates due to PBC are difficult to assess but, according to S. Sherlock, are probably on the order of 0.6 to 2% of those dying with cirrhosis. For clinical trial assessments on survival, comparison to experience with no pharmacologic interaction at the institution doing the research (i.e. Mayo Clinic) is probably a valuable approach.

Ninety per cent of PBC patients are female; most are middle-aged. In summary, PBC is characterized by chronic cholestasis with pruritus, jaundice, hypercholesterolemia, xanthomas, osteomalacia. In the later stages, portal hypertension, ascites, encephalopathy and other signs of liver failure may be seen. The diagnosis is confirmed by a raised AP level, sometimes a high serum IgM, a positive serum AMA_b test and diagnostic or compatible hepatic histology on needle biopsy. Visualization of the bile ducts by endoscopy or percutaneous cholangiography may be necessary in atypical patients.[16] Liver biopsy features favoring PBC as opposed to conditions difficult to rule out [ex. cholestatic sarcoidosis, chronic active hepatitis, PSC, chronic drug jaundice, etc.] include intact lobules, slight piecemeal necrosis, periseptal cholestasis and lymphoid aggregates. Widespread use of automated biochemical screening has resulted in an increasing number of patients being diagnosed when asymptomatic. The diagnosis must be made from other causes of a raised AP such as Paget's disease [the raised serum γ-GT usually makes the distinction; but other causes of chronic pruritus have to be considered].[17]

PBC is generally a progressive disease but the rate of progression varies greatly from one patient to another [M.M. Kaplan, NEJM 316:521-528 (1987)]. The terminal phase is characterized by hyperbilirubinemia (>100 μmol per

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[14]In addition to structural changes in the bile ducts, other ducts with a high concentration of HLA class 2 antigens or their epithelium such as the lacrimal and pancreatic ducts are involved.

[15]The antigens to which the antibodies are directed are localized on the inner mitochondrial membrane. The antigenic component specific for PBC-serum is M2 [P.A. Berg et al., J. Hepatol. 2:123 (1986)]. Four M2 antigen polypeptides have been identified, all components of the pyruvate dehydrogenase complex (PDC) of mitochondrial enzymes. E1 is a 50 kDa 2-oxoacid dehydrogenase complex, E2 is the 74 kDa complex of lipoamide acyl transferase, E3 is a 50 kDa 2-oxoglutarate complex. Protein X is a 52 kDa member of the PDC and cross-reacts with E2 [D.R. Fregeau et al., J. Immunol. 142:3815 (1989); S.P.M. Fussey, Proc. Natl. Acad. Sci. USA 85:8654 (1988); J. Van de Water et al., NEJM 320:1377 (1989)]. An ELISA test has been developed against E2 and protein X. It is 98% sensitive and 96% specific for the diagnosis of PBC. The titer correlates with histological stage of disease and prognostic variables such as serum BIL and serum albumin.

[16]These include males and those with a negative serum MA_b test, with inconclusive liver biopsy or with abdominal pain.

[17]For example, the diagnosis may be made in patients under investigation for a condition known to be associated with PBC, such as thyroid or collagen disease, or in the course of family surveys.

liter), a major decrease in the number of intrahepatic bile ducts, and extensive fibrosis or cirrhosis. Far advanced PBC, uniformly fatal until recent years, can now be salvaged with high degree of survival (ca. 80%) and rehabilitation rates in those that have access to liver transplantation [B.H. Markus et al., NEJM 320:1709-1713 (1989) (Mayo Clinic Study)]. Prognosis based on clinical data determines the best time for the operation, but the reasons for transplantation are not necessarily standardized from institution to institution. A number of predictors of survival as indicators of prognosis have been proposed. According to O. Epstein [Gut, 26:A1126 (1985)] and J.M. Shapiro et al., Gut, 20:137 (1979)], when **serum BIL values** are consistently greater than 100 μmol/l, **(6 mg/dl)**, the patient is unlikely to survive for more than 2 years. Autoimmune diseases such as thyroiditis, sicca syndrome or Raynaud's phenomenon also correlate with decreased survival [D.R. Beswick, Gastroenterology, 89:267 (1985)]. E. Christensen et al. [Gastroenterology, 89:1084 (1985)] and J. Roll et al. [NEJM 308:1 (1983)] have proposed other features predicting decreased survival. These include symptoms, advanced age, hepatosplenomegaly, ascites and serum albumin <3 g/dl. In the view of Sherlock, histologically, piecemeal necrosis, cholestasis, bridging fibrosis and cirrhosis correlate with the worst prognosis. G.J. Gores et al. [Gastroenterology, 96:1552 (1989) (Mayo Clinic)] showed that once varices have developed, 83% survive 1 year and 59% 3 years. Survival after the initial bleed is 65% at 1 year and 46% at 3 years. Varices are more likely to develop in those with a high serum BIL and with an advanced histological stage of the disease. Spread of disease from portal to periportal areas implies progression [S. Sherlock and J. Dooley, editors. In: Diseases of the Liver and Biliary System, Chapter 14: Primary Biliary Cirrhosis, Blackwell Scientific Public., Oxford, London, pp 236-248 (1993)]. According to R.G. Lee et al. [Gastroenterology, 81:983 (1981)] granulomas seen on an initial biopsy may predict longer survival.

D. Pharmacologically-related Drugs

Under this subheading, the reviewer is listing specific medical treatment(s) that can be recommended for the treatment of PBC. Presently, no drug has been approved for this indication, but many have been tested under a variety of conditions.

As pointed out by M.M. Kaplan [NEJM 316:521-528 (1987)] the search for specific therapy for patients with PBC has centered primarily on immunosuppressive agents in an attempt to blunt the immunologic attack on bile ducts. Below is a succinct appraisal of the main findings in trials with a variety of agents, already approved for other indications.

Drug	Main Findings/Conclusions
1. Colchicine A drug that interfers with collagen synthesis. It may also degrade collagen.	• Improved biochemical tests and survival. • Had no effect on symptoms or liver histology [M.M. Kaplan et al., NEJM 315:1448 (1986)]. • Relatively non-toxic; 0.6 mg b.i.d. may be given. • L-T benefit is uncertain.
2. Azathioprine An immunosuppressive drug evaluated in two double-blind studies.	• Had no effects on symptoms. • Improved survival but the publication [E. Christensen et al., Gastroenterology, 89:1084-1091 (1985)] did not give biochemical or histological data. (not now used).
3. Cyclosporin A An immunosuppressive agent widely used in the organ transplantation area.	• In limited trials it reduced symptoms and improved biochemical tests [R.H. Wiesner et al., NEJM 322:1419-1424 (1990) (Mayo Clinic)]. • Over 2 years, biopsies showed less progression. • Unsafe for L-T use: nephrotoxicity and hypertension are serious complications. • The high incidence of renal toxicity compromised the double-blind nature of the clinical trials. This introduced a potential bias in the evaluation, particularly with regards to symptoms [S.J. Munoz, NEJM, 323:1352 (1990)].
4. MTX An antimetabolite, folic acid antagonist, anti-inflammatory: it may inhibit the biological activity of IL-1 in pathological states, a function that is independent of its action as a folic acid antagonist.	• According to a report by M.M. Kaplan [Gastroenterology, 101:1332-1338 (1991)], 15 mg/week in one 24-h period reduced symptoms and improved biochemical tests. • Late-stage patients did not respond. • Complications included BM suppression and interstitial pneumonitis [although these were uncommon and reversible, it might be questioned whether a potentially hepatotoxic drug should be given over many years even in a small dose.]
5. D-penicillamine A copper-chelating agent. It is also an anti-inflammatory and can impair maturation of collagen.	• Reduced liver copper. • Although initial trials were favorable, these could not be confirmed by the Mayo Clinic investigators [E.R. Dickson, Gastroenterology, 86:A1062 (1984)]. • Side effects were serious and numerous. [This treatment has been abandoned]

6. Chlorambucil An antineoplastic drug; an alkylating agent of the nitrogen mustard group; also immuno-suppressive.	• Improved biochemical tests • Improved IgM concentrations. • Liver biopsies showed less inflammation [J.H. Hoofnagle et al., Gastroenterology 91:1327-1334 (1986)]. • L-T administration limited by potentially severe side effects: BM suppression was a complication. [This trial has been abandoned]
7. Corticosteroids Drugs with multiple pharmacological properties including anti-inflammatory.	• Induced less itching and fatigue, and a fall in serum AP and transaminases. • Improved liver function and histologic features. • But increased development of bone thinning (severe osteopenia) contraindicates this treatment [H.C. Mitchison et al. Hepatology 10:420-429 (1989)] [should not be used in PBC].

In summary, although several of the agents listed above have been associated with some improvement in biochemical tests and even histologic results, only two drugs, colchicine and azathioprine, appear to improve survival. But azathioprine is no longer used and the L-T benefits of colchicine are uncertain. Cyclosporine A may have comparable efficacy to UDCA but, owing to nephrotoxicity, the former appears unsafe for L-T use. From this summary review it is concluded that there is need for an effective **[subject to definition]** and safe therapeutic agent to adequately treat PBC.

PBC is a very complex disease, but at least two main processes appear to delineate the pathophysiology of this condition. One is the immune damage directed at the intrahepatic bile ducts. The other, reduced bile flow (cholestasis) and retention of endogenous BAs which cause cytotoxic damage to the liver [A.F. Hofmann and H. Popper, Lancet ii:398-399 (1987)]. There is accumulating evidence that UDCA may affect these pivotal mechanisms. The review in sections that follows, demonstrate that UDCA has immunonodulatory action, is less toxic than endogenous hydrophobic BAs, is choleretic and thereby improves hepatic excretion and is cytoprotective. Since the initial report by U. Leuschner et al. [Dig. Dis. Sci. 30:642-649 (1985)] there have been reports of potential benefits in a wide range of cholestatic and chronic inflammatory liver conditions [J.S. De Caestecker et al., Gut 31:1061-1065 (1991) but most is known about the use of UDCA in PBC [A.G. Lim et al., Gut, 37:301-304 (1995)]; [R.A. Rubin et al. Ann. Intern. Med. 121:207-218 (1994)]. The numerous literature publications now available show that - compared with other previously assessed treatments - UDCA is non-toxic, well-tolerated and suitable for L-T use. With UDCA, safety is not the main issue. The critical issue and the primary task of the MO review of NDA 20-675 is to assess and define the clinical efficacy of UDCA in PBC.

IV. PRECLINICAL PHARMACOLOGY

[NOTE: This section is the subject of an on-going pharmacology review. The information briefly summarized below was taken from the numerous literature publications. When possible, the BA conjugate (or free vs salt derivative is identified). For clarity of presentation, many literature references have been omitted. The subject matter is revisited in the section Human PK and Bioavailability.]

A. Primary Therapeutic Activity

1. In-vitro data

- TUDCA ↓ cholesterol uptake by rat jejunal membrane (unknown mechanism).
- In liver perfusion studies (rats and baboons), UDCA produced:
  - either unchanged or ↓ bile flow,
  - ↑ output of BAs and PLs
  - ↓ cholesterol specificity

  TUDCA caused:
  - slight output of plasma-membrane enzymes (5-nucleotidase and alkaline phosphodiesterase)[18]
- UDCA has a direct anticytotoxic effect on hepatocytes. Using the isolated perfused liver, the cholestatic effect of CDCA (as the taurine conjugate) was ↓ by the simultaneous infusion of UDCA [D.M. Heuman et al. Gastroenterology 96:A607 (1989)]. With isolated hepatocytes, UDCA protected against injury caused by added CDCA or DCA [A. Roda et al., Ital. J. Gastroenterol. 17:233 (1985)]. According to A.F. Hofmann, this effect can also be observed with isolated cultured mast cells, but the effect is less, suggesting that transport into the cell may be necessary for the anticytotoxic effects to occur. The mechanisms of these anticytotoxic effects of UDCA are yet to be clarified.
- Some controversial data have been published. For example, T. Tamura et al. [Gastroenterology 100:A342 (1991)] showed, on histopathologic examination, that bile duct obstruction in rats induced proliferation of bile ducts, periportal fibrosis and mild inflammation and occasional

[18]This may represent an important difference between the effects of UDCA and those of CDCA on the hepatobiliary system.

parenchymal necrosis. Although administration of UDCA reduced those changes, the difference was not striking (partial amelioration), perhaps due to the short period of observation (7 days). Also, K.J.R. Watson et al. [Gastroenterology 100:A810 (1991)] reported that UDCA (25 mg/Kg/day) by daily lavage had no protective effect on the development of increased rigidity of microsomal membranes, decreased microsomal enzyme activity or histological changes in the chronically bile duct-ligated rat.

- [Apoptosis is the fragmentation of a cell into membrane-bound particles that are then eliminated by phagocytosis (programmed cell death).] UDCA and 7-Keto-LCA (an obligatory intermediary during the epimerization of CDCA to UDCA), but not LCA, induced apoptosis in the colonocytes of their proximal colon of normal mice [R. Hanif et al., Gastroenterology 110:A526 (1996)]. This finding suggests a potential mechanism by which UDCA may exert its chemoprotective effect *in vivo*.

- In hepatocytes isolated from bile duct-ligated hamsters, B. Bouscarel et al. [Gastroenterology 110:A1156 (1996)] showed that during cholestasis, the glucagon-induced cAMP production is reduced without any decrease in the number of glucagon receptors. UDCA (and TUDCA) produced a decrease in cAMP production and BA uptake. UDCA may exert its hepatoprotective mechanism during cholestasis through regulation of cAMP synthesis (effect on signal transduction).

The effects in isolated systems briefly summarized above are similar to those in intact animals.

2. In vivo data

Intravenous and oral administration of UDCA to rats, rabbits, hamsters and dogs:

- ↑ bile flow
- ↑ total BA output; ↑ biliary UDCA concentration
- ↓ biliary concentration of CA
- Total biliary BA concentration was reported ↑, ↓ or unchanged.
- CDCA and DCA concentrations were either ↓ or unchanged.

- ↑ concentration and saturation of cholesterol (CHOL), while CHOL output was unchanged.

- ↑ phospholipid (PL) concentration and output.

- ↓ HMGCoA reductase and 7-hydroxylase activity, ↓ triglycerides (TG) and PL synthesis and ↑ CHOL synthesis in the liver.

- Inconsistent results have been reported from experiments in animal models of cholestasis or liver injury. Dietary UDCA (3.5 mg/300 g rat-day) did not protect against severe subacute hepatotoxicity induced by the combination CCl_4 + ethanol. The authors [V. Simko and E. Michael, Gastroenterology 100:A787 (1991)] proposed that the preventive potential of UDCA should be further tested in models with milder hepatotoxicity. These results are to be contrasted to those of S. Erlinger et al. [In: Bile Acids as Therapeutic Agents, G. Paumgartner et al., editors, Kluwer Academic Publishers, Dordrecht, Chapter 29, pp. 251-255 (1991)] who reported a protective effect of UDCA on two experimental models of cholestasis: bile duct ligation and ethinyl estradiol-induced.

- A.F. Hofmann [Folk Symposium 68, Basel, Switzerland, pp 143-160 (1992)] has reviewed the concepts of hypercholeresis and proposed the **cholehepatic shunt circulation hypothesis**. Hypercholeresis is the induction of a bicarbonate-rich bile flow by UDCA, to a much greater extent than would be expected from the recovery of BA in bile. The key determinant of whether cholehepatic cycle occurs is the affinity of the molecule (such as UDCA) for the canalicular transport system in relation to its affinity for the glucuronidation in the endoplasmic reticulum. The view that canalicular bile flow is initiated by the osmotic effects of secreted anions has been confirmed. The canaliculi and the biliary ductules comprise a secretory unit, analogous to the nephron. The biliary ductules absorb lipophilic molecules passively. Phase II bio-transformation steps play a key role in promoting biliary and intestinal excretion of xenobiotics and endobiotics.

B. Secondary Therapeutic Activity

- UDCA produced minimal or no effect on water and sodium excretion from the g.i. tract in rats and rabbits, and induced less damage than CDCA on the g.i. tract mucosa.

- The reviewer agrees with the sponsor that these findings tend to confirm the clinical and experimental observations that diarrhea is infrequent with UDCA.

- In mice and rats, UDCA ↑ hepatic AP without ↑ AP serum levels.

- UDCA lowering effect on CHOL serum levels (which has been seen in most clinical trials) may be explained by inhibition of hepatic CHOL synthesis and ↑ fecal excretion of sterols.

- UDCA may have an stimulatory effect on pancreas: it ↓ blood sugar levels in mice and ↑ the volume of pancreatic secretion in rabbits.

- UDCA had no significant effect on urinary volume and electrolytes in rats.

- UDCA showed no anticonvulsive properties in mice.

C. Metabolism (Figs. 1 and 2)

- UDCA is absorbed passively from the small intestine [F.A. Wilson, Amer. J. Physiol. 4:G83-G92 (1981)].

- UDCA reaching the terminal ileum might also be actively absorbed.

- Since UDCA is ingested in the protonated form and does not achieve appreciable solubility in water until the pH is nearly 8, it is likely that the compound is solubilized in micelles formed by the secretion of bile into the small intestine.

- UDCA is unlikely to be biotransformed during its transport through the enterocyte. It is likely to be absorbed entirely via the portal venous circulation. In portal blood, it will be highly protein bound (>99%), based on *in vitro* studies [R. Aldini, J. Lipid. Res. 23:1167-1173 (1982)].

- The absorption of UDCA is rapid as maximal plasma concentration were obtained within 30 min. in rats and monkeys.

- UDCA is almost exclusively distributed in the g.i. tract and the hepatobiliary system (EH-drug) and is almost completely excreted in the feces ($t_{\frac{1}{2}}$ = 2 days) (see human data).

- The uptake of UDCA by hepatocytes is effected through an active transport mechanism. **The first pass extraction by the liver is quite variable, especially in PBC patients.**

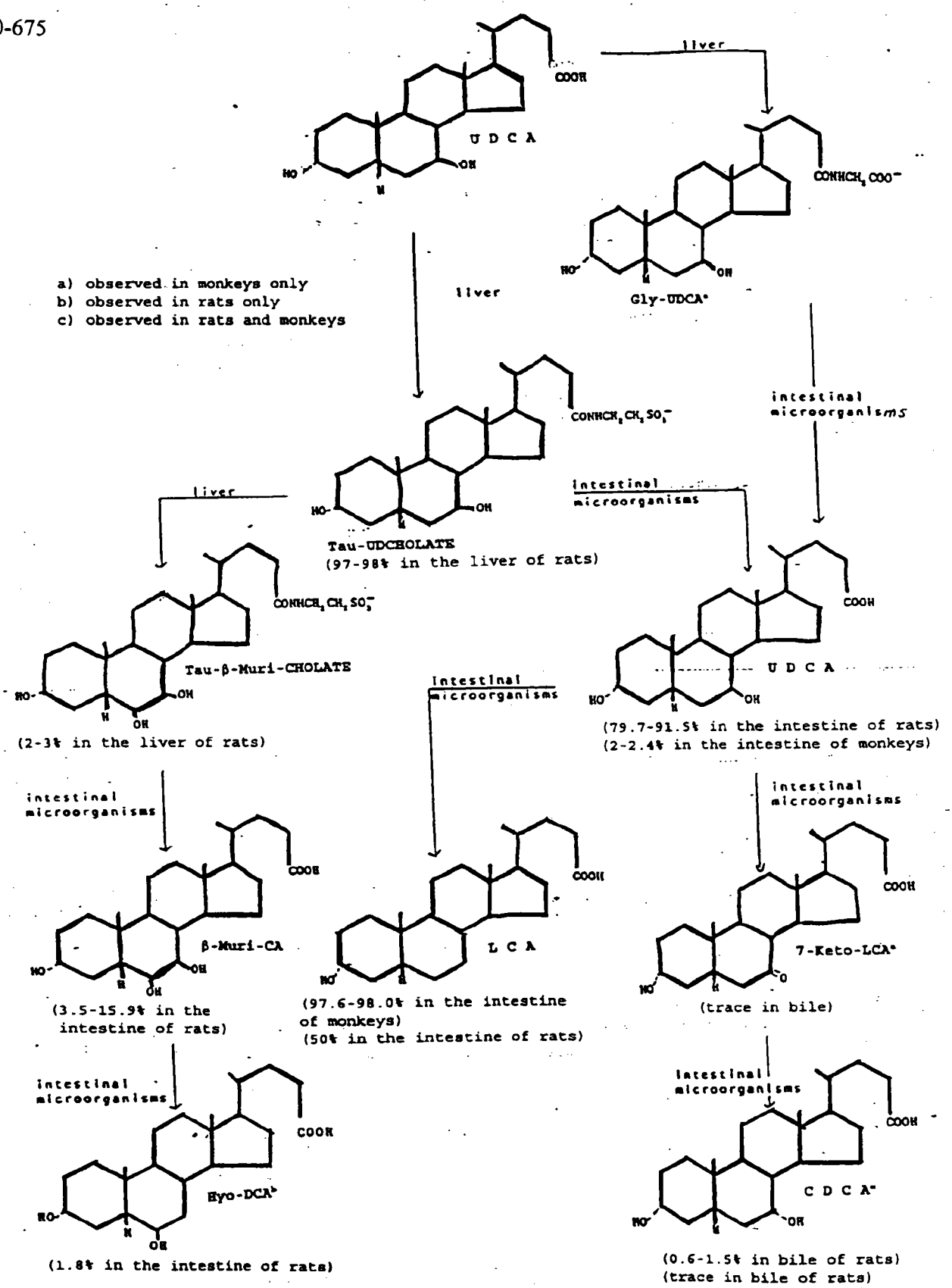

Fig. 1. - Metabolism of UDCA in animals (% of total BA composition in liver, bile or intestine, 24 h following single dose administration).

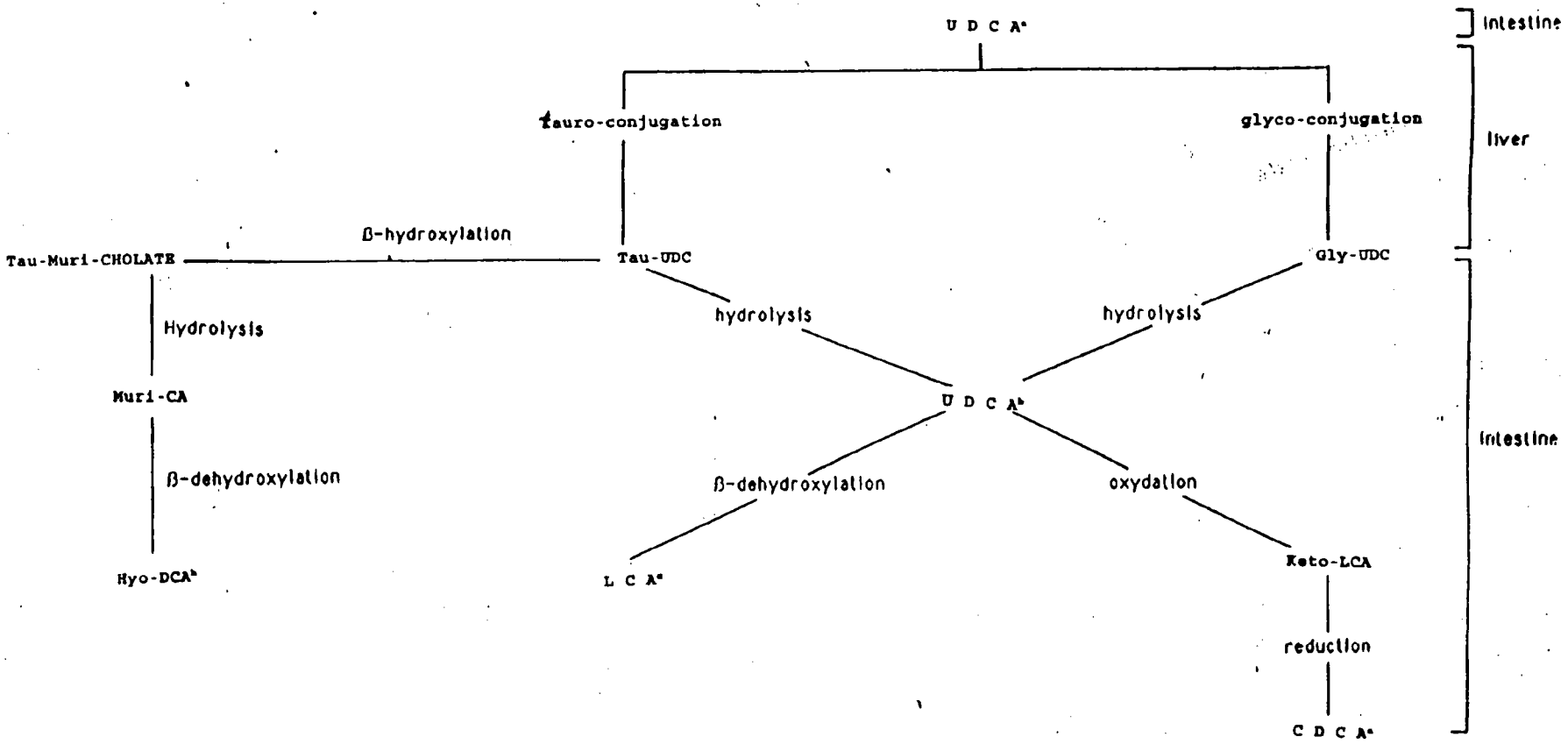

Fig. 2. - Metabolic pathway of ursodeoxycholic acid

a) active transport by hepatocytes
b) partly reabsorbed
c) poorly reabsorbed

Abbreviations:
UDCA = Ursodeoxycholic Acid
Tau-Muri-CHOLATE = Tauro-Muri-Cholate (salt)
Muri-CA = Muri-Cholic Acid (free acid)
Hyo-DCA = Hyodeoxycholic Acid
Tau-UDC = Tauro-Ursodeoxycholic Acid
LCA = Lithocholic Acid
Gly-UDC = Glyco-Ursodeoxycholic Acid
Keto-LCA = Keto-Lithocholic Acid
CDCA = Chenodeoxycholic Acid

- Upon entering the hepatocyte, UDCA forms a co-enzyme A derivative and is then amidated with glycine or taurine, to form glyco- or tauro-UDCA [GUDCA and TUDCA, respectively].

- TUDCA and GUDCA are secreted actively into canalicular bile.

- TUDCA is partly metabolized, through β-oxidation, into tauromuricholate (TMuriC).

- TMuriC, GUDCA and TUDCA are excreted into the intestine through the bile, where they are hydrolyzed into free (unconjugated) UDCA and muricholic acid by intestinal microorganisms.

- Some deconjugation may occur during ileal transit (see human data).

- Inside the body, while in the EHC and beyond, all BAs exist as **salts**, usually the Na^+ salt. They are identified as cholate, ursodeoxycholate, glyco-ursodeoxycholate, tauro-ursodeoxycholate, etc.

- Muricholic acid will subsequently undergo β-hydroxylation to produce hyodeoxycholic acid. β-dehydroxylation of UDCA will lead to lithocholic acid (LCA) [A.F. Hofmann, Clinics in Gastroenterology 6:3-24 (1977)].

- UDCA may also be oxydated into Keto-LCA which may subsequently be reduced into CDCA.

- A fraction of the LCA is absorbed, and returns to the liver where it is reamidated (to form GLCA and TLCA). In most mammals, except man, **LCA is not sulfated** and it is **toxic to the liver**.

- The reabsorption of LCA is poor in comparison to the also incomplete reabsorption of hyodeoxycholic acid, CDCA and UDCA, all of which undergo EPHBC.

V. PRECLINICAL TOXICOLOGY

A. Acute Toxicity

The sponsor noted:

- UDCA exhibited a low degree of toxicity after acute administration by oral, sc, i.p. and i.v. routes.

- Species differences. After p.o administration, hamsters [LD_{50} = 1.8(M) to 2(F) g/Kg] were more sensitive to UDCA than mice [LD_{50} >10 g/Kg], mongrel dogs [LD_{50} >10 g/Kg] or Wistar rats [LD_{50} >5 g/Kg].

  No significant sex differences were seen.

- Toxic signs observed in the various species included inhibition of autonomous movements, CNS toxicity (sedation, ataxia, convulsions) and g.i. tract disturbances (vomiting, salivation, inhibition of B_{WT} gain and decreased food consumption).

B. Chronic Toxicity

A summary of the results presented by the sponsor is given in Table 1. The oral administration of UDCA to rats and monkeys for 26 to 52 weeks, was associated with marked liver toxicity, in some cases resulting in death.

C. Carcinogenicity

A summary of the results presented by the sponsor is given in Table 2. From the data presented by the sponsor, as well as from correspondence sent from Dr. H. Falk to Dr. Bachrach (June 15, 1981), histologic findings of **preblastomatic dysplasia** were made in the urinary tract of rats treated lifelong with UDCA. The sedimentations consisted of calcium oxalate and calcium phosphate. The sponsor should be asked to provide a plausible explanation for the preblastomatic dysplasia as well as for the precipitation of UDCA in the renal pelvis of Sprague Dawley rats. Were these findings formulation-dependent? Were these rats given the sodium salt of UDCA or the free bile acid? As shown in Table 2, these findings of preblastomatic dysplasia were not reproduced in Fischer rats given higher amounts of test compound.

D. Mutagenicity

UDCA was not mutagenic in the following series of *in vitro* and *in vivo* tests: Ames test, mouse lymphoma forward mutation assay, sister chromatid exchange assay in human lymphocytes, germinative cells of mice, micronucleus test in bone marrow of Chinese hamsters and bone marrow cells of Chinese hamsters by chromosome metaphase analysis.

TABLE 1

UDCA: Chronic Toxicity Studies - Summary

SPECIES/ STRAIN	DOSE (g/Kg) (per group)	DURATION (weeks)	TOXIC EFFECTS
ORAL			
Rats Wistar 6 groups n=10 M 10 F per group	0 1% CMC (vehicle) 0.5 to 4.0	5 (6 days/week by gavage)	No effects noted.
Rats Sprague Dawley 4 groups n=25 M 25 F per group	0.8% (aqueous HPMC (vehicle) 0.1 0.5 2.5	26 (7 days/week by gavage)	All treated groups: Histological examination: Basophilic accumulations in convoluted tubules of the outer cortex of kidney. High dose group: ↓ absolute food intake (M&F). Significant inhibition of weight gain from week 10 on in M and from week 22 on in F. Macroscopic examination revealed ↓ lung weight in F and slightly ↓ heart, liver, spleen, kidneys, adrenals and brain weights in M & F (attributed to weight loss and associated ↓ in fat deposits).
Rats Wistar 6 groups n=20 M 20 F per group	0 1% CMC (vehicle) 0.5 1 2 4	26 (6 days/week by gavage)	Control groups: One death in untreated group at week 20 and one death in 0.1% CMC group at week 26. Treated groups: One death in each of the 0.5 and 2 g/Kg dose groups. Two deaths in 1 g/Kg and 3 in 4 g/Kg dose groups. All deaths occurred at week 20 or beyond and were attributed to pathological changes in lungs and intestine. 1, 2 and 4 g/Kg groups: Histopathological examinations revealed intrahepatic cholangitis, hyperplasia of the bile ducts and multiple small focal necrosis. 4 g/Kg group: Marked suppression of weight and ↑ water intake. ↓ weight of brain and thyroid and adrenal glands.
Monkeys Rhesus 5 groups n=2-5 M 2-4 F per group	0 0.04 CDCA 0.1 CDCA 0.04 UDCA 0.1 UDCA (as capsules)	26	CDCA groups; Liquid stools. ↑ serum AP, SGOT and SGPT. ↓ in the proportion of C and DCA in bile. CDCA became major biliary bile acid and proportion of LCA significantly ↑. Histology showed prominent portal inflammation with marked eosinophilia. Hypertrophy of smooth endoplasmic reticulum, dilation of bile canaliculi with some edematous microvilli bulging into the lumen forming blebs. UDCA groups: ↓ in the proportion of C and DCA in bile. Marked ↑ in UDCA and slight ↑ in LCA with no change in CDCA. Proliferation of the smooth endoplasmic reticulum noted at histology. All treated groups: Dose-related ↓ in HMG-CoA reductase activity with no change in total serum CHOL.

TABLE 1 (Cont'd)

Monkeys Rhesus 5 groups n=4 M 4 F per group	2% arabic gum solution (vehicle) 0.05 0.1 0.3 0.9 (by gavage)	52	0.1, 0.3 & 0.9 g/Kg dose groups: ↑ SGPT between 1st and 9th month of Tx with gradual return to normal. Conjunctivitis-like symptoms in the anterior part of eyes and abnormal retinal reflection and edema in posterior part of some animals. One M in high dose group and 5F died (two in each of 0.3 and 0.9 g/Kg dose groups and 1 in 0.1 g/Kg group). All deaths occurred at week 16 and beyond. All dead animals had anorexia and became less active 10 to 14 days before dying and presented with watery stools, dehydration, anemia and ↓ body temperature. 0.3 and 0.9 g/Kg: Inhibition of body weight gain from week 15 on (M) and week 10 on (F). ↓ in food and water intake (F). ↑ in SGOT between months 1 and 5 and in AP between months 3 and 6 followed by gradual return to normal. Phorocytosis throughout liver lobules (M & F), bile-duct proliferation (F). Small round-cell infiltration, vacuolar degeneration and necrosis of hepatic cells, phorocytosis and hepatic abscess in animals who died. Necrosis on stomach wall in dead M in 0.9 g/Kg dose group. 0.9 g/Kg: At start, some animals vomited the drug on administration. This resolved after 1 to 2 weeks. Increase in AP at month 3. Endoplasmic reticulum extension, increase in lysosomes with irregular myelin-figure and cell necrosis (M & V). Proliferation of epithelial cells of bile-duct mixed with inflammatory and necrotic cells in the interstices of proliferated fasciculus of collagen fibers in M.
INTRAPERITONEAL			
Rats Wistar 6 groups n=10 M 10 F per group	0 1% CMC (vehicle) 0.0625 0.125 0.250 0.500	5 (6 days/week)	0.0625 g/Kg: Slight cellular infiltration in portal field. 0.125 and 0.25 g/Kg: Cholangitis and cholangial proliferation in portal field of liver lobule and near intralobular veins. Numerous small necrotic foci in lobules showing necrosis of hepatic cells, cellular infiltration and proliferation of connective fibers. 0.25 and 0.5 g/Kg: Two deaths (1M & 1F) and 10 deaths (6M & 4F) respectively. Pathological findings included adhesion of viscera (especially intestine), abscess formation in kidneys and retention of ascites. 0.125, 0.250, 0.5 g/Kg: Inhibition of B_{WT} gain. ↓ in body weight during week 1 (M & F). ↓ food intake, Hct and Hb values. Slight ↓ in specific gravity of blood in 1 M (0.25 g/Kg) and in 2 F (0.125 and 0.5 g/Kg). ↑ liver and spleen weights (M & F) and in adrenal gland (F).

TABLE 2

UDCA: Carcinogenicity Studies - Summary

Species Strain	Dose g/Kg	Duration (weeks)	Deaths M	Deaths F	Toxic Effects
ORAL					
Mice Charles River CD-1 n=50 M 50 F 5 groups	0 0 0.025 0.150 1.000 (via the diet)	104	24 19 19 22 36	26 18 22 23 27	1 g/Kg: Corneal opacites from wk 60 on. ↑ mortality (M). Hypertrophic enlargement of liver parenchymal cells in 24 of 50 M and slightly ↑ incidence of karyomegaly in the liver (M). No neoplastic or other proliferative lesions. ↑ incidence of hepatocellular carcinomas and adenomas and liver hyperplastic nodules in (M).
Mice B6C3F$_1$ n=80 M 80 F 4 groups	0 0.038 - 0.05 0.166 - 0.146 0.362 - 0.459 (via the diet)	104	7 9 8 3	11 13 3 11	Low and medium dose groups: Slight inhibition of B_{WT} gain at wks 1 & 2 (M) Medium dose group: ↑ liver weight (wk 26) and liver/B_{WT} ratio (wks 26, 52 and 78) in M. High dose group: Inhibition of B_{WT} gain from wk 1 to 64 (M), ↑ food consumption from wk 8 to 52 (M) and wk 11-70 (F) and slight ↓ in food efficiency from wk 1 to 52 (M). ↑ liver weight (wks 26, 52, 78) in M & F, liver/B_{WT} ratio (wks 26 & 52) in M. Calcification in kidneys (more pronounced in M than F). Hyperplasia of gallbladder epithelium (F). All treated groups: Hyperplasia of bile duct.
Rats Fischer n=90 M 90 F 4 groups	0 0.023 - 0.029 0.077 - 0.098 0.239 - 0.300 (via the diet)	104	19 26 27 27	12 14 13 18	Medium dose: Inhibition of B_{WT} gain from wk 32 to 100 (F). ↑ liver weight (F) and liver/B_{WT} ratio (M & F). High dose: Inhibition of B_{WT} gain from wk 12 to 90 (M) and from wk 15 to 104 (F). ↑ food consumption up to wk 104 (M) and from wk 1 to 52 (F). Slightly ↓ food efficiency from wk 1 to 52 in M & F. ↑ liver and adrenal weights, liver/B_{WT} ratio (M & F) and ↑ kidney/B_{WT} ratio (M). All treated groups: Bile duct hyperplasia and fibrosis.
Rats Sprague Dawley n=50-100 M 10- 50 F 6 groups	0[a] 0.017 - 0.022[b] 0.059 - 0.064 0.164 - 0.201 (via the diet)	126 (M) 138 (F)	67 39 40 42[d]	69 37 43[c] 38	All treated groups: Test compound (UDCA) precipitated in the renal pelvis causing deposits, epithelial metaplasia, pyelitis and fatty degeneration in the kidneys and hyperplasia in the adrenals. High dose: ↑ in number of benign pheochromocytomas in (F) considered incidental.

a) 100M; 10F b) 50M 0F c,d) $p<0.05$ vs control

E. Reproductive Studies

1. Fertility and reproductive performance/Segment I

- UDCA administered to rats at doses up to 2.7 g/Kg/day by gavage, had no significant effect on the fertility and breeding capacity.

- Slight to moderate decreases in maternal and fetal body weights and an increase in the resorption rate were however noted at a dose of 2 g/Kg/day.

2. Teratology/Segment II

- UDCA did not show any teratogenic effect following oral administration at daily doses up to 1.5 g/Kg in mice and 4.0 g/Kg in rats as well as following i.p. administration at daily doses up to 0.2 g/Kg in both mice and rats.

- ↓ in maternal and fetal body weights were however noted following oral dosing in both mice and rats as well as an ↑ in the number of resorption sites in rats dosed at 2.0 g/Kg.

- Similar findings were reported following i.p. administration.

- Rabbits were more sensitive to the toxic effects of UDCA.

  - Pregnant rabbits treated with doses of 0.1 and 0.3 g/Kg/day showed decreased food consumption, body weight gain and motor activity.

  - Coma and death occurred in 4 animals of the high dose group.

  - No malformed fetuses were found.

  - UDCA, therefore, did not show any teratogenic potential in rabbits at doses up to 0.3 g/Kg/day.

  - The lowest toxic dose for pregnant animals ranged from 0.033 to 0.1 g/Kg/day.

3. Perinatal and postnatal study/Segment III

- A peri- and post-natal study was carried out in pregnant rats treated at daily doses of 0.25, 1 and 2 g/Kg from day 17 of gestation to 21 post-delivery.

  - A slight decrease in maternal body weight was noted in the high dose group.

- No external anomalies were observed in newborns (F_1).

- Mortality, mean body weight, physical maturation and gross findings in nurslings and weanlings from treated F_0 did not differ from controls.

- There were no skeletal anomalies and no influences of UDCA on the reproductive performance of the F_1 generation and on the growth of newborns of the F_2 generation.

VI. HUMAN PKs AND BIOAVAILABILITY-SUMMARY

The sponsor explains that PK and bioavailability studies have not been conducted with UDCA tablets, 250 mg. But the ADME profile of UDCA has been extensively reviewed in the literature. A summary of these reported findings is presented in part A. This includes PD actions, with emphasis on human data and those PD effects that may be applicable to PBC. Effects related to gallstone dissolution are not emphasized. In part B of this subsection, matters related to the formulations of UDCA used in the pivotal clinical trials are addressed.

A. Overview of BA Metabolism in Humans, Including the Biosynthesis of UDCA

NOTE: This subsection is based, primarily, on the publication by A.F. Hofmann, "Pharmacology of Ursodeoxycholic Acid, an Enterohepatic Drug" [Scand. J. Gastroenterol. 29(Suppl.204):1-15 (1994)].

In humans, the biosynthesis of UDCA starts from CHOL. Following 7α-hydroxylation in the liver, CHOL is transformed into two primary BAs: CDA and cholic acid [(CA), resulting from the hydroxylation of CHOL at both the 7α and the 12α positions of the ring]. After biosynthesis, these two primary BAs are conjugated with either TAU or GLY at their carboxylic acid group. The four primary conjugated BAs (TCA, GCA, TCDCA and GCDCA) are secreted into bile and absorbed efficiently from the small intestine by both active and passive mechanisms.[19]

- In the jejunum GLY dihydroxy-BAs are likely to be absorbed passively in the protonated form [A.F. Hofmann et al., Gastroenterology 93:693-709 (1987)].[20]

- In the ileum, conjugated BAs, especially cholyl conjugates, are absorbed by a sodium-dependent cotransport system that has been cloned [M.H. Wong et al., JBC 269:1340-1347 (1994)].

APPEARS THIS WAY ON ORIGINAL

[19] A.F. Hofmann, Intestinal absorption of bile acids and biliary constituents: the intestinal component of the enterohepatic circulation and the integrated system. In: L.R. Johnson et al., editors. Physiology of the gastrointestinal tract.

[20] This mechanism of absorption is enhanced by the transient episodes of acidic intraluminal pH in the jejunum, as well as by the acidic microclimate that is present at the luminal surface of the jejunal enterocyte. In the guinea pig, there appears to be a sodium-independent carrier in the jejunum for conjugated BAs that differs from the sodium-dependent ileal transporter. Whether this is present in humans is not known, although there is convincing evidence for absorption of dihydroxy-bile acid conjugates in the proximal small intestine. In the pig, jejunal conservation of BAs is greater than ileal conservation of BAs [C. Juste et al., Dig. Dis. Sci. 33:67-73 (1988)].

- In the distal intestine, conjugated BAs undergo some deconjugation and a fraction of the unconjugated BA that is formed is absorbed. But the amount of UDCA being absorbed from the colon is small.

- The unconjugated BA returns to the liver where it is "re" conjugated.

- Thus, for the primary BA amidates, there is continuous deconjugation-reconjugation during enterohepatic cycling of the molecules.

- In the colon, deconjugation is completed; 7-dehydroxylation occurs, mediated by anaerobic bacteria. CA is converted to DCA; CDCA is converted to LCA.[21]

- Both DCA and LCA are absorbed to some extent from the colon and returned to the liver. About a fifth to a half of the DCA that is newly formed is absorbed from the colon; absorption is passive [A.F. Hofmann, Gastroenterology (locus cited) (1987)]. The absorbed DCA undergoes conjugation with GLY or TAU in the liver and is then secreted into bile. Its amidates are absorbed from the small intestine and mix with the amidates of the primary BAs.[22]

- LCA is absorbed less efficiently from the large intestine than DCA [R.N. Allan et al., Gut 17:413-419 (1976)], probably because its insolubility at body temperature, its greater hydrophobicity, which is likely to promote absorption to the immense area of bacterial membranes that is present in the colonic lumen.

- In the human hepatocyte, LCA is not only conjugated with GLY or TAU but also with sulfate (at the 3 position) [A.E. Cowen et al., Gastroenterology 69:59-66 (1975)]. The sulfolithocholylglycine and sulfolithocholyltaurine that are thus formed are secreted into bile.

  NOTE: In contrast to the amidates of CA, CDCA and DCA, the sulfated lithocholyl amidates are poorly conserved by the small intestine, and these compounds are rapidly lost from the body [A.E. Cowen et al., Gastroenterology 69:67-76 (1975)]. Sulfation is not complete with a single hepatocyte passage [S.S. Rossi et al., J. Lipid. Res. 28:589-595 (1987)] but unsulfated lithocholyl amidates are likely to be efficiently absorbed from the small intestine and will be sulfated (again in part) during their next transit through the hepatocyte.

- There is a small portion of UDCA in biliary BAs in most humans [A.F. Hofmann et al., Gastroenterology 83:738-752 (1982)]. The origin of this UDCA has not been definitely established. It is likely to be formed in the colon by bacterial epimerization of the 7-OH group of CDCA[23] and then absorbed passively.

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[21] Removal of the 7-OH group involves a 3-oxo-$\Delta^4\Delta^6$ resonating intermediate [J.P. Coleman et al., JBC 262:4701-4707 (1987)]; formation of such a resonating intermediate cannot occur in the C ring and 12-dehydroxylation does not occur.

[22] The end result is the accumulation of a pool of DCA amidates that circulates with the amidates of primary bile acids. A fraction undergoes deconjugation and is absorbed in part in unconjugated form. In the liver, the unconjugated bile acid is reconjugated and joins those primary and secondary bile acid amidates that were returned intact from the small intestine.

[23] In principle, it could be formed directly from cholesterol, that is, it could be a primary bile acid, as has been shown to occur in the bear and the nutria. However, its absence from biliary fistula bile makes this unlikely. It also seems unlikely that it is formed in the liver from the 7-oxo derivative of CDCA, which in turn was formed in the colon by bacterial 7-dehydrogenases. In humans, 7-oxo-LCA is reduced preferentially to CDCA rather than UDCA. Were UDCA to be formed via this route, 7-oxo-LCA should be present in systemic plasma. This does not appear to be the case. Thus, in humans it is likely that UDCA is formed in the colon, and then absorbed passively.