I. BACKGROUND/INTRODUCTION/RATIONALE

XENICAL™ (Ro 18-0647; orlistat) is a chemically synthesized derivative of lipstatin, a natural product of *streptomyces toxytricine*. Lipstatin, its hydrogenated derivative, tetrahydrolipstatin (THL) and orlistat are all inhibitors of pancreatic lipase.[1] Orlistat main inhibitory effects are on the lipases involved in human gastrointestinal fat digestion: pancreatic gastric (there is no lingual lipase), carboxylester lipase and human milk bile salt-stimulated lipase (BSSL). Colipase-dependent lipase is the major lipolytic enzyme secreted from the exocrine pancreas. This enzyme is one of the most studied and characterized lipases, and in the adult it is considered to be responsible for quantitative digestion of all TG.[2] Pancreatic lipase is positionally specific, i.e. the sn-2 position of TG molecules is absolutely resistant to hydrolysis. Thus, colipase-dependent lipase hydrolyses both TG and DG molecules releasing two or one FA respectively [B. Borgström et al., J. Lipid Res. 5:522-531 (1964)]. For optimal activity, colipase-dependent lipase is dependent on a 9KD protein cofactor, colipase[3], also secreted by the pancreas.[4] Gastric lipase is entirely a product of the chief cells of the gastric mucosa ["lingual" and pregastric (pharyngeal) lipases are the same as gastric lipase] that is not expected to make major contributions to intraduodenal fat digestion in healthy human adults. However, in patients with pancreatic insufficiency,[5] e.g., cystic fibrosis, the intestinal environment may favor the function of gastric lipase. The major products found in stomach contents during gastric lipolysis are diacylglycerol and FA. The most important physiological functions of gastric lipase seem to be to generate FA for the following reasons: (1) to induce binding between the colipase-dependent lipase-colipase complex and the emulsion surface (vide infra), (2) to be "emulsiogenic" when they become partially ionized in small intestinal contents (vide infra), and (3) to release CCK and GIP. Because FA induce rancidity, food products contain only trace amounts of FA. Human milk bile salt-stimulated lipase (BSSL) is a constituent of human milk, that is activated by primary and secondary bile salts that retains its potential activity during passage through the stomach. This is a non-specific lipase as it hydrolyzes not only TG but also DG and MG, CE- and fat-soluble vitamin esters [L. Bläckberg and O. Hernell, FEBS Lett, 157:337-341 (1983)]. The carboxyl ester hydrolase (CEH),[6] secreted from the human pancreas, is closely related to BSSL.

There are three additional lipases involved in human GI fat digestion. Pancreatic phospholipase $A_2$ is the sole intestinal luminal source of phospholipase activity.[7] With phosphatidylcholine (lecithin), the physiological reaction products are 1 lyso-PC (1-lysolecithin) and 1 FA. The saturated chain in phospholipid is usually in the *sn*-1 position, therefore palmitoyl and stearoyl lysolecitins are formed. The Paneth cells are believed to contain phospholipases of A class, which are particularly active against phosphatidylglycerol and phosphatidylinositol (PI) [C.M. Mansbach et al., J. Clin. Invest. 69:368-376 (1982)]. They are also reported to contain cholesteryl esterase activity [P. Lechêne de la Porte, 86:211-214 (1986)]. Their physiological roles are unclear but it is conceivable that Paneth cells may act in human fat digestive/absorption as the "diffuse pancreas of the GI

BEST POSSIBLE

tract". In additional studies[8] cholesteryl esterase activity was also found in enterocytes and intercellular spaces, suggesting that luminal (pancreatic) or plasma enzymes may be absorbed into the lamina propria. Binding of CEH to epithelial cells occurs and may promote lipid-product absorption into enterocytes.[9] Regarding microbial lipases, the (phospho) lipases of the anaerotic flora of the human colon are heterogenous and poorly characterized.[10] They cleave all 3 FA ester linkages of TG and both fatty and other ester linkages of phospholipids. The enzymes only become important in malabsorption syndromes when they hydrolyze FA ester bonds and hydroxylate double bonds, thereby releasing long-chain hydroxy FA that (in addition to steatorrhea) can induce diarrhea. Long-chain FA (LCFA) are only sparingly absorbed from the colon, but SCFA (acetic, butyric, propionic, and valeric) are well absorbed. The latter are produced in abundance from microbial fermentation of the monosaccharide and peptide units of microbially digested dietary fiber, resistant starch, proteins, sloughed cells, and mucus.[11]

From the information provided it is not clear to this consultant whether the effect of orlistat on lipases in the human GI tract is universal (are all lipases inhibited?) or whether some lipases escape inhibition. Also, is the orlistat effect on colipase-dependent lipase reversible or irreversible? All of this information is important when trying to understand intraluminal events following the administration of orlistat. Orlistat also inhibits lipoprotein, hepatic, hormone-sensitive and diacylglycerol lipases *in vitro*. However, because orlistat's poor bioavailability from the GI tract, the inhibition of these enzymes *in vivo* appears to have little (if any) clinical relevance.

The potent, irreversible inhibition of human gastrointestinal lipases by orlistat results in an inhibition of fat absorption and, as a consequence, weight reduction.[12] A detail description of the digestion and absorption of fat, with the sequential and concerted action of lipases, the physical-chemical states of dietary lipids in postprandial intestinal contents [including "steady-state" stage of duodenal fat digestion] is beyond the scope of the present consult. This information can be found in the excellent review by Carey and Hernell [locus cited (1992)] where, in addition, there is a section on new concepts on aqueous diffusion, passive apical membrane penetration, and possible active transport of lipid products. However, an important piece of information, apparently germaine to the consult, is information of duodenal lumen during established fat digestion in humans. This information is summarized in Table 1. These data are presented here because it seems important to establish what is being offered to the colon that should have been absorbed in the small intestine. A balance between what is being ingested, what is being absorbed from the small intestine/colon and what is being excreted in the stool should ideally be assessed. But the available clinical tools do not allow such a balance to be reliably done. As we will see from now on, because of the sparse information on intraluminal/ mucosal colonic events, the interpretation of findings like the ones assessed in the present clinical report is very difficult and, by necessity, must be based on a certain degree of speculation.

TABLE 1

Chemistry of Duodenal Lumen During
Established Fat Digestion in Healthy Humans

| Species | Duodenal Concentrations (mmol/L) |
|---|---|
| Sodium | 105 ± 4.2 |
| Potassium | 14.4 ± 1.0 |
| Calcium | 14.9 ± 2.6 |
| Bile salt | 14.5 ± 8.8 |
| Phospholipid | 4.8 ± 1.8 |
| Cholesterol | 2.4 ± 2.0 |
| Triglyceride | 36.6 ± 44.5 |
| Diglyceride | 7.3 ± 6.3 |
| Monoglyceride | 5.4 ± 4.5 |
| Fatty Acid | 20.4 ± 14.0 |
| Cholesterol Ester | 0.2 ± 0.4 |
| Pancreatic Lipase | 3-5 μM |
| Pancreatic Colipase | 3-5 μM |
| Pancreatic Phospholipase $A_2$ | 3-5 μM |
| Pancreatic Carboxyl ester Hydrolase | 3-5 μM |

[From: O. Hernell et al., Biochemistry 29:2041-2056 (1990)]

NOTE: Within the colon the bacterial concentration is $10^{11}$ to $10^{12}$ cfu/ml; nearly one-third of the fecal dry weight consists of viable bacteria [Simon and Gorbach, Chapter 64, Intestinal Flora and Gastrointestinal Function, IN: Physiology of the Gastrointestinal Tract, Second Edition, edited by Leonard R. Johnson, Raven Press, New York, pp. 1729-1747 (1987)]



From their review on the matter, Carey and Hernell [locus cited (1992)] concluded that the most logical classification of fat malabsorption syndromes should fall into four categories: (1) faulty digestion (intraluminal), (2) faulty dispersion (also intraluminal), (3) faulty penetration (mucosal) and (4) faulty transport (lamina proprea, lymphatic). It is worth noting, however, that fat malabsorption syndromes often have a mixed pathophysiologic basis, such as a) when there is both faulty digestion and faulty dispersion in the acidic small bowel that accompanies chronic pancreatic insufficiency, the clinical condition that parallels the effects of orlistat the most, as well as in gastric hypersecretion syndromes and probably in many otherwise healthy newborns[13]; b) when there is both a gut enteropathy plus endocrinopathy as in celiac sprue,[14] and probably in Whipple's disease and AIDS, and c) when there is both a gut enteropathy and faulty dispersion as in the "blind-loop" syndrome.[15] It is also of interest to mention that orlistat is also expected to decrease the absorption of cholesterol as can be predicted by the Simmonds et al. data [J. Clin. Intest. 46:874-890 (1967)].

The background/introduction section of this consult review includes also a brief summary of the fate of fat in the colon and fecal fat. Most of this information was taken from the review by Carey and Hernell [(locus cited) information was taken from the review by Carey and Hernell [(locus cited) (1992)]. Approximately 400 different species of anaerobic bacteria in viable concentrations of $10^{11}$/g dry weight of colonic contents (40% to 55% of solids) carry out all the hydrolytic digestive functions of the human colon. The bacterial lipases, phospholipases $A_1$ and $A_2$ (phospholipase B), lysophopholipases, and phosphodiesterases (phospholipases C and D) are widespread among the normal colonic flora. In contrast to the mammalian small intestinal lipases and phospholipases, bacterial lipases possess certain characteristics. These characteristics are summarized in Table 2.

TABLE 2

Characteristics of Bacterial Lipases

| | |
|---|---|
| 1) | Do not exhibit positional specificity. |
| 2) | Are not inhibited by BAs. |
| 3) | Do not exhibit selectivity for physical state, i.e., can hydrolyze monomers albeit slowly. |
| 4) | Their rates of hydrolysis are not generally dependent on carbon number or degree of unsaturation. |
| 5) | They have their pH-optima at neutral or slightly acidic pH, which is the common pH of the healthy human colon.[a] |
| 6) | No cofactors are required. |
| 7) | Certain colonic bacteria also produce a variety of oxido-reductases that can attack the double bonds of unsaturated FA[b] as well as the steroid rings of acidic[c] and neutral sterols.[d] |

a) D.F. Evans et al., Gut 29:1035-1041 (1988)
b) These mechanisms are important because fecal FAs, particularly hydroxy FAs are mediators of diarrhea.
A.T. James et al., Biochem. J. 78:333-339 (1961)
Y.S. Kim and N. Spritz, NEJM 279:1424-1426 (1968)
C.S. Soon et al., Gastroenterology, 63:748-757 (1972)
H.J. Binder, Gastroenterology 65:847-850 (1973)
c) P.B. Hylemon: Metabolism of bile acids in intestinal microflora, in Danielsson H., Sjövall J (eds): Sterols and Bile Acids, New Comprehension Biochemistry, vol 12, Amsterdam, Elsevier, pp 331-343 (1985)
d) N.B. Myant: The Biology of Cholesterol and Related Steroids. London, England, Heiermann, pp 136-137 (1981).

BEST POSSIBLE

In the human colon, there is rapid and total hydrolysis of TG and all other complex lipids such as phospholipids and Ch-esters by microbial lipases and, even in the most florid steatorrhea, glycerides are uncommon in stool.[16] For example, TG is hydrolyzed to three FA plus glycerol, phospholipid, is hydrolyzed to two FA plus glycerol, phosphate and choline and CE produces one Ch. plus one FA. Complex lipid hydrolysis by colonic bacterial lipases explains why it is generally impossible to distinguish pancreatic from mucosal malabsorption by an examination of stool for glycerides.[17] Unsaturated FA (UFA) may be reduced at the double bonds by bacterial reductases to form saturated FA,[18] and about 10% to 20% of unsaturated FA are oxidized by bacterial oxidases at their double bonds to form hydroxy FA.[19] For example, the common monounsaturated FA, oleic acid (18:1n-9), is transformed to 10(9)-hydroxystearic acid. Hydroxy FA are powerful colonic cathartics[20] just like 12-hydroxyoleic (mislabelled stearic) acid, the principal FA esterified in castor oil's TG.[21]

The physical-chemical as well as chemical states of normal fecal fat are listed in Table 3. These analyses were performed after addition of an equal volume of water, homogenization, and centrifugation. Feces separated into three phases: oil, aqueous, and solid and each had total compositional analyses. Most fecal FA are present as monovalant ($Na^+$,$K^+$) and divalent ($Ca^{2+}$,$Mg^{2+}$) soaps. About 10% to 20% of the FA are hydroxylated depending on diet and colonic factors. No glycerides (MG, DG or TG) are detected. The remainder of fecal fat is composed of neutral (from Ch and plant sterols) and acidic (bile acid) steroids and membrane lipids of bacteria. Even in severe fat malabsorption in adults, it is highly unusual to find intact glycerides in stool fat[4] [J.B. Thompson et al., JLCM 73:521-530 (1969)].

TABLE 3

Physical-Chemical and Chemical States of
Normal Fecal Fat (4 to 5 g/24 h)

| Chemical | Phase | % of Total Fat |
|---|---|---|
| Fatty acids (including OH-FA) | Oil or solid | 70 |
| $NA^+$ + $K^+$ soaps | Aqueous or solid | |
| $CA^{2+}$ + $Mg^{2+}$ soaps | Crystalline solids | 10 |
| Glycerides (TG, DG, MG) | Oil | 0 |
| Sterols | | |
| Neutral (Ch) | Solid | |
| Acidic (Bile Acids) | Aqueous | 15 |
| Other, bacterial Phospholipid, etc. | Oil or solid | 5 |
| [From Carey and Hornell's review (locus cited) (1992)] | | |

BEST POSSIBLE

Finally, because of orlistat's mechanism of action, through the inhibition of TG hydrolysis, larger than normal quantities of fat (mainly unhydrolyzed TG) are being offered to the colon. In this situation, BA and FFA concentrations may be increased. Were this to be the case, there would be concern because increased secondary BAs (DCA and LCA) and FFAs in the colon are thought to be one of the factors promoting colon cancer in animals and humans.[22] In reality, this is another very difficult area where knowledge is incomplete. One of the reasons fostering uncertainty here is that even now, 1997, some BA found in the human stools have not been chemically/structurally identified.

Epidemiological data have repeatedly associated high intake of dietary fat and low intake of dietary fiber with the development of neoplasia in the human large bowel. In a study by F.A. Macrae et al. [Gastroenterology 108:A501 (1995)] a low fat diet with wheat bran supplement significantly reduced the occurrence of larger adenomas [these interventions may delay transition from smaller to larger adenomas, a step that may critically define those adenomas likely to progress to malignancy]. High intake of fat stimulates hepatic secretion of primary BAs that are required for fat solubilization. Primary BAs not reabsorbed in the terminal ileum are bacterially degraded into secondary BAs in the large bowel. BAs are capable of causing damage to colorectal cell membranes due to their physicochemical properties. From animal studies it is evident that especially the secondary BAs, DCA and LCA, have a cytotoxic potential and promote tumor development in the large bowel. DCA has *greater* proliferative effects on neoplastic colonocytes compared to differentiated cell *in vitro* [D. Peters et al., Gastroenterology, 110:A576 (1996)].[23,24,25] In addition, increased concentrations of BAs have been demonstrated in the intestinal contents of populations at high risk for colorectal cancer, who typically consume a diet high in fat, and in subjects with colorectal neoplasia. The consumption of dietary fiber probably reduces colorectal cancer risk. One of the physiological mechanisms that have been considered in this respect is that the consumption of dietary fiber results in the generation of SCFAs by bacterial fermentation in the colon.[26]

One factor thought to be important to evaluate is the cell proliferation of the colorectal epithelial cell because marked changes in cell proliferation occur in the early stages of carcinogenesis. A proliferative gradient exists in cells of colonic mucosa. Differentiated mitotically inactive luminal mucosal cells are formed from undifferentiated mitotically active basal cells in the crypts of Lieberkuhn. The luminal cells that are normally sloughed daily are replaced by cells that migrate from the base of the crypt, and this allows the use of biomarkers, such as BrdU or PCNA that can be attached to cells at different proliferative stages. This thesis proposes that for different premorphological abnormalities, quantification of the biomarkers is related to different cancer risk levels. But, as we will see below, this "increased proliferation" thesis is not universally accepted.

Theoretically, in the case of orlistat administration, increased cell proliferation, were to occur, may be due to either the fat material(s) or the unabsorbed compound, in original or derivative form being presented to the colonic mucosa. In a study in 30 normal subjects by Stadler et al. [Gut 29:1326-1331 (1988)], increasing amounts of supplementary fat correlated with increasing colonic epithelium hyperproliferation. Changes in proliferative biomarkers in man have been reported with several nutritional interactions including a) 2-week supplementary fat and omega-3 FA consumption [n=10; J. Stadler et al. (locus cited) (1988); M. Anti et al., Gastroenterology 103:883-891 (1992)] and b) 12-week calcium supplementation [n=8; G. Steinbach et al., Gastroenterology 106:1162-1167 (1994)] and c) 12-week caloric restriction [n=15; G. Steinbach et al., Cancer Res. 54:1194-1197 (1994)].

In rats fed nutritionally unbalanced diets (40% of calories from fat and 0.1% $Ca^{++}$) in addition to orlistat administered for a period of nine days, Meier [Res. Report #B-156860, April 16 (1993)] reported an increased mucosal cell turnover in a dose-related manner. However, orlistat administration for up to 18 months to rats, when combined with a regular diet did not result in any morphological or functional changes to the rectal mucosa. The inference is that in the case of orlistat studies, the observed cell proliferation in rats (which was not reproduced upon L-T administration of the compound), is due to dietary components (fat et al.) rather than the compound itself. Some previous observations are worth mentioning here. In another study by the sponsor [D.E. Beattie et al., Res. Report B-113404, October 18 (1993)], apparently healthy subjects received dietary fiber and fat in addition to orlistat, 80 mg t.i.d. (lower than the recommended dose) for 7 days. As expected, total fat and FFA content of the feces increased in those subjects. However, the daily total fecal fat excretion increased 7-fold while the daily fecal FFA excretion (the fraction of concern) increased only 2.5-fold. Moreover, the concentration of total fecal fat increased ~~by 3-to-5-fold, while~~ the concentration of fecal FFA (mg/g feces) was unchanged.

Aside of the meaning of colonic epithelial cell hyperproliferation, the effect of orlistat on human colonocyte cell proliferation is not defined. This has prompted the sponsor to carry out a study in 12 otherwise healthy obese individuals administered orlistat at the proposed recommended doses (120 mg t.i.d.). The experimental subjects were consuming a hypocaloric diet (1900 Kcal) with a proportionally normal fat content (ca. 30%). The study was set to show if orlistat's inhibition of fat absorption leads to an increased exposure of the colonic mucosa to total fat, FFAs and bile acids. The study also assessed mucosal cell turnover rate.

BEST POSSIBLE

## II. BRIEF DESCRIPTION OF THE DESIGN, EXECUTION AND MAIN FINDINGS IN THE STUDY

This trial was carried out by Hal Nelson, M.D. (Natl. Jewish Center for immunology and Resp. Med., Denver, CO), under Protocol NP15138. The study was well-designed and apparently well-executed. Twelve M and 12 F obese, otherwise healthy subjects were hospitalized for colonoscopy followed by a seven day run-in (baseline) period during which no medication was taken and all feces were collected. Not included in the trial were subjects with potential confounders such as history of cancer, presence of any condition that causes fat malabsorption or of lactose intolerance and food allergies. Also excluded from the trial were subjects who were on a special diet or those who could not fulfill the dietary requirements (1900 Kcal/day including ca. 70 g of fat per day; with ca. 30% of calories derived from fat and the calcium content from ca. 824 to 899 mg/day), those who had diarrhea (>2 liquid stools/day) during one week prior to the study, or constipation (> days duration) within the last 2 weeks prior to the trial, those with G.I. surgery for weight reducing purposes or a history of post-surgical adhesions, and those with a history or current presence of bulimia or laxative abuse. Concomitant medications and/or treatments that may be confounding were proscribed during the trial.

The design was that of a double-blind, randomized, parallel study comparing the effects of orlistat (120 mg capsules, Ro 18-0647/090, Batch No. PT 2157 T 48, Clinical Order No. C-177153; given three times a day with breakfast, lunch and dinner for 42 days) to those of PL (matching PL capsules, Batch No. PT 2160 T 31, Clinical Order No. C-175063 given/taken as the orlistat capsules). The standardized, calorie-controlled diet continued through 42-day duration of the trial. At the end of the 10-day period at the study site (7 days of run-in and 3 days of randomized treatment) the subjects were released for a 29-day out-patient period. Subjects continued receiving orlistat or PL and the nutritionally balanced hypocaloric diet. Subjects were readmitted at the study center for an additional 10-day period for completion of test medication administration. Fecal samples were collected during the last 7 days. A colonic biopsy was repeated on Day 43.

Biopsy samples[27] were taken during colonoscopy on day -7 and day 43, after an overnight fast. Ca. one hour before the colonoscopy procedure, at least one tap water enema was administered. Eight biopsy specimens of 2.8 mm were taken from the rectum ca. 8 to 10 cm from the anus. These 8 biopsy specimens were used to measure cell proliferation by the techniques of BrdU incorporation (3 biopsy samples) PCNA immunochemistry (3 biopsy samples) and WCMC (2 biopsy samples). After homogenization, fecal samples[28] were analyzed for total fat, FFA and BA content and pH. In addition, the laboratory provided analysis of total $Ca^{++}$ content. Fecal water was analyzed for FFA, BA concentrations and pH.

The PD data were analyzed descriptively. Mean, SDM and range were calculated for the baseline mean, treatment mean value and mean change from baseline (treatment-baseline = Δ) for the following parameters.

**Fecal material**[a]: Total fat, FFA and BA content, pH (also calcium and fecal weight)

**Fecal water**[b]: FFA and BA concentration, pH

a) The mean daily value for the baseline (days -7 to -1) and treatment intervals (days 36 to 42) was defined as the total amount obtained during the collection period divided by the number of days in the collection period; the endpoint was defined as the last day in which a fecal sample was obtained.

b) The total volume of fecal water was calculated by multiplying the amount of fecal water (mL per g of feces) by the weight of the 24h fecal sample (g per 24h). For each fecal water parameter, for example CA, the total amount (mg) of CA was collected on the database. This was then divided by the total fecal water per day, to arrive at the concentration of CA per mL of fecal water for each 24h period.

BEST POSSIBLE

The consultant agrees with the sponsor that since fecal water parameters and fecal pH are concentrations, it would not be valid to record a zero for an absent daily value. Therefore, to calculate the mean concentration for the whole collection period, the daily concentrations were summated, then divided by the number of days in which a sample was provided.

The PD results (sponsor's Tables 4 through 8, and Fig. 1 through 5) can be summarized as follows:

**Analysis of Fecal Material**

- There was a statistically significant difference ($p<0.05$) between the orlistat and PL groups for fecal weight, total fecal fat, and fecal FFAs, excretion of which increased to a greater degree with orlistat treatment than with PL treatment.

- Less total BAs were excreted ($p<0.05$) and the pH of the feces was more acidic ($p\leq0.05$) after orlistat than after PL treatment.

- Significantly less LCA and DCA were excreted in the feces with orlistat than with PL treatment.

**Analysis of Fecal Water**

- During orlistat treatment, the increase in fecal water FFAs approached statistical significance ($p=0.06$) while total BAs were significantly decreased ($p<0.05$) relative to the changes observed following PL treatment.

- The pH became significantly more acidic ($p<0.05$) during orlistat than during PL treatment.

- Significantly less DCA was excreted during orlistat than during PL treatment.

**Biomarkers of Cell Proliferation**

BEST POSSIBLE

- Crypt compartment analysis for each of the biomarkers (BrdU, PCNA, WCMC) demonstrated no significant difference in values between the test treatment groups at baseline or significant change after treatment.

- No significant differences ($p>0.05$) was shown in statistical tests intended to correlate changes in fecal material and fecal water parameters with changes in the three biomarkers (BrdU, PCNA and WCMC).

**Safety**

- A total of 77 AEs were reported in this study by 22 subjects: 47 AEs in the 12 subjects in the orlistat-treated group and 30 in 10 out of 12 subjects in the PL-treated group.

- The majority (42) of these AEs were g.i. system related and were more common in the orlistat than in the PL-treated group (31:11) of these, the most frequent were:

  - fatty/oily stools (8:0)
  - flatulence (8:3)
  - soft stools (5:2)

- 3 severe AEs were of the g.i. system (flatus with discharge, fecal urgency, nausea) rated by the investigator as possibly related to orlistat.

## III. POSSIBLE INTERPRETATION OF PD FINDINGS

An introductory note is in order. The available information on colonic function is very incomplete. The identification and characterization of factors that modify colonic function are poorly understood. Therefore, interpretation of changes in both the colonic lumen and the colonic wall is a very difficult task. Attempting to explain PD data may lead to more questions than answers and only a few (if any) definite conclusions. With regard to potential safety concerns, the important findings in study W-144999 are related to:

### A. Intraluminal Changes

**Fecal weight**

**Fecal water:** Increases in FFAs, what happens to the CDCA family and the CA family of bile acids.

### B. Colonic Epithelial Cell Findings

Pertinent questions are:

- Appropriateness of biopsy site
- Appropriateness of endpoints
- Is there cell hyperproliferation
- Is there a risk of cancer?

**Fecal Weight**



The increase in total weight induced by orlistat (53 g/24h) was moderate in comparison to that induced by PL (17 g/24h). This indicates that total weight did not increase accordingly, in comparison to the increase in total fat [19 g/24h for orlistat-treated vs virtually no change (0.6 g/24h) for PL-treated patients). In this instance it can be presumed that the fat being offered to the colon is of normal composition (normal TG). But there is no information on the relative proportion of any DG and/or MG and therefore, it is not known if the fat(s) being offered to the colon are being subjected to the action of bacterial lipase. In this regard, the situation after orlistat treatment would resemble what is seen in other clinical conditions, such as pancreatic insufficiency. This and other conditions such as celiac sprue and jejunal by-pass are accompanied by normal lipolysis. But the latter conditions are present with watery diarrhea because of high amounts of FFAs.

Under orlistat treatment, the increase in FFAs in the fecal material was also moderate (5.9 g/24h in comparison to PL, 0.5 g/24h). This finding, together with modest increase in the concentration of FFAs in fecal water (1.4 μmol/mL vs no change with PL) suggest that, at least from quantitative considerations, FFAs are not expected to be a big problem after administration of orlistat. One possibility is that the FFAs present is the G.I. lumen are being normally absorbed, but there is no experimental support of this proposal.[29] Indeed, the available data are very incomplete. The nature of these FFAs is not known. As mentioned above, hydroxy FA are powerful colonic cathartics (could this explain some of the AE observed after administration of orlistat?). SCFAs, ~~such as~~ butyric acid (no information on this ~~study) appear to be~~ protectant to the epithelial cell of the colon.

One finding of interest in the fecal matter analyses was a decrease of total BAs with orlistat treatment (-64 mg/24h), a change that went in the opposite direction to that seen with PL (increase of 52 mg/24h). The observed changes in total BA were primarily due to changes in the secondary BAs, DCA and LCA, both of which **decreased** with orlistat treatment (-19 and -38 mg/24h, respectively) in comparison to **increases** with PL (43 and 13 mg/24h, respectively). [The changes in the primary BAs, CA and CDCA were minor; UDCA virtually did not change after administration of either orlistat or PL.] A possible explanation for the changes in total and individual (secondary) BAs is given under Comments for fecal water.

**Fecal Water**

BEST POSSIBLE

Although approximating statistical significance ($p=0.06$), the changes in FFA concentration under orlistat treatment (increase of 1.4 μmol/mL) were minor in comparison to those seen with PL (0 μmol/L). Measurements of BAs in fecal water are expected to reflect an important aspect of safety because it is the soluble BAs, i.e. those in fecal water, that would be expected to interact with colonic epithelium. In an attempt to highlight the main pharmacodynamic effect on BAs in fecal water observed in this study, i.e. a decrease in DCA, the reviewer has assembled Table 4. This is a composite of sponsor's Tables 6 and 7. Since UDCA did not

19 mg/24h) with orlistat in comparison to PL (increase of 13 mg/24h) in the solid phase of the stool.

TABLE 4

Fecal Water Data

Changes in Total BAs and in the Two Main Families of BAs in Obese Subjects Taking a Hypocaloric Diet (1900 kcal/day; ca. 30% fat) and orlistat (120 mg t.i.d.) for 6 Weeks

Depicted are changes (Δ = Treatment-Baseline)

| PL | | ORLISTAT | | p-value for Δ ORLISTAT vs PL |
|---|---|---|---|---|
| I. CHANGES IN TOTAL BAs (µg/mL) | | | | |
| -11 | | -146 | | S[a] |
| II. CHANGES IN THE CHOLIC ACID FAMILY (µg/mL) | | | | |
| CA | DCA | CA | DCA | |
| -14 | 11 | -13 | -107 | S[b] |
| III. CHANGES IN THE CHENODEOXYCHOLIC ACID FAMILY (µg/mL) | | | | |
| CDCA | LCA | CDCA | LCA | |
| -7 | -3 | -8 | -7 | NS[c] |

a) Statistically significant differences also shown in the solid phase of the stool
b) Statistically significant differences also shown in the solid phase of the stool
c) Statistically significant differences shown in the solid phase of the stool



Possible explanations for these findings include:

1) **Decreased liver synthesis of BAs**

After all, the presence of fat in the diet is the best stimulus to circulating BAs. In addition, the rate limiting step in the biosynthesis of BAs is cholesterol. With orlistat, there is a decrease in cholesterol absorption (which may account for the observed hypocholesterolemia) mainly due to inhibition of the enzyme carboxyl ester hydrolase (CEH). This results in a decrease of hydrolysis of cholesterol esters (palmitate, oleate, linoleate and arachidonate) and less free cholesterol available for intestinal absorption. These considerations presuppose that, in order to explain the findings in the colon one needs to look into the effect (if any) of orlistat in the entero-porto-hepatic-systemic circulation of BAs. Although this was not done in the present trial, another study (Report N-138712/July 31, 1996), four weeks of treatment with orlistat (120 mg t.i.d. with meals) in addition to a hypocaloric diet, did not change cholesterol saturation of bile or gallbladder motility compared with PL and diet alone. In that study, orlistat-treated subjects had unchanged BA composition (total BA, CA, CDCA, LCA, DCA or UDCA). On the other hand, bile phospholipids, total BAs and the primary BA, cholate decreased in the PL group. Incidentally, study N-138712 also showed that orlistat and its primary metabolite M1 underwent biliary excretion. But it is worth clarifying that no EHC of the drug or metabolites is expected because, aside of the BAs, a molecule is yet to be described that undergoes EHC.

So, decreased liver synthesis of BAs does not appear to be the explanation for the observed decreased total BAs and DCA in the liquid (and solid) phase of the stool.

**2) Decrease in DCA due to colonic events**

BEST POSSIBLE

From the above it seems reasonable to assume that the decrease in DCA is a consequence of colonic events. Because DCA goes down and the synthesis of its precursor CA, is not going down relatively speaking (although the total BA is decreasing), one concludes that <u>there seems to be no reciprocal increase in CA</u>. What appears to be occurring is a decrease in the bacterial enzyme (the dehydroxylating enzyme) that normally converts the primary into secondary BAs in the colon. Whatever the mechanism, the decrease in DCA in fecal water is considered good, not bad from the safety viewpoint.

The finding of a significant decrease in DCA in both the liquid and the solid phase of the stool is important for a number of reasons. Regarding colon cancer, recent efforts focus on earlier case finding and developing methods of chemoprevention.[30] Many publications have reviewed the evidence for the existence of a relationship between microbial metabolism, diet and carcinogenesis. None of the evidence is direct or definitive but it is strongly suggestive. A large number of studies have shown that diet can alter the metabolism of the intestinal flora, and this change, in turn, affects the production of carcinogens and cocarcinogens. The mechanisms by which diet influences carcinogenesis are very complex. One area that has received considerable attention over the last two decades has been the possibility that BAs in stool play a contributory role in the development of colon neoplasms. A wide variety of lines of evidence have produced converging results that support this concept. Secondary BAs, particularly DCA, can act as promoters of colon cancer, are mutagenic, and adversely effect DNA as well as critical cell signal transduction pathways. Studies from Europe have shown that levels of DCA in blood are elevated in patients with colon cancer and adenomatous polyps compared to normals. It has recently been shown that BA induced colonic irritation stimulates intracolonic nitric oxide release in humans [F. Casellos et al., Gut <u>38</u>:719-723 (1996)]. Also, various epidemiological studies have shown that factors related to colon cancer, such as low fiber/low calcium diets (positive relationship with colon cancer) and high fiber/high calcium diets (negative relationship with colon cancer) specifically affect solubility of BAs in colon contents. An increase in dietary fiber and calcium reduces the solubility of DCA in fecal water, whereas low calcium/low fiber diets increase the solubility of DCA, making it more available to interact with colonic epithelial cells.

Reddy et al. has proposed a unifying mechanism that could explain a beneficial effect on cancer risk from change in dietary components. This is the reduction of concentration in stool of cytotoxic BAs which promote development of cancer [JNCI 56:441-442 (1976)]. In the colon, DCA induces mucosal damage manifest by disruption of intraepithelial cell tight junctions, enhanced mucosal permeability and anion secretion. DCA is also an effective promoter of experimental colon cancer. Reducing its concentration by dietary administration of calcium (precipitated DCA as insoluble salt) or fiber (binds DCA and reduces its solubility by causing more acidic stool) has been shown to reduce cancer formation in animal model of colon cancer. Although in these studies, other factors than just concentration of BAs in stool may be involved, a contributing effect of DCA to the process of cancer formation is widely supported. It has also been demonstrated that UDCA, a BA that appears to have therapeutic effect in protecting against formation of adenomas and colon cancer in persons determined to be at risk for this common neoplasm, may act through a decrease in DCA production. UDCA competitively inhibits the fecal bacterial enzyme (7α-dehydroxylase) responsible for dehydroxylation of CA to form DCA. Treatment with orlistat appears to have the same effect on DCA as that seen with UDCA.

BEST POSSIBLE

Incidentally, the changes (decreases) in DCA in fecal water observed in this study appear to be the action of orlistat and not of the hypocaloric diet. This is because the changes in DCA were not seen among PL-treated patients who received the same diet. Little is known about the fate of orlistat in the g.i. lumen and colon. As shown by Hartmann et al. [JBC 266:2021-2027 (1991)], orlistat binds covalently to the putative active site serine-152 of pancreatic lipase. This means that what is being offered to the colon is not free orlistat but orlistat bound covalently to serine (orlistat-serine) or to a small peptide (orlistat-serine-histidine; orlistat-SER-HIST-GLY, etc.). Such covalent binding would decrease even further the systemic bioavailability of this lipase inhibitor. Therefore, AEs beyond the g.i. tract are expected to be minimal after administration of orlistat.

Based on the findings in Res. Report W-144999 and the currently accepted theories on the role of BAs especially secondary BAs, in colon cancer, the consultant recommends that the sponsor consider doing a cancer prevention study in a standard rat model of colon cancer.

### B. Colonic Epithelial Cell Findings

In study W-144999, orlistat (120 mg t.i.d.) treatment of obese subjects for six weeks did not change the proliferative status of colonic epithelium. Proliferation was measured by three techniques: identification of crypt cells