THE EFFECTS OF PREGNANCY AND FEMALE SEX STEROIDS ON GALLBLADDER EMPTYING, BILIARY LIPID OUTPUT AND SMALL BOWEL TRANSIT TIME

by

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SUMMARY

THE EFFECTS OF PREGNANCY AND FEMALE SEX STEROIDS ON GALLBLADDER EMPTYING, BILIARY LIPID OUTPUT AND SMALL BOWEL TRANSIT TIME

In Western populations gallstones occur in approximately 10 percent of men and 20 percent of women by the age of 65. The majority of gallstones are predominantly composed of cholesterol. The mechanisms leading to cholesterol gallstone formation are poorly understood, but prequisites include supersaturation of biliary lipids with excess cholesterol, the presence of nucleating factors and the retention of precipated cholesterol crystals. The greater incidence of gallstones in women is probably related to hormonal factors.

Risk factors in women for gallstone formation include pregnancy and the ingestion of oral contraceptive steroids. The increased probability of gallstones correlates with the number of pregnancies and women who take oral contraceptive steroids or conjugated oestrogens double their risk of developing gallstones. The mechanisms by which pregnancy and oral contraceptive steroids increase the risk of cholesterol gallstones are poorly understood but several mechanisms which include increased biliary cholesterol secretion and retention of precipitated cholesterol crystals have been implicated. A factor contributing to biliary cholesterol saturation in pregnancy may be the observed decrease in the number of enterohepatic cycles during pregnancy. This observation could be caused by slow transit of bile acids through the small intestine, perhaps secondary to progesterone or other neurohormonal effects on small intestinal muscle. Female steroid hormones and pregnancy may also influence gallstone formation by altering the motility of the gallbladder.

The aims of this thesis were to (a) quantitate gallbladder volumes throughout the day in non-pregnant and pregnant subjects as well as in subjects taking oral contraceptive steroids or oestrogens alone, (b) assess the

influence of gallbladder volume and small intestine transit time on biliary lipid composition (c) study lipid composition of gallbladder bile in women taking oral conjugated oestrogens (d) assess orocaecal transit time in pregnancy and (e) examine the relationship between gastric emptying and gallbladder emptying and time to refilling.

Abdominal ultrasound was used to measure gallbladder volume throughout the day and night and during ingestion of standard meals in pregnant and postpartum women and oral contraceptive users. Results were compared with a control group who were studied in both the follicular and luteal phases of the menstrual cycle. Increases in gallbladder volume in pregnancy were correlated with serum progesterone. Evidence of altered gallbladder motility in pregnancy was found. The gallbladder of pregnancy was sluggish. Fasting volume, the residual volume after meals and the volume remaining in the gallbladder throughout the day doubled during pregnancy and these changes correlated with increases in serum progesterone. In contrast, emptying of the gallbladder was not altered by the phase of the ovulatory cycle or by the ingestion of oral contraceptive steroids. Gallbladder refilling in the day did not occur in normal subjects ingesting three standard meals per day.

The role of the enterohepatic circulation in biliary lipid secretion was studied as this may be an important mechanism by which altered motility of the gallbladder during pregnancy and the ingestion of contraceptive steroids predispose to cholesterol saturated bile and gallstone formation. The rate of biliary secretion was measured in human female volunteers during naso-gastric infusion of both weak and potent stimuli of gallbladder contractility (amino acids and fat respectively) and upper small intestinal bile was simultaneously collected. Changes in the enterohepatic circulation were monitored using abdominal ultrasound to quanitate gallbladder volume and breath hydrogen levels after administration of the non-absorbable carbohydrate lactulose to estimate small intestine transit time. Serum levels of

pancreatic polypeptide were measured during each test. Continuous intraduodenal infusion of a solution of amino acids that is known to maximally stimulate pancreatic secretion was less potent in stimulating contraction of the gallbladder than intraduodenal infusion of fat. Bile was relatively more saturated with cholesterol when the gallbladder contracted at a slower rate and the small intestine transit time was slow. The effects of Premarin (Ayerst), a mixture of conjugated oestrogens prepared from the urine of pregnant mares, on gallbladder emptying and biliary lipid secretion in postmenopausal women were studied using the techniques described above. No difference was found in biliary lipid secretion or gallbladder emptying on or off Premarin.

The lactulose breath test was used to measure orocaecal transit time throughout pregnancy and in the postpartum period. The results were compared with serum progesterone. Orocaecal transit time was delayed in late pregnancy and returned to normal postpartum. The pattern was similar to the pattern of the sluggish gallbladder of pregnancy suggesting a common neurohormonal mechanism.

To investigate if the rate of emptying of solids from the stomach controlled the rate of gallbladder emptying and time to refilling, the relationship between gallbladder and gastric emptying rates was studied in healthy volunteers. Following the ingestion of a standard radioactively labelled meal gastric emptying was measured scintigraphically while gallbladder volumes were monitored sonigraphically until gallbladder refilling occurred. The rate of gallbladder emptying in normal volunteers after a regular meal was dependent upon the rate of gastric emptying of the meal. Therefore, some of the delay in gallbladder emptying seen in late pregnancy could be due to delayed gastric emptying.

The findings of this study provide valuable information on normal biliary physiology and a plausible rationable for pregnancy as a gallstone risk factor by demonstrating the presence during gestation of prolonged periods

of gallbladder stasis. The aetiology of this stasis is unlikely to be related to high circulating oestrogen levels and is more likely due to progesterone effects.

Supporting evidence for the latter hypothesis comes from the observation of the lack of influence of exogenous oestrogens on gallbladder and biliary lipid kinetics. The data also suggests that contraceptive steroids are more likely to predispose to cholelithiasis by inducing changes in biliary lipid metabolism rather than changes in gallbladder function.

STATEMENT

This thesis contains no material which has been accepted for any other degree or diploma in any University and does not contain any material previously published or written by another person, except where due reference is made to such material in the text.

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LITERATURE REVIEW

A. GALLSTONE INCIDENCE

The incidence of gallstones is 10% in men and 20% in women by the age of 50 to 65 (Cleland 1953). In the USA gallstones are responsible for about 500,000 operations each year and approximately 8,000 deaths. The direct costs have been estimated to be more than two billion dollars (Kern 1975).

B. GALLSTONE CLASSIFICATION

Gallstones can be classified by the following three methods:

(i) Radiology

Stones can be classified as:

(a) Radiopaque - This feature accounts for 20% of stones and indicates a relative calcium content of greater than 4% by weight.

(b) Radiolucent - Accounts for the remaining 80% of stones indicating a relatively lower calcium content.

(ii) X-ray Diffraction of Stone Powders

Using x-ray powder diffraction, the crystalline constituents of gallstones removed from the gallbladder can be broadly classified as cholesterol and calcium salts (Sutor and Wooley 1973). On this basis there are three main types of stones:

(a) cholesterol (<5% calcium) 60%

(b) mixed stones 27%

(c) pure calcium 13%

By this method it was found that men are just as likely as women to form pure calcium stones but both calculi of cholesterol and those of cholesterol with calcium salts are more common in women.

(iii) Cholesterol vs Pigment

Gallstones can be classified from direct chemical analysis as cholesterol or pigment (Trotman, Petrella, Soloway et al 1975).

(a) Pigment

Understanding of the pathogenesis of pigment stones has not progressed very far. The lack of clear understanding of their chemical composition has retarded research progress. Although certain clinical associations are noted below most pigment stones in the Western world and in the Orient occur in their absence.

(b) Cholesterol

Factors thought to be important in the aetiology of cholesterol gallstones include the following:

1. Infection
2. Bile Stasis
3. Changes in the composition of hepatic bile

Originally it was thought that infection of the gallbladder wall could allow bile salts to be passively absorbed leaving relatively more

CHOLESTEROL VS PIGMENT STONES

	CHOLESTEROL	PIGMENT
Incidence	70-90%	10-30%
Morphology	Multiple, 2-30mm in diameter, smooth or faceted, laminated or crystalline on cross section	Multiple, 2-5mm, irregular or smooth, black or brown, amorphous or crystalline. Black - confined to gallbladder. Bilirubin polymer. Brown - found in common bile duct. Calcium bilirubin.
Composition	60-90% Cholesterols - cholesterol, monohydrate, anhydrous cholesterol and cholesterol II - account for 71% of the total crystalline material in gallstones (Sutor and Wooley, 1971). Other - pigment, precipitated bile acids, mucoproteins, other proteins or calcium salts	Bilirubin 35% (range 10-50) Bile acids 2 Cholesterol 2 Calcium 9 Carbonate 7 Also heavy metals, proteins, sulphates, magnesium salts, calcium soaps, polypymole polymers and mucin.
Clinical features	Female predominance, obesity, female sex steroids, conditions reducing bile salt pool	Associated with cirrhosis, chronic hemolytic anaemia - black type, stasis and infections (ascaris, clonorchis, typhoid) - brown type

cholesterol in the gallbladder to nucleate and form cholesterol gallstones but this now seems unlikely. In addition to the role of infection in providing potential nucleating agents it is likely that infection and inflammation of the gallbladder allow precipitation of calcium salts to initiate or accelerate gallstone formation.It is now generally accepted that gallstones may form without infection and biliary lipid research has concerned itself with the latter two aspects.

C BILIARY LIPID BIOCHEMISTRY IN CHOLESTEROL GALLSTONE DISEASE

Since 1968 progress has been made in understanding the pathogenesis of cholesterol gallstones but many uncertainties remain. The clarification of the physical state of the lipids in bile in the mid 60's and the demonstration of the importance of the relative molar percentage of each lipid were key findings (Admirand and Small 1968).

Gallstones are formed from organic components of bile.The major organic components of bile are bile acids, cholesterol and phospholipid.Bile salts are formed in the liver from cholesterol. The rate limiting reaction in this sequence is the 7-alpha hydroxylation of cholesterol (Mendelsohn, Mendelsohn, Staple 1965). Bile salt synthesis may be regulated by a negative feedback system such that bile salts returning to the liver inhibit 7-alpha hydroxylase, and the incorporation of acetate and mevalonate into cholesterol (Shefer, Hauser, Bekersky et al 1970). Removal of these inhibitions may increase bile salt synthesis several fold. Both cholesterol and phospholipid are insoluble in water, but they are soluble in bile because it is a micellar solution. Phospholipid (lecithin) has a hydrophobic and a hydrophilic portion similar to bile acid and is present in bile in high concentrations (350-600mg/100ml) (Figure 1). It greatly expands the size of bile acid micelles and increases their capacity to

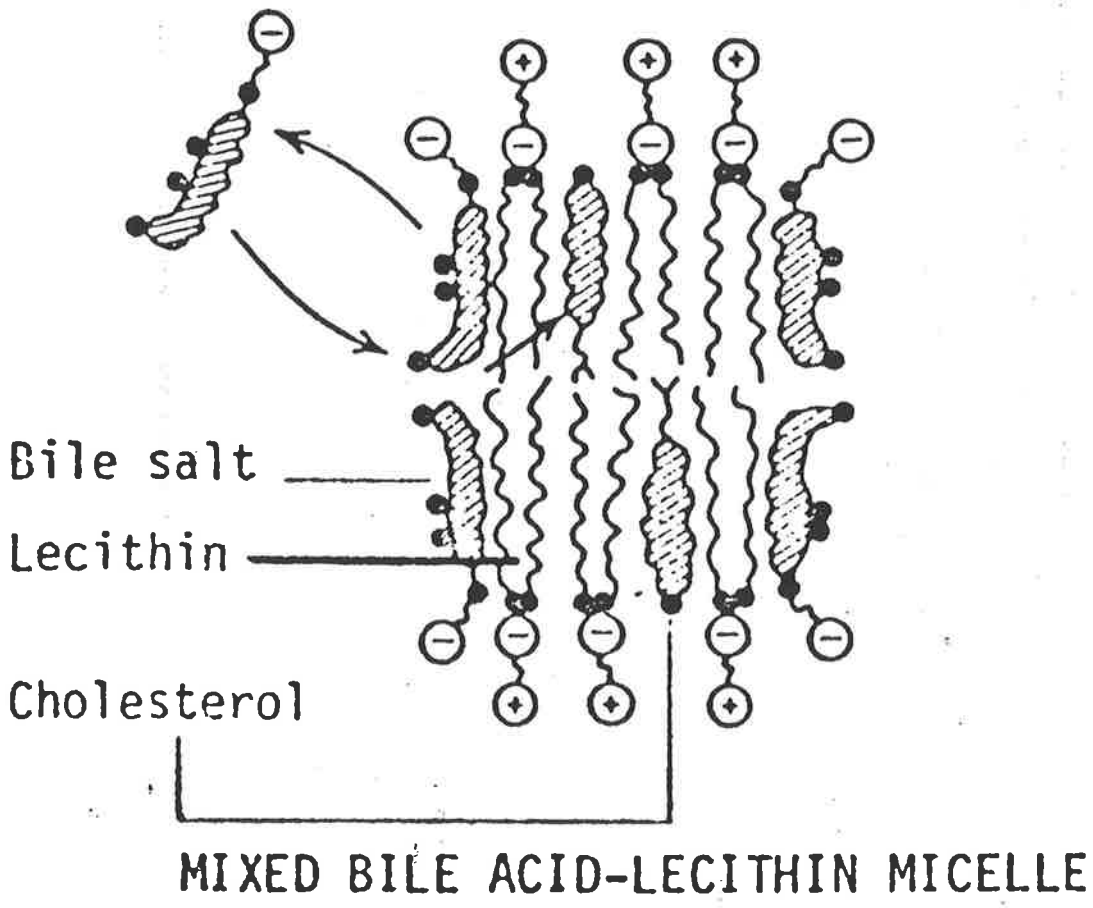

FIGURE 1

Diagram showing the accepted structure of bile salt-lecithin (phospholipid) micelles.

solubilise cholesterol. These lipids are present normally in the molar ratios of bile salts 75: phospholipid 20: cholesterol 5. When the cholesterol percentage is increased to greater than 10%, either because of increased cholesterol secretion or decreased bile acid secretion, cholesterol may precipitate in the gallbladder and initiate stone formation. It is now recognised that cholesterol gallstones will form only when bile is supersaturated with cholesterol, as it contains more cholesterol in a single phase solution than can be solubilized in the bile salt - phospholipid micelles present. The water insoluble cholesterol is maintained in solution with bile salts, providing a sufficient concentration of the swelling amphipath. Phospholipid is present to increase the size of the mixed micelles. Excess phospholipid disrupts the micellar structure. The inter-relationship of the concentrations of these three lipids is most clearly demonstrated using the triangular co-ordinates described by Admirand and Small (1968). This representation assumes that the water content is fixed at 90% by weight. A zone of concentrations for these three lipids in which a liquid micellar phase is maintained can be demonstrated (Figure 2). However, there has been considerable disagreement as to the exact definition of the line of supersaturation and its significance (Metzger, Heymsfield, Grundy 1972).

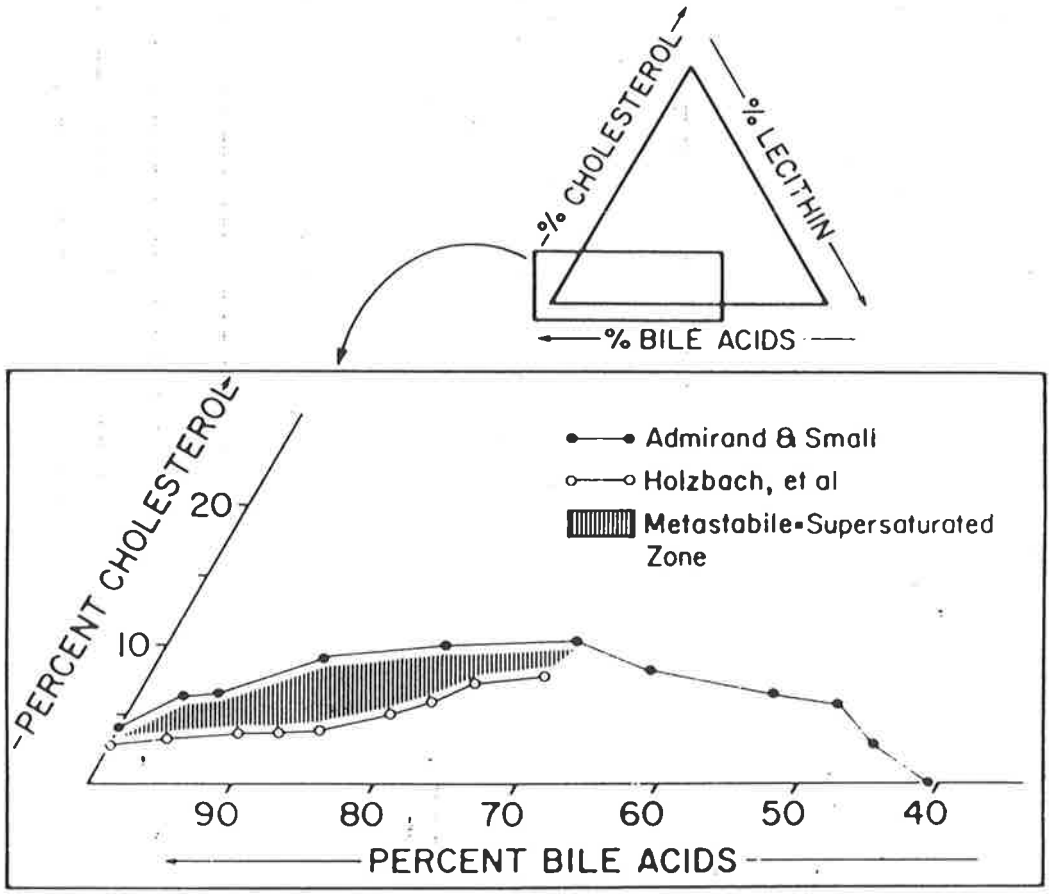

FIGURE 2:

The relative molar proportion of biliary lipids plotted on triangular co-ordinates. The axes represent the percentage of the total moles of bile acids, lecithin (phospholipids) and cholesterol constituted by each of these components which total 100 percent. The limits of cholesterol solubility are shown as defined by Admirand and Small (1968) and Holzbach, Marsh, Olszewski et al (1973) with the metastable supersaturated zone between them.

Admirand and Small (1968) focussed attention on the liver as the site of active bile secretion and biliary lipid production. They hypothesized that a difference in the ability to solubilize cholesterol must exist between human control biles and those from cholesterol gallstone patients and that cholesterol insolubility probably represented the first step in gallstone formation. They and subsequently others (Metzger, Adler, Heymsfield et al, 1973) found that in patients with cholesterol gallstones, bile secreted by the liver is supersaturated with cholesterol. In the same patients, bile stored in the gallbladder is 100% saturated with cholesterol in micellar solution and also contains cholesterol crystals. They postulated that during storage in the gallbladder the supersaturated bile was "seeded" or nucleated by precipitated, bile pigment or other material, and that cholesterol crystals precipitated from solution and initiated gallstone formation.

Many investigators have found supersaturated or lithogenic bile in gallstone patients and have identified the basis for the abnormal biliary lipid composition in several groups of patients who are at high risk of developing stones. For example, obese subjects secrete excessive biliary cholesterol (Bennion and Grundy 1975, Grundy, Duane, Adler et al 1974) and patients with ileal resection, disease or bypass secrete reduced amounts of bile salts (Cohen 1971). In both disorders the relative molar percent cholesterol in bile is increased and the incidence of gallstones is high.

Vlahcevic and co-workers (Vlahcevic, Bell, Buhac et al 1970; Swell, Bell, Vlahcevic 1971) were the first to report a small bile salt pool size in patients with cholesterol gallstones and to implicate this small pool size

in the pathogenesis of stones. They and others (Mok, Von Bergmann, Grundy 1977) hypothesized that the bile salt pool size was small because of an overly active negative feedback regulation of bile salt synthesis. This presumed defect in regulation, thought by some to be genetic, was proposed primarily because other mechanisms causing a small bile salt pool size could not be demonstrated. Specifically, the patients did not lose excess bile salts in the stool and they had normal hepatic synthetic function.

A number of other observations of biliary lipid composition and bile salt pool size cast some doubt upon the importance of the hypothesis just described in the pathogenesis of stones.

The observations are;-

1. Both hepatic bile secreted in the fasting state and bile stored overnight in the gallbladder, can be supersaturated with cholesterol in normal subjects without gallstones (Metzger, Adler, Heymsfield et al 1973).The large overlap in biliary lipid composition between gallstone patients and controls suggests that supersaturated bile alone is not sufficient for gallstone formation.

2. Small bile salt pool sizes are also found in many subjects without gallstones. Indeed, some investigators find no difference in mean pool size between control and gallstone patients (Northfield and Hofmann 1975).

These findings raise an important question.What factors in addition to supersaturated bile are necessary for gallstone formation?.

D.THE ENTEROHEPATIC CIRCULATION

(i) The Role of the Gallbladder and Small Intestine:

The enterohepatic circulation (EHC) of bile occurs via the liver, biliary tree, gallbladder, jejunum, ileum and portal vein. The secretion of cholesterol and lecithin into bile is dependent upon bile acid secretion which is in turn dependent on an intact EHC.

In healthy individuals, ileal bile acid absorption from the intestinal lumen (Krag and Phillips 1974) and hepatic bile acid extracton from portal blood are highly efficient (Reichen and Paumgartner 1980). Because bile acid pool size is constant in the steady state and no bile is stored in the liver, the rate of hepatic bile acid secretion is directly proportional to the frequency of enterohepatic cycling, which is largely determined by the rate of delivery of bile acid from the extrahepatic biliary tree to the terminal ileum. The delivery of bile acid to the terminal ileum in its turn is determined by two mechanical pumps, the gallbladder and small intestine. The gallbladder, in its reservoir capacity, determines the delivery of bile acid to the intestinal lumen. Bile acid, the major component of biliary secretion, is required for fat digestion and absorption through the formation of micelles. During fasting about 50% or more of the bile acid pool is stored in the gallbladder and bile acid secretion into the intestine is relatively low (Metzger, Adler, Heysmfield et al 1973; Northfield and Hofmann 1975; Mok, Von Bergmann, Grundy 1980). During feeding the gallbladder contracts and bile acid secretion into the intestine increases in relation to the degree of gallbladder emptying, which,in turn, is primarily a function of the content of the meal (Rock, Malmud, Fisher 1981; Ladas, Isaacs, Murphy et al 1984). High fat meals

induce maximum gallbladder contraction. To integrate and optimise the process of digestion, gastric emptying of solids should regulate gallbladder contraction through the release of humoral mediators from upper small bowel and pancreas. Increased storage of bile acid in the gallbladder or slowed small intestinal transit could slow the delivery of bile acid to the terminal ileum and lower the bile acid secretion rate. Biliary bile acid secretion rate is lower when stimulated by intermittent feeding (Mok, Von Bergmann, Grundy 1979; Northfield and Hofmann 1975; LaRusso, Szcepanik, Hofmann 1977; Mok, Von Bergmann, Grundy 1980; La Russo, Hoffman, Hofmann et al 1975) than by continuous intraduodenal infusion (Grundy and Metzger 1972; Mok, Von Bergmann, Grundy 1979,Von Bergmann, Mott, Howard 1980, Grundy, Von Bergmann, Grundy 1979, Grundy, Duane, Adler et al 1974; Bennion and Grundy 1975; Mabee, Meyer, Den Besten et al 1976, Valdivieso, Palma, Nervi et al 1979, Mok, Von Bergmann, Grundy 1978) of a standard liquid formula. Because bile contains relatively more cholesterol when bile acid secretion rate is low, the lower rates of bile acid secretion during gallbladder storage and slowed intestinal transit could be expected to increase cholesterol saturation of bile.

The importance of the gallbladder and small intestinal transit in regulating the enterohepatic cycle and pool size is suggested by several observations:-

1. Some (Shaffer and Small 1977, Redinger 1976) but not all (Adler, Metzger, Grundy 1974; Shaffer,Braasch, Small 1972), investigators have noted a decrease in pool size after cholecystectomy, attributed to nearly continuous cycling of bile acids and nearly continuous inhibition of hepatic bile acid synthesis. These studies are difficult to interpret because the storage function of the gallbladder before surgery was usually not known.

2. In the dog (Parkin, Smith, Johnston 1973) and in man (Inberg and Vuorio 1969; Faberberg, Grevsten, Johansson et al 1970) truncal vagotomy was followed by increase in gallbladder volume and increase in bile acid pool size. Gallbladder motor response to standard stimuli seemed unimpaired by vagotomy but Inberg and Vuorio (1969) noted a larger residual volume in the gallbladder after a fatty meal. Stempel and Duane (1978) more recently found that the pool size of both primary bile acids, (cholic acid and chenodeoxycholic acid) increased after vagotomy in eight male patients. They also noted a decrease in cholesterol saturation of bile after vagotomy and a significant inverse correlation between pool size and change in molar percent cholesterol. Their findings suggested that vagotomy does not predispose to cholesterol gallstones, but others have proposed that retention and stratification of bile in the gallbladder results in certain layers having a disproportional percentage of cholesterol (Thurebon 1966; Nakayama and Van Der Linden 1975) leading to nidus formation, precipitation of cholesterol and an increased incidence of gallstones.

3. In patients with coeliac disease Low-Beer, Heaton, Heaton et al (1971) described enlarged gallbladders and decreased gallbladder contractility after a fatty meal, with decreased bile acid turnover and increased bile acid pool size. The changes were attributed to diminished CCK release by damaged small intestinal mucosa and reduced stimulation of the gallbladder.

4. Hepner (1975) reduced gallbladder emptying in healthy subjects by feeding a 95% carbohydrate diet for several days and then studied bile acid kinetics. He found a similar increase in pool size of both cholic acid and chenodeoxycholic acid and a decrease in the secondary bile acid deoxycholic acid pool size. The traditional turnover rate of both primary bile acids decreased slightly but significantly during the experimental diet. The

synthesis rates were unchanged. A similar diet in cholecystectomised patients had no effect on bile acid kinetics.

5. Duane and Hanson (1978) used indocyanine green as a marker of gallbladder content and related its rate of emptying after a standard meal to bile acid kinetics. They found significant negative correlations between rates of gallbladder emptying and pool sizes of the primary bile acids plus significant positive correlations between the traditional turnover rate of each bile acid and gallbladder emptying rates. Gallbladder emptying was not significantly related to synthesis rates of either bile acid but synthesis rates did correlate positively with intestinal transit time measured by the breath hydrogen method (Bond and Levitt 1975; Mok, Von Bergmann, Grundy 1977). They also found an inverse correlation between pool size and cycling frequency.

6.Recent studies in man (Mok, Von Bergmann, Grundy 1980) indicate that considerable amounts of bile secreted at night by pass the gallbladder contrary to earlier belief. Similar conclusions had been reached by Stanley (1970) on the basis of his studies on the faecal excretion of bile acids in obese subjects undergoing a prolonged fast and by Small et al (Small, Dowling, Redinger 1972) in their primate studies. Von Bergmann et al (Von Bergmann, Mok, Grundy 1976) described the use of bilirubin output after stimulation of the gallbladder to estimate gallbladder storage capacity and reported 37-86% of the bile acid pool in the gallbladder after an overnight fast.

7.Small intestinal transit is an important determinant of bile acid cycling frequency (Einarsson, Grundy, Hardison 1979) and thus can alter the rate of biliary lipid secretion (Valdivieso, Palma, Nervi et al 1979; Mok, Von Bergmann, Grundy 1977). Faster small intestinal transit increases bile acid

cycling frequency and the rate of biliary lipid secretion. Slow small intestinal transit would slow delivery of bile acid to the terminal ileum and lower bile acid secretion rate. Because bile contains more cholesterol when bile acid secretion rate is low (Northfield and Hofmann 1975; Lindblad, Lundholm, Schersten 1977; Wagner, Trotman, Soloway 1976, Nilsson and Schersten 1969; Metzger, Adler, Heymsfield et al 1973), the lower rate of bile acid secretion during slow intestinal transit would be expected to increase cholesterol saturation of bile. Small intestinal transit time is related to the type of stimulus infused into the bowel lumen. Ruckebush and Fioramonti (1975) observed that different types of equicaloric test meals produced different durations of disruptions of myoelectrical activity in the rat small intestine. Longer lasting digestive activity was noted with oleic acid compared with amino acids or glucose. De Weaver, Eeckhout, Vantrappen et al (1978) showed in the dog that the duration of disruption of the small intestine migrating myoelectric complex after a meal depends much more on the physicochemical composition of the food that on its volume or amount of calories. Therefore the physiochemical composition of food may regulate small bowel motor activity and in turn biliary lipid secretion.

Thus an abnormality of motor function of the gallbladder could result in a decrease in the number of enterohepatic circulations causing a decrease in bile salt secretion rates leaving relatively greater amounts of cholesterol in bile increasing the chance of crystal and stone formation. During pregnancy the total bile acid pool is increased (Kern, Everson, De Mark et al 1981). Pooling of bile acids within the small intestine and gallbladder could contribute to an expansion of the total bile acid pool.

During pregnancy, total bile acid pool size and gallbladder volume are increased (Kern, Everson, De Mark et al 1981; Braverman, Johnson, Kern

1980; Ylostalo, Kirkinen, Heikkinen 1982). In addition, the size of the bile acid pool directly correlates with the increases in gallbladder volume (Kern , Everson, De Mark et al 1981). It is likely that a hormone, probably progesterone, inhibits smooth muscle contraction, and induces the increase in gallbladder volume. Subsequently bile acids are sequestered in the gallbladder, slowing their enterohepatic circulation, secondarily stimulating bile acid synthesis and expanding the bile acid pool to a new steady state level. However total bile acid pool size is significantly increased in the first trimester at a time when gallbladder volume is not significantly increased. Thus in early pregnancy, the increased bile acid pool is either an effect of one or more hormonal or metabolic changes in bile acid synthesis or bile acid pool sequestration is occurring in another site.

The main non-gallbladder site within the enterohepatic circulation where bile acid sequestration can occur is the small intestine. Administration of propantheline bromide to healthy subjects results in prolonged gastrointestinal transit time and expansion of bile acid pool size (Duane and Bond 1980). In addition, orocaecal transit is reversibly prolonged in both the third trimester of pregnancy (Wald, Van Thiel, Hoechstetter et al 1982) and in the luteal phase of the ovulatory cycle (Wald, Van Thiel,Hoechstetter et al 1981). Thus it is possible that gastrointestinal transit is prolonged in early pregnancy and therefore contributes to the increased bile acid pool by sequestering bile acids within the intestinal lumen.

(ii)Interruption of the Enterohepatic Cycle - Effect on Biliary Lipids

Thurebon (1962) by careful experiments on patients with occludable common duct T-tubes demonstrated that interruption of the EHC for one to three hours produced a decrease in lecithin and bile salt concentration. However cholesterol secretion by the liver was unchanged causing the formation of bile saturated with cholesterol. Refeeding of bile collected during the interruption returned cholesterol saturation of bile to normal. Thurebon concluded that later in the storage cycle, the gallbladder fills with hepatic bile that has changed its composition due to natural interruption of the EHC creating favourable conditions for layer formation, stratification and cholesterol gallstone formation. Possible causes for this interruption could include dietary factors (e.g. predominance of carbohydrates) or prolonged periods of fasting. Other observers have shown that interruption of the EHC by fasting (causing sequestering of the bile acid pool in the gallbladder) increases the cholesterol saturation of bile (Metzger, Adler, Heysmfield et al 1973; Mok, Von Bergmann, Grundy 1978)

E. THE ROLE OF THE GALLBLADDER IN CHOLESTEROL GALLSTONE FORMATION

A stasis theory for gallstone formation was first suggested by Meckel von Helmsbach (1865). The idea that incomplete gallbladder emptying contributed to cholesterol gallstone formation was supported by Montgomery (1866) and Ord (1879). They showed that cholesterol stones could form from spheroliths which resulted from layering of liquids of different density in the gallbladder. Steinman (1889) and Rous,McMaster and Drury (1924) felt that nucleation or nidus formation by bile pigment spheroliths was encouraged by gallbladder stasis, producing laminated gallstones. They also suggested that impaired gallbladder emptying allowed stratification within

the gallbladder of solids of differing specific gravity, thus favouring the aggregation and subsequent additional layering of stones in a milieu containing proteinaceous material or layers of bile. Poor gallbladder emptying or biliary lipid mixing could prevent the natural passage of early minute stones into the duodenum (Rains 1962).

In recent years however, most emphasis has been on biliary lipid composition and there have been few studies of the gallbladder in relation to the pathogenesis of stones. Nevertheless, it is apparent that the gallbladder must be involved in the formation of gallstones for several reasons:

(a) Cholesterol stones are rare after cholecystectomy

(b) Precipitation of cholesterol crystals from a supersaturated solution requires time (Holan, Holzbach, Hermann et al 1979). Bile retention in the gallbladder must be sufficiently prolonged not only for crystal formation but for crystal agglomeration and stone formation.

(c) If gallbladder contraction is complete, lithogenic bile and cholesterol crystals, if present, will be expelled into the duodenum where they are harmless. Stasis of bile in the gallbladder, because of ineffective or incomplete contraction, might be the additional necessary condition for gallstone formation.

F. FEMALE SEX STEROIDS AND SMOOTH MUSCLE MOTILITY

(i) Non-Biliary Smooth Muscle

There is good evidence that the ovarian hormones, oestrogen and progesterone, can affect the motor activity of extra-uterine smooth muscle. Van Wagenan and Jenkins (1939) demonstrated increased dilatation of the

ureters of monkeys in the immediate post partum period. Hundley, Diehl and Diggs (cited Kumar 1962) showed decreased ureteric peristaltic activity in non-pregnant women given progesterones. Activity increased with oestrogens. Similar results were found with in vivo intestinal motility studies by Tsutsulopulos (cited by Yoshida and Mori 1969). Twenty years later Kumar (1962) demonstrated that progesterone in vitro had a marked inhibitory effect on the spontaneous contractility of ureter, large bowel and stomach. Schatzman (1961) studied the guinea pig ileum and concluded that a decrease in muscle tone occurred with the administration of progesterone or oestradiol. The previously conflicting results with oestrogens were probably related to the high concentrations used. Yoshida and Mori (1969) found two different levels of oestrogen effects on in vitro intestinal motility. With lower concentrations there was an excitatory effect and with higher concentrations an inhibitory effect. Nagler and Spiro (1961) studied oesophageal motility by manometry in controls and pregnant women. Non-propulsive motor activity was seen more consistently in pregnant subjects. This finding led the way for further investigations of the effects of sex steroids on gastrointestinal smooth muscle in human subjects.

(ii) Gallbladder and Small Intestinal Motility

There is evidence that contraceptive steroids increase biliary cholesterol secretion in women (Bennion, Mott, Howard 1980; Bennion, Grinzberg, Garrick et al 1976; Pertsemilidis,Paneveliwalla, Ahrens 1974),but information on the affect of female sex hormones and pregnancy on gallbladder function is scant. In separate cholecystographic studies Gerdes and Boyden (1938) and Potter (1936) suggested that impaired gallbladder emptying and concentrating ability occur late in pregnancy. Nilsson and Stattin (1967) also used oral cholecystography to measure gallbladder

emptying after bolus injection of cholecystokinin (CCK) in both phases of the menstrual cycle in each of 10 subjects. They found slower emptying in the luteal phase in eight of them. However the phase of the cycle was not accurately documented by serum progesterone levels.

Pregnancy and female sex steroid intake are major risk factors for cholesterol cholelithiasis (Bennion and Grundy 1978; Kern, Erfling, Simon et al 1978; Friedman, Kannel, Dawber 1966). In the pathogenic sequence of cholesterol cholelithiasis proposed by Small (Small 1980; Small 1974; Shaffer and Small 1976) a prerequisite for cholesterol gallstone formation is the hepatic secretion of lithogenic bile. In addition major physiochemical changes, including nucleation (Holan, Holzbach, Hermann et al 1979), crystal formation (Sefaghat and Grundy 1980; Walton 1967), and adherance of crystal must occur in the gallbladder to generate cholesterol gallstones. Because these changes require time, retention of lithogenic bile in the gallbladder is necessary for these events to occur. If gallbladder emptying were prompt and complete, lithogenic bile and any cholesterol crystals that may have formed would pass uneventfully into the duodenum, preventing the sequence leading to gallstones. Previous studies in pregnant women have suggested that the volume of the gallbladder is increased and its contraction is sluggish (Braverman, Johnson, Kern 1980; Potter 1938; Gerdes and Boyden 1938). Gallbladder emptying in the luteal phase may be impaired (Nilsson and Stattin 1967) but women taking oral contraceptive steroids seem to have normal gallbladder volumes and emptying after a liquid meal (Braverman, Johnson, Kern 1980). However emptying during continuous intraduodenal amino acid infusion is slower (Kern, Everson, De Mark et al 1982). The latter finding implies that gallbladder contractility might be impaired in women taking contraceptive steroids.

Progesterone and oestrogen levels increase progressively in pregnancy. It

appears that progesterone rather than oestrogen causes the inhibition of gallbladder emptying and the delay in orocaecal transit time is noted in the third trimester of pregnancy (Wald, Van Thiel, Hoechstetter et al 1982). Studies in experimental animals suggest that oestrogens actually enhance smooth muscle activity. Ryan and Pellecchia (1982) showed that oestrogen pretreatment in the in vitro male guinea pig gallbladder significantly decreased the threshold acetycholine requirements for contraction. Progesterone had the opposite effect. Datta, Hey and Pleuvry (1974) found that oestradiol pretreatment causes a dose-dependent increase in intestinal propulsion when administered to oopherectomised mice. A mixture of progesterone and oestradiol had no significant effect. There are no studies in man to confirm or refute these experimental findings of gallbladder and intestinal smooth muscle excitation by oestrogen.

G. FEMALE SEX STEROIDS AND BILIARY LIPID SECRETION

Bile of women taking birth control pills has often been shown to be considerably more saturated with cholesterol than that of other women (Bennion, Mott, Howard 1980; Kern, Everson, De Mark et al 1982). It has been established that the higher the cholesterol saturation among patients without gallstones, the greater the prevalence in the population from which the patients were chosen (Redinger and Small 1972). For instance the Masai of East Africa and the Japanese have a very low cholesterol saturation and as a consequence a low prevalence of cholesterol gallstones, whereas young North American Indian Women have a high prevalence of supersaturated bile (Thistle and Shoenfield, 1971) and an exceptionally high prevalence of gallstones(Sampliner, Bennett, Comess 1970). Thus, the higher the cholesterol saturation, the greater the risk of gallstones.

According to some epidemiological studies, women on birth control pills (Boston Collaborative Drug Surveillance Programme 1973) and postmenopausal women (Boston Collaborative Drug Surveillance Programme 1974) on oestrogens have a risk of surgically documented gallstone disease that is 2.0 to 2.5 times that of control groups. However more recently the Royal College of General Practitioners' oral contraceptive study (1982) suggested

that the use of oral contraceptives confers no increased risk but simply accelerates gallbladder disease only in women susceptible to it. In addition, this study suggested that the acceleration may be associated with the dose of oestrogen in the combined oral contraceptives. These latter new observations may be due to current "low dose" oestrogen contraceptives. "High dose" contraceptives are prescribed only when the lower dose oestrogen contraceptives prove inadequate. Many of the physiological changes in biliary lipids observed with high dose oestrogen preparations may not occur with lower doses. In addition most studies performed in different phases of the menstrual cycle have failed to show significant differences in biliary lipid output and in particular, the cholesterol saturation index (Kern, Everson, De Mark et al 1981, Williams, Scallion, McCarthy 1980; Whiting, Down, Watts 1981).

A few studies have examined the effects of oestrogens on biliary lipids in humans.

Oestrogens have several effects on hepatic biliary lipid metabolism which result in the formation of lithogenic bile. These effects include the following:-

1. Women taking oral contraceptive steroids have an increase in cholesterol saturation index and the proportion of women with supersaturated bile is increased (Bennion, Ginsberg, Garrick et al 1976; White, Howat, Schoefield 1976; Pertsemilidis, Panveliwalla, Ahrens 1974). The mechanism is probably by hepatic enhancement of cholesterol secretion in bile (Bennion, Mott, Howard 1980).

2. An increase in molar percentage cholesterol in T-tube bile from three women taking Premarin and Provera (conjugated equine oestrogens ;

medroxyprogesterone acetate) along with a decrease in cholicacid synthesis and pool size (Pertsemilidis, Panveliwalla, Ahrens 1974).

3. Reduced bile acid synthesis and secretion in rats (Davis and Kern 1976; Davis and Kern 1977; Stramentinoli, DiPadova, Jualano et al 1981) and hamsters (Bonnorris, Coyne, Chung et al 1977) so that bile becomes supersaturated

4. In hamsters the rate limiting enzyme 7-alpha-hydroxylase which converts cholesterol to bile acids is reduced by ethinyl oestradiol (Bonnorris, Coyne, Chung et al 1977).

5. The physical characteristics of the lipid composition of the hepatic microsomal membrane, the site of cholesterol conversion to bile acids is altered in the rat by ethinyl oestradiol (Davis and Kern 1977).

Suggestive evidence of an oestrogen effect comes from the following observations:-

1. Bennion, Drobny, Knowler et al. (1978) found a smaller total bile acid pool and a smaller cholic acid pool in Indian and white women compared with men. Biliary cholesterol saturation was inversely correlated with chenodeoxycholic acid pool size.

2. Bennion, Knowler, Mott et al. (1979) studied biliary lipids and bile

acid kinetics in young Pima Indian boys and girls before, during and after puberty. Prior to puberty the bile acid pool size was the same in both sexes. It became larger in males but not females at the time of puberty. Biliary lipid saturation increased in both sexes especially females at puberty and correlated positively with urinary oestrogen excretion.

3. Bennion (1977) studied one young woman before and after bilateral oophorectomy. After operation the bile acid pool size doubled without change in distribution of bile acids and the molar percent cholesterol in the bile decreased.

4. In male volunteers a seven day course of a 30ug oral dose of ethinyl oestradiol caused a significant rise in the cholesterol saturation index of gallbladder bile (Anderson, James, McDonald et al 1980). However more recently, Down, Whiting, Watts et al (1983) showed that the daily ingestion of 30ug ethinyloestradiol alone in women had no significant effect on lithogenic index. The reason for this sex difference in unclear, but it may be related to the 50% fall in the levels of circulating testosterone which also accompanied the use of ethinyl oestradiol in men.

Criticism has been directed at the animal studies because of the large doses of oestrogens required to produce biliary lipid changes. Also physiological changes in natural sex hormones during a normal menstrual cycle have been shown to have little (Low-Beer, Wicks, Heaton et al 1977) or no effect (Bennion, Grinzberg, Garrick et al 1981; Whiting, Down, Watts

1981, Kern, Everson, DeMark et al 1981; Williams, Scallion, McCarthy 1980).

With a variety of synthetic oestrogen/progestin mixtures Bennion, Mott and Howard (1980) showed that contraceptive steroid use could increase both the molar percent cholesterol in human female gallbladder bile and hepatic secretion of cholesterol. Kern, Everson, De Mark et al (1982) showed the same changes in healthy young women taking a contraceptive steroid combination of 50ug of mestranol and 1mg of norethnidrone.

Recently Down, Whiting, Watts et al (1983) found evidence in healthy young women that the progestagen, norgestrel and not the oestrogen, ethinyl oestradiol is responsible for the increase in bile cholesterol saturation which accompanies the use of oral contraceptives. They suggested that the overall oestrogen to progestagen potency ratio of an oral contraceptive may be more important in determining its effect on the biliary cholesterol saturation index than the dose of each sex hormone.

The origin of the increased cholesterol secreted into bile is unknown, but there are five main possibilities:-

(i) increased dietary intake

(ii) enhanced absorption

(iii) increased synthesis

(iv) increased hepatic uptake of lipoprotein cholesterol

(v) decreased breakdown of cholesterol to bile acids

The first two possibilities seem unlikely. In rats the rate of cholesterol synthesis is greater in females than in males. (Mukherjee and Gupta 1967; Carlson, Mitchell, Goldfarb 1978). Pregnancy markedly stimulates hepatic cholesterol synthesis in the rat and the saguines fusciollis monkey (Feingold, Wiley, Moser et al 1983). Oestrogen administration stimulates the activity of hepatic 3-hydroxy 3-methylglutaryl coenzyme A reductase, the rate limiting enzyme in cholesterol synthesis. However, Turley and Dietschy (1979) have clearly shown in rats that the rate of biliary cholesterol secretion is independent of the rate of hepatic cholesterol synthesis. Other studies in rats show that a large dose of ethinyl oestradiol (5mg/kg/day) increases the specific binding of low density lipoprotein (LPL) by rat liver membrane (Kovanen, Brown, Goldstein 1979) and greatly augments the uptake and catabolism of plasma LDL (Chao, Windler, Chen et al 1979). Those findings may be relevant to the observation that ethinyl oestradiol (150ug/day) increases the turnover rate of serum cholesterol in human subjects (Nestel, Hirsch, Couzens 1965) and to studies suggesting that plasma lipoproteins serve as the major source of biliary cholesterol (Schwartz, Halloran, Vlahcevic et al 1978; Schwartz, Berman, Vlahcevic et al 1978). It seems possible therefore that the excess biliary cholesterol secreted by users of contraceptive steroids could be derived from accelerated hepatic uptake and catabolism of plasma LDL cholesterol, but direct study of this problem is needed.

The relation between serum lipids and lipoproteins and biliary lipid and bile acid composition and secretion has been studied.Patients with type IV hyperlipidaemia appear to have an increased incidence of cholesterol gallstones (Kadziolka,Nilsson, Schersten 1977), Einarsson and co-workers have described an increase in bile acid synthesis and pool size, especially cholic acid (Einarsson, Hellstrom, Kallner 1974) increased hepatic HMG CoA reductase activity (Angelin, Einarsson, Hellstrom 1976) and increased cholesterol saturation of bile. Pregnancy (Williams, Simons, Turtle 1976) and oral contraceptive steroids (Odell and Molitch 1974) are associated with similar changes in serum lipids - an increase in triglyceride concentration, a slight variable increase in serum cholesterol and an increase in very low density lipoproteins. Elevated high density lipoprotein (HDL) levels have been associated with a lower incidence of gallstone disease (Scragg, McMichael, Seamark 1984). The effect of contraceptive steroids on HDL levels varies with their composition, oestrogens causing an increase and progestagen a decrease (Bradley, Wingerd, Petitti et al 1978). Schwartz, Berman, Vlahcevic et al (1978) reported that HDL cholesterol is the major precursor for biliary cholesterol and bile acid synthesis, but this issue cannot be regarded as completely settled. Furthermore the role of lipoprotein remnants and the rate of metabolism of serum lipids in biliary lipid synthesis and secretion have not been adequately studied.

The possibility of altered gallbladder function in response to oestrogens has been incompletely studied. In addition effects of oestrogens upon gallbladder storage capacity, concentrating ability and contractility have not been well characterised.

The gallbladder has enormous concentrating capacity (Makhlouf 1979) which has not been studied in intact man except by cholecystography. Concentration of bile is dependant upon sodium and water absorption, probably mediated

by the putative sodium pump Na+, K+ ATPase (Van Os and Slegers 1971).

Na+. K+ ATPase is an enzyme which supplies the energy for the movement of sodium out of the hepatic cell into the canalicular bile and is the putative mediator of bile acid independant flow (Schwartz, Lindenmayer, Allen 1975, Erlinger, Dhumeaux, Berthelot et al 1970; Charney, Silva, Besarab et al 1974; Simon, Sutherland, Accatino 1977).

Sodium enters gallbladder epithelial cells passively across the luminal membranes down an electrochemical gradient maintained by Na+ extrusion across the basolateral membrane by a Na+ K+ - ATPase pump. Movement of water is believed to be passive and secondary to active solute movement. Cyclic Amp (c Amp) has been proposed as a second messenger for the effects of several mediators (Wood and Svanvik 1983). Secretin and extracts of islet cell tumours but not CCK or gastrin have been reported to inhibit fluid absorption by the isolated rabbit gallbladder (Leyssac, Bukhave and Frederiksen 1974). VIP is a potent stimulant of c Amp production by human gallbladder cells (Dupont, Broyart, Broery et al 1981). VIP infused intravenously in the cat inhibits gallbladder water and electrolyte transport and reverses its direction to a net secretion. Female sex hormones in pharmacological doses have been reported to inhibit gallbladder fluid absorption in vitro (France, Men on, Reay et al 1977).

It is not known whether gallbladder function is influenced by different phases of the ovulatory cycle. Riegel, Ravdin, Morrison et al(1935) studied the composition of gallbladder bile in women at term and found changes consistent with impaired gallbladder concentrating function. This effect could be due to oestrogen or progesterone. Historical reports suggest that bile becomes dilute in the later phases of pregnancy (Riegel, Ravdin, Morrison et al 1935; Gerdes and Boyden 1938; Potter 1936). In the pregnant hamster there is decreased bile acid independant bile flow with an associated fall in Na+K+-ATPase activity in liver homogenates (Reyes and Kern 1970). Oestrogen administration also decreases the bile acid independent fraction of bile and hepatic Na+K+-ATPase activity (Reichen and Paumgartner 1977).

It is possible that the enlarged gallbladder noted in pregnancy by Kern, Everson, De Mark et al (1981) could be primarily due to reduced water absorption without increased retention of bile acids, although this seems unlikely. The significance of the increased volume of the gallbladder in pregnancy can be interpreted correctly only with additional information about whether bile acid mass retained in the gallbladder is also increased.

H. GASTROINTESTINAL PEPTIDES AND GALLBLADDER MOTILITY

Gastrointestinal peptide effects on extrahepatic biliary motility are multiple and their integration is complex. It has been generally accepted that CCK is the candidate hormone for gallbladder contraction. (Broden 1958, Sturdevant, Stern, Resin et al 1973).

(i) Cholecystokinin

In 1928 Ivy and Oldberg used the name cholecystokinin (CCK) to describe the active principle responsible for the contraction of the gallbladder. Some 15 years later Harper and Raper (1943) showed that intestinal extracts stimulated secretion of pancreatic enzymes and named their active factor pancreozymin. Subsequently, Jorpes and Mutt (1968) at the Karolinska Institute, Stockholm showed that these active principles (CCK and pancreozymin) were one and the same regulatory peptide capable of eliciting both contraction of the gallbladder and secretion of pancreatic enzymes.The original peptide of CCK contained 33 amino acids (CCK-33). Subsequent studies demonstrated that only the octapeptide (CCK-8) located at the carboxy-terminus of CCK-33 was required for contraction of the gallbladder and relaxation of the sphincter of Oddi. In addition, CCK-8 was shown to be 3 to 10 times more potent than CCK-33 in stimulating contraction of the gallbladder. CCK released from the small intestinal I cells may regulate gallbladder contraction (Wiener, Inoue, Fagan et al 1981) in response to luminal stimuli. Guinea pig gallbladder contraction in response to CCK is a result of a direct action on smooth muscle cells (Yau, Makhlouf, Edwards et al 1973). CCK-induced small bowel contraction is mediated via neural pathways (Hedner 1970). Continued release of CCK from upper small bowel may be stimulated by slow gastric emptying of solids which may in turn control

the rate of gallbladder contractions. During feeding the gallbladder contracts allowing bile acids to enter the intestine to emulsify fat and transport cholesterol in micelles. Gallbladder contraction is dependent on the content of the meal which releases CCK from the jejunum and it should therefore relate directly to the rate of delivery of ingested meals into the upper small intestine. Thus factors controlling the rate of gastric emptying could indirectly control the rate of gallbladder emptying.

The sphincter of Oddi has the potential for altering bile partitioning between the gallbladder and small intestine. Meltzer (1917) advanced the concept of reciprocal innervation to explain the simultaneous contraction of the gallbladder and relaxation of the sphincter in response to intraduodenal magnesium sulphate. McMaster and Elman (1926) also demonstrated that sphincter resistance decreased as the gallbladder contracted and that differential pressures betwen the extra and intra hepatic biliary tree and the gallbladder could account for gallbladder refilling (Elman and McMaster 1926). It is relevant to note from previous studies that in addition to its direct gallbladder effect, CCK relaxes the sphincter of Oddi (Sandblom, Voegtlin, Ivy 1935, Hong, Magee, Crewdon 1956).

The role of CCK in co-ordinating gallbladder contraction and sphincter of Oddi relaxation is well established (Walsh 1978). Guinea pig gallbladder contraction in response to CCK is a result of a direct action on smooth muscle cells. The sphincter of Oddi in the dog (Sandblom, Voegtlin, Ivy 1935) cat (Behar and Biancani 1978) baboon (La Morte, Gaca, Wise et al 1980) and man (White and Bourde 1970) relaxes in response to CCK, probably by the action of noncholinergic, nonadrenergic inhibitory neurons (Behar and Biancani 1980). However other studies have demonstated that CCK increases muscular activity in the sphincter of Oddi in opossum and rabbit

species (Becker and Moody 1978; Sarles, Bidart, Devaux et al 1976; Honda, Toouli, Dodds et al 1983).

Autonomic control of the gallbladder plays an important role in potentiation of the pressure effect of CCK and the maintenance of gallbladder tone (Pallin and Skoglund 1964).

(ii) Pancreatic Polypeptide

Pancreatic Polypeptide (PP), a 36 amino acid polypeptide, is found almost exclusively in the pancreas (Lonovics, Devitt, Watson et al 1981). In vivo studies in humans, dogs and the pig have demonstrated that PP administration results in gallbladder relaxation with increased storage of bile (Lin and Chance 1974; Adrian, Mitchenere, Sagor et al 1982; Greenberg, McCloy, Adrian et al 1978; Greenberg, McCloy, Chadwick et al 1979). Although the effects of PP on the biliary tree are opposite to CCK, both peptides rise in parallel after a meal and purified CCK is an effective humoral releaser of PP in humans (Lonovics, Guzman, Devitt et al 1980).

(iii)Motilin

Motilin, a polypeptide hormone of 22 amino acid residues has been isolated from the duodenum and jejunum of man and several animals (dogs, pigs, baboons). It is not similar in structure to any member of either gastrin or secretin families of hormones (Domschke 1977).

Motilin is released during Phase III and IV activity of the interdigestive (fasting) myoelectrical complex and in man is associated with bile flow into the duodenum (Keane, DiMagno, Dozois et al 1980). Periodic contractions of the gallbladder occur in close association with the interdigestive migrating contractions in the stomach and both can

simultaneously be reproduced by exogenous administration of motilin. (Itoh and Takahashi 1981).Thus motilin may regulate biliary motility in the period between meals.

Pancreatic Polypeptide and motilin have little effect on in vitro preparations of human, rabbit and dog gallbladder muscle strips (Pomeranz, Davison, Shaffer 1983, Lonovics, Devitt, Rayford et al 1979). Therefore both PP and motilin appear to exert their respective actions at sites remote from the gallbladder without directly affecting gallbladder muscle.

(iv) Opioids

Receptors that bind opioids are found on mucosal cells, smooth muscle fibres and on both the body and terminals of nerve cells of the gut. At present there is little data on the distribution of either cells that produce endogenous opioids or cells that contain opioids receptors within the biliary tree. Thus, the physiological role of opioids in regulating biliary motility is undefined.

Emptying of the gallbladder during an infusion of CCK-8 and administration of either saline, morphine, enkephalin or naloxone (an opioid-antagonist) has been evaluated in man by real time ultrasonography (Worobetz, Baker, McCallum et al 1982). Saline and naloxone did not alter the normal pattern of emptying of the gallbladder during intravenous infusion of CCK-8. On the other hand, emptying during the infusion of CCK-8 was completely blocked by morphine or enkephalin. After administration of morphine or enkephalin, the gallbladder assumed a round shape and its volume significantly increased. When naloxone was administered after morphine or enkephalin, the gallbladder emptied rapidly. These findings suggest that the opioids stimulate the sphincter of Oddi and the pressure of the sphincter

increases. As a result the resistance to the flow of bile out of the gallbladder and common bile duct into the duodenum increases. The gallbladder cannot empty even though it contracts. Blockade of the opioid effect by naloxone relaxes the sphincter and the contracting gallbladder empties quickly.

I NEURONAL DETERMINANTS OF EXTRAHEPATIC BILIARY MOTILITY

(i) Influence of the parasympathetic autonomic nervous system.

Autonomic contributions to gallbladder tone have been examined by nerve stimulation and pharmacologic methods. The parasympathetic nervous system is clearly involved in the maintenance of gallbladder tone (Pallin and Skoglund 1964); conversely vagotomy decreases resting gallbladder pressure (Liedberg 1969) Gallbladder pressure can be elevated by intravenous acetylcholine and pilocarpine and this effect is blocked by atropine in all animal species studied (Halpert and Lewis 1930; Winkelstein and Achsner 1924; Winkelstein and Aschsner 1926; Menguy, Harlenbeck, Bollman et al 1958).

Parasympathetic receptors appear to be present in the gallbladder and the parasympathetic system probably interacts with hormonal stimuli in regulating gallbladder tone.

Foesel and Sewing (1978) showed that contraction of the gallbladder caused by a stimulation of its intrinsic parasympathetic fibers could be enhanced

by a subthreshold concentration of CCK. This observation strongly favoured a contibuting role of cholinergic innervation in the control of gallbladder motor function. Yau and Youther (1984) more recently performed direct transmural field stimulation on guinea pig in vitro gallbladder preparations and showed acetlycholine-mediated contraction. The contractions were sensitive to atropine and tetrodotoxin, indicating that the activity of cholinergic neurons were responsible for the muscle contraction.

In humans, Rock, Malmud and Fisher (1981) showed that sham feeding resulted in emptying of 50% of gallbladder volume. The effect was abolished by atropine. The amount of bile in the fasting gallbladder is doubled in humans after truncal vagotomy compared with selective vagotomy and the amount of residual bile after emptying is greater (Inberg and Vuorio 1969). While human gallstones placed in a canine gallbladder usually dissolve promptly one study has shown that truncal vagotomy significantly retards dissolution (Barnett and Hilburn 1966). Sequestration of bile into a large stagnant gallbladder could result in diminished bile acid secretion and increased cholesterol saturation of bile through interruption of the enterohepatic circulation. However, studies have shown a decrease in biliary cholesterol saturation when the gallbladder leaves the enterohepatic circulation either by disease or surgery (Shaffer and Small 1977; Redinger 1976).

Westphal, Gleichmann and Joik (1931) reported that electrical stimulation of the vagus could increase water absorption by the dog gallbladder. Their results were not consistent and more recently vagal stimulation in the cat failed to influence the rate of fluid transport in the gallbladder using a perfusion technique (Bjorck, Jansson and Svanik 1983).

The influence of vagotomy on the sphincter of Oddi is controversial. Some papers have reported sphincter relaxation. (Dowling 1971; Beneventano, Rosen, Schein 1969). In dogs results are variable (Amdrup and Griffith 1970) while in the rabbit, vagotomy does not change electromyographic patterns (Gerolami and Sarles 1977). In the conscious dog, metacholine has been reported to elicit spasm of the common duct (Lin 1975)

More importantly CCK appears to inhibit phasic contractions and decrease spincter tone by stimulation of non-adrenergic non-cholinergic inhibitory neurones (Behar and Biancani 1980).

(ii) Influence of the sympathetic autonomic nervous system

Sympathetic stimulation by way of the right splanchnic nerve has been demonstrated to cause a relaxation of the gallbladder and this effect is most readily seen on a gallbladder that is in a contracted state either induced hormonaly or neuraly. Such adrenergic pathways are mediated via beta receptors (Persson 1972). However the exact functional implications of this are still unclear.

It has also been found that electrical stimulation of the splanchnic nerves stimulates the rate of water absorption by the gallbladder of the cat, an effect which can be abolished by alpha adrenergic receptor blockade. The precise site of action of the sympathetic nerves on water transport by the gallbladder is unknown. Apart from a possible direct effect on mucosal cells, sympathetic nerves may act on local ganglia to inhibit release of a neurotransmitter which inhibits water absorption. A possible transmitter released by local reflexes is vasoactive intestinal peptide (VIP).Vasoactive intestinal peptide is a hormone which is a member of the secretin family of

gastrointestinal hormones. Vasoactive intestinal peptide may function physiologically to inhibit contraction of the gallbladder. In several animals (guinea pig, opossum, cat and rabbit), physiologic concentrations of VIP inhibit the contraction of the smooth muscle of the gallbladder induced by acetylcholine and CCK (Lonovics, Devitt, Rayford et al 1979). In addition, VIP has been isolated from nerve fibres within the muscle layers of the gallbladder of both man and cat (Sundler, Alumots, Hakanson et al 1977).

The effect of pregnancy, the menstrual cycle or female sex steroids on the autonomic nervous system has not been studied with regard to gastrointestinal smooth muscle activity. In addition no studies have examined the interaction of female hormones with cholinergic or adrenergic receptors. Therefore it remains a possibility that these hormones produce some of their effects through autonomic pathways and receptors.

J. THE RELATIONSHIPS OF GASTRIC EMPTYING TO GALLBLADDER EMPTYING

For a thorough understanding of post prandial gallbladder emptying it is important to examine the complex regulatory mechanism of gastric emptying which are poorly understood.

The emptying of solids and liquids from the human stomach is tightly regulated by chemoreceptive mechanisms that are capable of selective response to meal components. For example, these mechanisms respond strongly to long chain but not short chain fatty acids (Hunt and Knox 1968), to tryptophan but not glycine (Stephens, Woolsen, Cooke 1975), and more strongly to HCl than citric acid (Hunt and Knox 1969). The mechanisms are not only selective but are also quantitative, regulating the quantity of acid entering the duodenum, independent of its concentration (Hunt and

Knox 1962) and regulating the number of calories emptying from the stomach per minute despite wide variations in the calorie density of liquid mass (Hunt and Stubbs 1975). Although relevant chemoreceptors have been localised in the proximal small intestine (Cooke 1977) little is known of receptor mechanisms through which they control the flow of nutrients from the stomach.

Solids and liquids entering the duodenum might trigger mechanisms that inhibit propulsive forces. For example, both neuronal and hormonal mechanisms are known to be capable of regulating the tone of the stomach (Stephens, Woolsen, Cooke 1976). Thus, the release of intestinal hormones or the triggering of nervous reflexes by chemoreceptors in the intestine might slow gastric emptying by diminishing gastric tone and thereby reducing gastroduodenal pressure gradients. The final mechanism may operate through the vagus nerve. Phasic contraction of the antrum is known to be inhibited via vagal mehanisms by fat, tryptophan, glucose, or acid in the duodenum (Kelly and Code 1969; Meyer and Jones 1974; Roze, Couturier, Chariot et al 1977; Thomas, Crider, Morgan 1934). Such inhibition might reduce propulsive pumping by the antrum.

As a motor unit the stomach is classicaly considered as having two functional areas,consisting of a proximal receptacle the fundus and body, and a distal pump the antrum. The antrum mixes and churns gastric contents and propels them into the duodenum..

Antral contractions determine the delivery rate of gastric contents into the dudenum. Frequency, direction and velocity of antral contractions are determined by the gastric slow waves and their superimposed spike activity. Spike activity is required for contractions to occur (Cooke

1975).

Mixed solid liquid meals may separate into different physical phases which may each empty from the stomach at an independent rate. Liquids leave the stomach more rapidly than solids (Heading, Tothill, McLoughlin et al 1976). As they are emptied into the duodenum, they are continuously replaced by newly secreted gastric juices, thus exposing the remaining solids to fresh gastric secretions. Liquids of high solute content have slower gastric emptying rates than isotonic liquids (Cooke 1975). However they generally empty from the stomach at rates more rapid than fats or solids (Moore, Christian, Coleman 1981).

Digestible solids empty slower probably because the antrum acts as a sieving apparatus. Suspended particles can pass through the pylorus only when they have been ground down to particles less than 1mm in diameter (Meyer, Ohashi, Jehn et al 1981). Particles greater than this size are returned to the antrum for further grinding (Weiner, Graham, Reedy et al 1981). Non-digestable solids were thought to remain in the stomach until the rest of the meal is emptied. However, Camilleri, Brown and Malagelada (1986) using non-digestible labelled bran recently showed that emptying of these particles is also a function of antral peristalsis. While the labelled bran was selectively retained by the stomach relative to liquid, it did appear to empty at similar rates to other solid components of the meal which had been ground into small particles by the antrum. In support of these findings Holt, Reid, Taylor et al (1982) observed similar emptying patterns for both larger non-digestible solids and titurable liver cubes. If gastric emptying of solids is responsible for continued stimulation of gallbladder contraction then gallbladder refilling should occur when gastric emptying of solids is complete.

More recently King, Adam, Pryde et al (1984) have drawn attention to the function of antral contractions and the role that peristaltic activity of the distal stomach plays in regulating gastric emptying of solids. Using real-time ultrasound they showed that emptying of gastric contents through the pylorus tended to occur in short episodes when the terminal antrum, pylorus and duodenum were relaxed. The authors concluded that this pattern of movement was unlikely to be related to antral peristalsis or to be attributed to constant intragastric pressure alone. They also observed that most gastric peristaltic cycles were accompanied by retrograde flow at the pylorus.

It is possible that any changes in gallbladder emptying seen with increased endogenous and exogenous female sex steroids may be secondary to changes in gastric emptying. However recent studies suggest that gastric emptying is not altered in either the second trimester of pregnancy (Schade, Pelekanos, Tauxe et al 1984) or the luteal phase of the ovulatory cycle (Horowitz, Maddern, Chatterton et al 1984).

Thus in the last century the search for the aetiology of gallstones has gone full circle. Initially the cause of gallstone formation was thought to relate to inflammation of the gallbladder wall and incomplete gallbladder emptying. When it was realised that biliary cholesterol saturation was vital for stone formation, attention was directed to the liver. The role of altered gallbladder motility in the genesis of lithogenic bile and cholesterol cholelithiasis is still relatively unexplored and attempts to describe this aspect of biliary physiology will improve our understanding of the multifactorial pathogenesis of cholesterol cholelithiasis.

Much remains to be learned about the effects of female sex hormones on biliary motility and the metabolism of bile acids and cholesterol.

CHAPTER II: CURRENT KNOWLEDGE AND UNANSWERED QUESTIONS REGARDING GALLBLADDER MOTOR FUNCTION

A. Gallbladder Function - Current Concepts in Pregnancy

B. Unanswered Questions Regarding Gallbladder Function

C. Thesis Aims

CURRENT KNOWLEDGE AND QUESTIONS UNANSWERED

A. GALLBLADDER FUNCTION - CURRENT CONCEPTS IN PREGNANCY

Studies by the Gastroenterology Unit of the University of Colorado have already shown that after the 13th week of pregnancy (i) the size of the gallbladder in the fasting state is twice that of non-pregnant controls (2) the time of contraction stimulated by a standard liquid meal, is longer than in controls (3) the maximum percent emptied after the meal is less and (4) the residual volume after maximum emptying is nearly three times as great as in controls. In early pregnancy (first 12 weeks) the only significant alteration is slower emptying after the liquid meal. These alterations in gallbladder function could contribute to precipitation of cholesterol crystals and their retention in the gallbladder. Further, they could account in part for the increased bile acid pool size and for some of the changes in bile acid synthesis observed in pregnancy.

The interrelation of the various components of the enterohepatic circulation (EHC) of bile acids is exceedingly complex. However, to understand cholesterol gallstone formation it is apparent that knowledge of mechanism altering bile acid EHC is important.

The storage of a large proportion of bile acid pool in the gallbladder during the night would be expected to produce fasting hepatic bile that is more lithogenic, whereas the storage of only a small proportion of the pool would probably be associated with less lithogenic fasting hepatic bile. In the latter situation most of the bile acid pool would be more or less continuously circulating through the intestine and liver, causing inhibition of bile acid synthesis and a smaller pool size, as found in many

patients after cholecystectomy.

If a large portion of the pool is stored in the gallbladder overnight, synthesis would be stimulated and the pool size increased. On the other hand, synthesis rate and pool size might be determined largely by the completeness of gallbladder emptying. If the gallbladder emptied completely, a large load of bile acid eventually returning to the liver would be expected to suppress synthesis, but if gallbladder emptying were incomplete and a substantial portion of the bile acid pool was retained, less would return to the liver and synthesis would be stimulated. Results from Colorado have strongly suggested a central role for the gallbladder in the enterohepatic circulation of bile acids and bile acid kinetics and support the hypothesis that altered gallbladder function in pregnancy might play an important role in cholesterol gallstone formation (Kern, Everson, De Mark et al 1981).

The hypothesis described assumes that bile acid absorption efficiency from the small intestine is the same during pregnancy, that intestinal transit of bile acids is not significantly altered and that there is no effect of pregnancy on the capacity of the liver to respond appropriately to changes in intracellular bile acid concentration. Alterations in the intestinal bile acid absorption or hepatic response are not suspected, but if experimental results indicated otherwise then direct studies would be necessary. Intestinal absorption of bile acids can be studied by ileal perfusion, but techniques for direct studies of hepatic response to changes in intracellular bile acid concentration in human subjects do not now exist. Intestinal transit time might be slowed as pregnancy progresses by a progesterone effect or other neurohormonal alteration. It is also conceivable that gallbladder emptying could be impaired by reduced gastric emptying by food stimulus to CCK release and/or reduced production of CCK

by the intestinal mucosa. Since the gallbladder response to intraduodenally-infused amino acid is diminished in pregnancy, gastric emptying may not be an important factor (Kern, Everson, De Mark et al 1982) At the time of the present studies, reliable means for measuring CCK were not generally available. CCK (or its octapeptide) cannot be given to pregnant women for ethical reasons but,if necessary to study the question of insufficient endogenous CCK vs impaired gallbladder response, it can be given to the other groups being studied, consisting of normal women and those on oral contraceptive steroids

A hypothesis of this thesis is that the increased size of the gallbladder after an overnight fast and after a meal in the latter two-thirds of pregnancy has two separate causes: (a) decreased concentration of bile, probably due to inhibition of gallbladder epithelial Na+K+ ATPase activity resulting from high serum oestrogen concentrations and (b) decreased tone and contractility of the gallbladder due to its exposure to high concentrations of progesterone.

It is essential to know whether the increased gallbladder volume contains an increased amount of bile acid or whether the volume increase is due entirely to water. Oral cholecystography has shown decreased concentration of contrast material by the gallbladders of pregnant women (Gerdes and Boyden 1938). One study has suggested that oestrogens inhibit gallbladder mucosal Na+K+ ATPase activity (France, Menon, Reay et al 1977), the enzyme responsible for its absorption of Na+ and water. The concentration of gallbladder bile cannot be measured directly but can be estimated from simultaneous measurements of volume changes by ultrasound and bile acid output into the duodenum. The latter can be calculated from data obtained with the non-absorbable marker technique during gallbladder contraction.

Progesterone is a smooth muscle relaxant. Both pregnancy and progesterone administration have been shown to improve gallbladder emptying in several animal species. This feature of gallbladder physiology will be studied in human subjects by measuring the effect of administration of progesterone containing compounds on gallbladder emptying in response to various stimuli and by correlating gallbladder motor activity with serum progesterone levels during pregnancy.

B. UNANSWERED QUESTIONS REGARDING GALLBLADDER FUNCTION

In this thesis the planned studies were designed to answer several questions about gallbladder function, the relation of gallbladder function to the bile acid kinetics of the enterohepatic circulation, the mechanism of observed changes, and the detailed character of alterations associated with pregnancy and female sex hormones. The following questions were examined:

(1) In pregnancy, is there more bile in the gallbladder throughout the day than in non-pregnant control subjects?

(2) Does administration of contraceptive steroids and oestrogen alone affect gallbladder function in human subjects?

(3) Do increased volumes of the gallbladder influence biliary lipid composition?.

(4) Is gallbladder bile in the fasting state less concentrated in menopausal women before and after taking oestrogens?

The increased pool size of the primary bile acids and the decreased number

of enterohepatic cycles during pregnancy could be caused in part by slow transit of bile acids through the small intestine, perhaps secondary to a progesterone or other neurohormonal effect on small intestinal smooth muscle. Thus an additional question asked in this thesis was:-

(5) Is small intestinal transit time prolonged in pregnancy?

As the work of this thesis developed it was observed that the gallbladder failed to refill in normal subjects ingesting three meals per day. A proposed mechanism for this observation was that the slow emptying of solids from the stomach into the upper small bowel controlled gallbladder emptying and refilling. Therefore a further question asked was:-

(6) What is the relationship between the rate of gastric emptying and gallbladder emptying?

C THESIS AIMS

The aims of this thesis were designed to answer the preceding questions and are as follows:-

(1) To measure gallbladder storage and emptying throughout the day and night during ingestion of meals in four similar groups of healthy, non-obese women: (a) controls in the follicular and luteal phases of the ovulatory cycle (b) pregnant women (c) post-partum women (d) contraceptive steroid users.

(2) To investigate the role of the gallbladder and small intestine in regulating the rate of biliary lipid secretion and biliary lipid

composition.

(3) To determine if chronic administration of the conjugated equino-oestrogen premarin alters gallbladder fuction, biliary lipid composition and secretion in post menopausal women.

(4) To determine if gastrointestinal transit time is prolonged in early pregnancy and to measure serial changes in gastrointestinal transit by studying the same individual throughout pregnancy and post-partum.

(5) To measure the time course of gallbladder emptying and refilling after ingestion of a standard breakfast, to relate the rate of gallbladder emptying to the rate of gastric emptying of solids, and to relate gallbladder refilling to both completion of gastric emptying of solids and reduction in humoral stimulation.

CHAPTER III: METHODS AND RESULTS

A. Gallbladder Function in the Human Female: Effect of the Ovulatory Cycle, Pregnancy and Contraceptive Steroids

- (i) Methods (a) Subjects
  - (b) Analytical Techniques
  - (c) Indices of Gallbladder Function
  - (d) Analysis of Data
- (ii) Results (a) Effects of the Ovulatory Cycle
  - (b) Effects of Pregnancy
  - (c) Effects of Contraceptive Steroids

B. Gallbladder and Small Intestinal Regulation of Biliary Lipid Secretion During Intraduodenal Infusion of Standard AminoAcid and Liquid Formula Stimuli

- (i) Methods (a) Subjects and Procedures
  - (b) Analytical Techniques
  - (c) Analysis of Data
- (ii) Results (a) Biliary Lipid Secretion
  - (b) Gallbladder Emptying
  - (c) Small Bowel Transit Time
  - (d) Human Serum Pancreatic Polypeptide

C. The Effects of Chronic Oestrogen Administration on Biliary Lipid Secretion, Bile Acids and Gallbladder Function in Post-Menopausal Women

- (i) Methods (a) Subjects
  - (b) Gallbladder Concentration and Emptying After IV CCK
  - (c) Analytical Techniques
  - (d) Biliary Lipid Secretion

(e) Bile Acids

(f) Gallbladder Volume and Emptying with Standard Meals

(g) Analysis of Data

(ii) Results (a) Gallbladder Emptying

(b) Gallbladder Concentration

(c) Biliary Lipid Secretion

(d) Bile Acids

D. Orocaecal Transit Time in Pregnancy

(i) Methods (a) Subjects

(b) Orocaecal Transit Time

(c) Analysis of Data

(ii) Results

E. Co-ordination of Gastric and Gallbladder Emptying After Ingestion of a Regular Meal

(i) Methods (a) Subjects

(b) Gastric Emptying

(c) Gallbladder Emptying

(d) Gastrointestinal Transit Time and Serum HPP

(e) Analytical Techniques

(f) Analysis of Data

(ii) Results (a) Gallbladder Emptying

(b) Gastric Emptying

(c) Serum HPP

METHODS AND RESULTS

A. GALLBLADDER FUNCTION IN THE HUMAN FEMALE: EFFECT OF THE OVULATORY CYCLE, PREGNANCY AND CONTRACEPTIVE STEROIDS

(i) METHODS

(a) Subjects

The characteristics of subjects are shown in Table 1. There was no difference in height, weight, percent ideal weight (Metropolitan Life Insurance Company. Statistical Bulletin, 1960) age, history of contraceptive use, or history of pregnancy between follicular and luteal phase controls. Although the pregnant group was heavier than the control group, the difference was consistent with normal fetoplacental development. Compared with controls, half as many pregnant women had prior history of taking contraceptive steroids, while twice as many had had a previous pregnancy. Women taking contraceptive steroids were slightly but not significantly heavier than controls. All of them had been taking contraceptive steroids continuously for 0.5-6 yr (median 3 yr) and none had been pregnant.

No subjects had any known illness and none was taking any medication (except contraceptive steroids). All subjects had normal fasting serum levels of aspartate amino transferase, alkaline phosphatase, and bilirubin.

Eleven studies were done in 9 women taking contraceptive steroids. The phase of the ovulatory cycle in controls was determined from menstrual

TABLE I

CHARACTERISTICS OF SUBJECTS

Group	n	Height(cm)	Weight(kg)	Percent ideal body weight[a] %	Age(yr)	Prior History CS[b]%	Pg[c]%	Prog(ng/ml)
CONTROLS								
All	22	164.4±5.1	52.5±4.7	94.3±7.4	26.9±3.6	67	27	4.5±6.0
FP	11	164.4±5.6	52.0±4.3	93.4±7.4	27.0±4.0	50	30	0.7±0.4
LP	11	164.3±4.9	53.0±5.2	95.2±7.6	26.8±3.2	88	20	8.0±6.7
Pregnant Women[d]								
All	22	165.0±6.1	59.9±7.3		21.9±3.2	33	55	65.0±35.7
TM1	7	167.6±6.0	56.5±9.2		21.7±3.0	33	71	29.8±10.1
TM2	10	163.7±5.2	56.5±5.6		22.5±3.7	20	60	58.8±17.4
TM3	8	165.1±7.2	65.0±5.9		21.3±2.6	50	38	110.7±25.6
Contraceptive Steroid Users[e]								
All	9	168.8±	57.6±5.4	97.8±4.7	25.4±3.9		0	

Values given as mean ± SD. Abbreviations: n=number of subjects, Prog=progesterone, FP=follicular phase, LP=luteal phase, TM1=first trimester, TM2=second trimester, TM3=third trimester, [a]determined from height-weight table of the Metropolitan Life Insurance Company. No comparable table exists for pregnant subjects. [b]Proportion with a previous history of contraceptive (CS) steroid use. [c]Proportion with a previous history of pregnancy (Pg). [d]One subject was studied in all trimesters and 1 subject was studied in the second and third trimester. [e]All subjects had been taking contraceptive steroids continuously for 0.5-6 yr.

history (ovulation was considered as occurring 15 days before menstruation) and a single serum progesterone level. It was 0.15 - 1.7 ng/ml in the follicular phase, and 2-17ng/ml in the luteal phase. All subjects had regular cycles, 11 were in the follicular and 11 in the luteal phase of the ovulatory cycle. The duration of pregnancy was determined from the date of the last normal ovulatory cycle. Seven subjects were in the first trimester, 10 in the second, and 8 in the third. The contraceptive steroids used in this study contained 1mg of the progestational compound norethindrone, and either 35, 50 or 80mg of the oestrogenic component, mestranol. Studies were done on days 12 to 21 of pill administration.

At 8.00 p.m. the evening before study, subjects ingested half a turkey sandwich and 250 ml of whole milk, and then fasted overnight. The day of study their diet contained 2238 cal, 15% protein and 46% fat. They ate at 8.30 a.m., 12.00 noon, 5.00p.m. and 8.00 p.m. Breakfast consisted of one egg, two slices of bacon, two pieces of buttered toast, 100 ml orange juice, 40 ml low fat milk, and 200 ml of coffee or tea (610 cal, 12% protein, 44% fat) All subjects ingested the entire diet as monitored by a dietitian.

(b) Analytical Techniques

Gallbladder size and emptying were determined by real time ultrasonography (see Appendix). They were obtained during fasting at 15 min after the start of breakfast, and every 5-10 min for 90 min. Thereafter, sonographs were taken hourly until midnight.

Subjects ate breakfast upright, reclined at a 15-30 degree angle for the next 90 min, but they engaged in normal physical activity the rest of the day.

(c) Indices of Gallbladder Function

Gallbladder volumes were calculated from sonographs. Fasting volume (FV) was the volume before breakfast. Residual gallbladder volume (RV) was the lowest volume achieved in the first 90min after breakfast. The percent of emptying (%E) was equal to (1-RV/FV) x 100%. The average hourly volume (HV) was the average of gallbladder volumes measured every hour from 11.00 am to midnight.

Although changes in gallbladder volume can result from bile entering the gallbladder, bile leaving the gallbladder, absorption of water, and secretion of water, only gallbladder volume was measured. Emptying is defined as the net decrease in volume that occurs over time. Gallbladder emptying was assumed to obey the first-order exponential function, Vt/Vo - e-(bt), where Vt is gallbladder volume at time t, Vo is initial gallbladder volume and b is the rate constant of emptying. Since commencing this study another group using the more easily reproducible radionuclide scanning technique with intravenous ^{99}Tc-EHIDA, has shown that gallbladder emptying after a mixed solid/liquid meal is best described by a double exponential function similar to the observations reported here (Baxter, Grime , Critchley et al 1985). Rate constants of gallbladder emptying after breakfast were calculated from ln/linear regression of gallbladder volume vs time. Serum progesterone levels were measured by radioimmunoassay at Endocrine Sciences (Tarzana, California).

(d) Analysis of Data

Group differences were evaluated by Student's t-test for unpaired data. The relationship of indices of gallbladder function to other variables were evaluated by linear regression analysis. Group differences in slopes of regression lines were evaluated by F statistics using the extra sum of squares principle (Draper and Smith 1966).

It was intended to evaluate each control subject in both phases of the ovulatory cycle, each pregnant subject in each trimester of pregnancy and

postpartum and each subject taking contraceptive steroids before and after contraceptive use. Most subjects however declined repeated studies. Only 2 pregnant women were studied in more than one trimester of pregnancy, and only 5 were studied post partum. No control was studied in both phases of the ovulatory cycle and no contraceptive steroid user was studied before starting this agent. Accordingly, differences in gallbladder function induced by the ovulatory cycle, pregnancy, or contraceptive steroids were analysed by comparing group mean values, a less sensitive technique to detect differences than paired analysis.

(ii) RESULTS

(a) Effects of the Ovulatory Cycle

All indices of gallbladder function were similar in the follicular and luteal phases of the ovulatory cycle (Table 2). Neither the day of the ovulatory cycle nor serum progesterone level correlated with any index of gallbladder function in the control group. Accordingly, data from control subjects were pooled for comparison in other groups.

(b) Effects of Pregnancy

Fasting, residual and average hourly volumes were increased in all trimesters of pregnancy (Table 2). In addition, gallbladder volume was significantly greater in pregnant women at each hour of the day (Figure 3). Thus, bile retention in the gallbladder was greater in pregnancy at all times. Fasting and residual gallbladder volume (Figure 4A and 4B) increased linearly during pregnancy from the first to third trimester. The linear increase in fasting and residual gallbladder volumes correlated directly and significantly with week of pregnancy and serum progesterone levels

TABLE II

INDICES OF GALLBLADDER FUNCTION DURING INGESTION OF A REGULAR DIET

Group	n	Indices of gallbladder function					
		FV(ml)	RV(ml)	b(min^{-1}) Early	b(min^{-1}) Late	%E(%)	HV(ml)
Controls							
All	22	17.2±5.2	4.2±1.8	0.022±0.003	0.009±0.002	74.1±12.2	3.5±1.6
FP	11	16.8±5.8	4.3±2.0	0.020±0.006	0.007±0.002	71.2±14.9	4.0±1.8
LP	11	18.0±4.5	4.1±1.7	0.024±0.005	0.011±0.002	77.0± 8.6	3.1±1.3
p		NS	NS	NS	NS	NS	NS
Pregnant women.							
All[a]	25	30.5±9.1	9.2±3.9	0.022±0.003	0.005±0.001	69.9± 9.3	7.5±3.0
p		≪0.001	≪0.001	NS	<0.005	NS	≪0.001
TM1	7	23.7±6.3	6.7±2.5	0.025±0.006	0.007±0.002	71.8± 7.2	7.0±3.8
p		<0.04	<0.04	NS	NS	NS	<0.06
TM2	10	33.7±9.0	9.7±4.5	0.026±0.005	0.005±0.002	70.8±12.3	7.4±3.0
p		≪0.001	≪0.001	NS	NS	NS	≪0.001
TM3	8	32.4±9.0	10.7±3.5	0.018±0.003	0.004±0.002	67.0± 6.6	8.3±3.4
p		≪0.001	≪0.001	NS	<0.005	NS	≪0.001
Contraceptive-steroid users							
All	9	23.8±5.2	5.0±4.7	0.022±0.003	0.010±0.002	80.2±15.8	3.5±3.9
p		<0.01	NS	NS	NS	NS	NS.

Values given as mean ± SD. Abbreviations: See legend for Table 1. FV=fasting volume, RV=residual volume, b=rate constant of emptying, %E=percent emptied, HV=hourly volume. [a]Total studies in 22 women. The p value under the control data refers to the comparison of follicular and luteal phases. All other p values refer to the comparison of the respective group to all controls.

(Figures 5A and 5B).

Average hourly volumes did not correlate significantly with either week of pregnancy or serum progesterone levels (Figures 4C and 5C).

Gallbladder emptying after breakfast is shown in Figure 6. Two rates of gallbladder emptying were identified. The initial rate of emptying to 50% of fasting volume, was identical in pregnant and control subjects (-0.022/min and -0.022/min respectively; p = NS). The second rate of emptying in the pregnant group was one-half that of the control group (-0.004/min vs. -0.009/min, respectively; F(n1-2, n2-2) 8.514; p $<$0.005).

Four women were studied in the third trimester of pregnancy and again after delivery. Fasting, residual,and hourly volume decreased in the postpartum period in each case (Table 3). Gallbladder volumes returned toward normal as soon as 2 weeks postpartum. In all subjects only one rate of emptying was observed after breakfast (Table 3) One subject, KLic, was studied at 18 weeks of pregnancy, 2 days postpartum, and 4 weeks postpartum. At 2 days postpartum, all gallbladder volumes were large and emptying was extremely slow (Table 3). Throughout the day her gallbladder achieved only 48% emptying. This marked degree of bile retention and slow emptying rate approached the normal range 4 weeks later.

(c) Effects of Contraceptive Steroids

Fasting gallbladder volume was increased in this group, but residual and hourly volumes were not (Table 2). Early and late rates of gallbladder emptying were almost identical to control (early =-0.022/min and late= -0.010/min). The amount of oestrogen in the preparation did not correlate

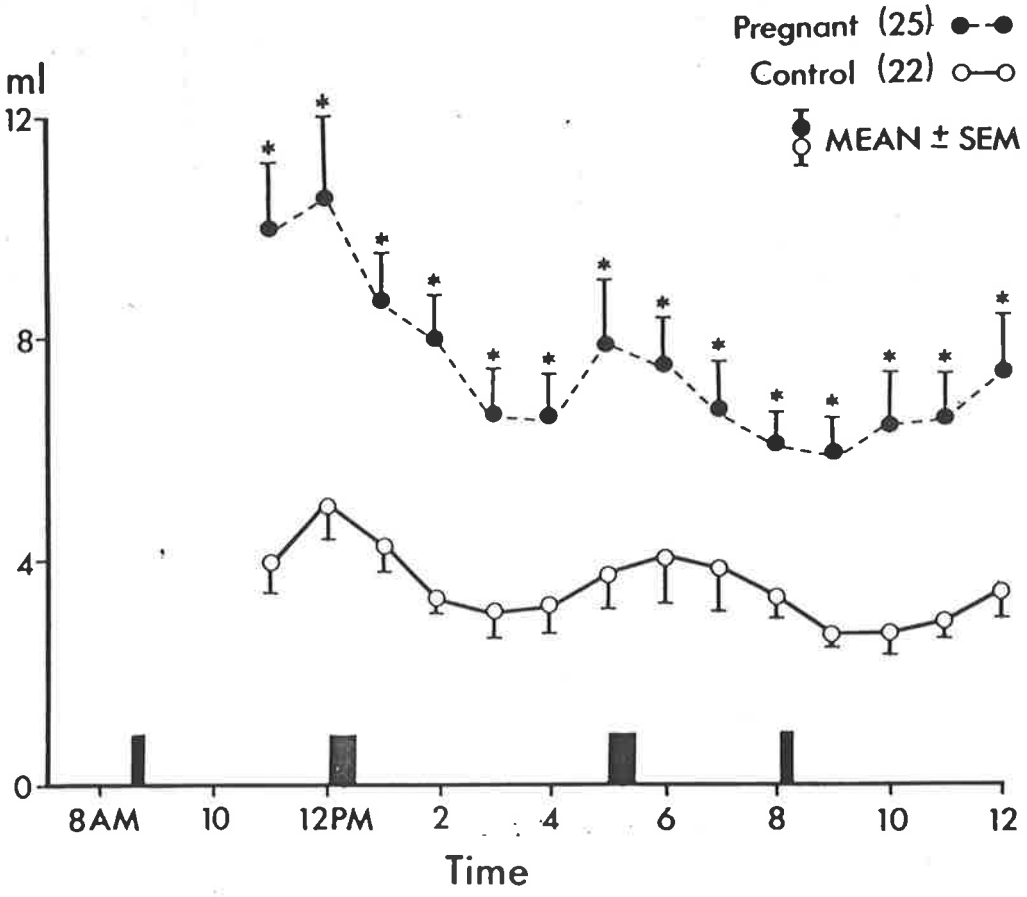

FIGURE 3: The hourly gallbladder volumes of pregnant and control subjects are plotted against time of day. The solid bars on the time axis represent periods of meal ingestion. The difference in gallbladder volumes between control and pregnant subjects was significant at each hour of the day and night (* = $p < 0.01$). Note the very small change in volume after meals. SEM = standard error of the mean.

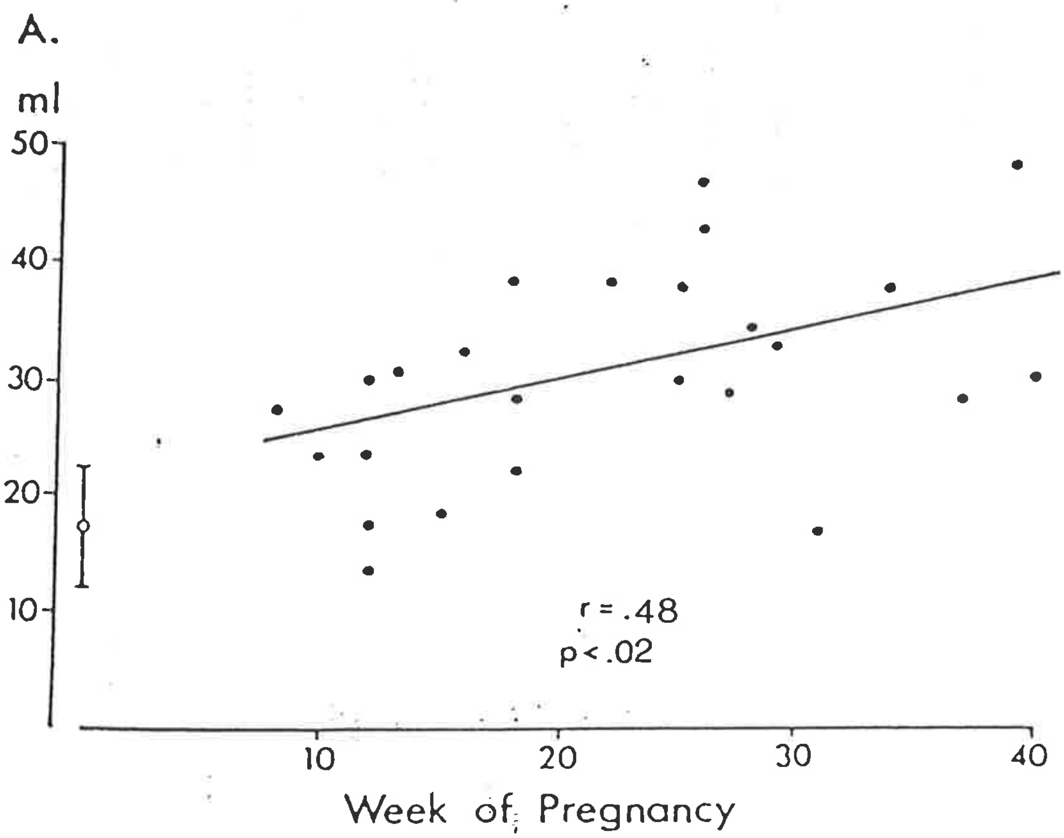

FIGURE 4A: Fasting gallbladder volumes are plotted against week of pregnancy. Fasting volumes (panel A) significantly correlated with week of pregnancy.

⊥ = mean ± standard deviation of controls.

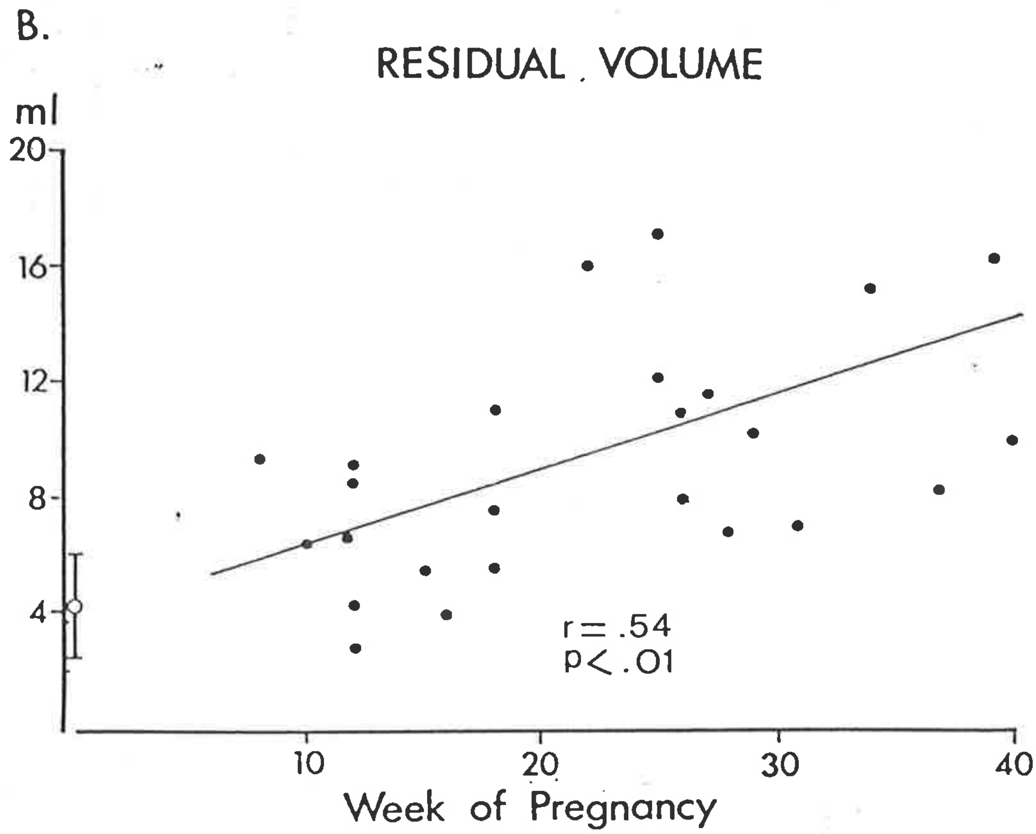

Figure 4B: Residual gallbladder volumes are plotted against week of pregnancy. Residual volumes (panel B) significantly correlated with week of pregnancy.

Ϙ = mean ± standard deviation of controls.

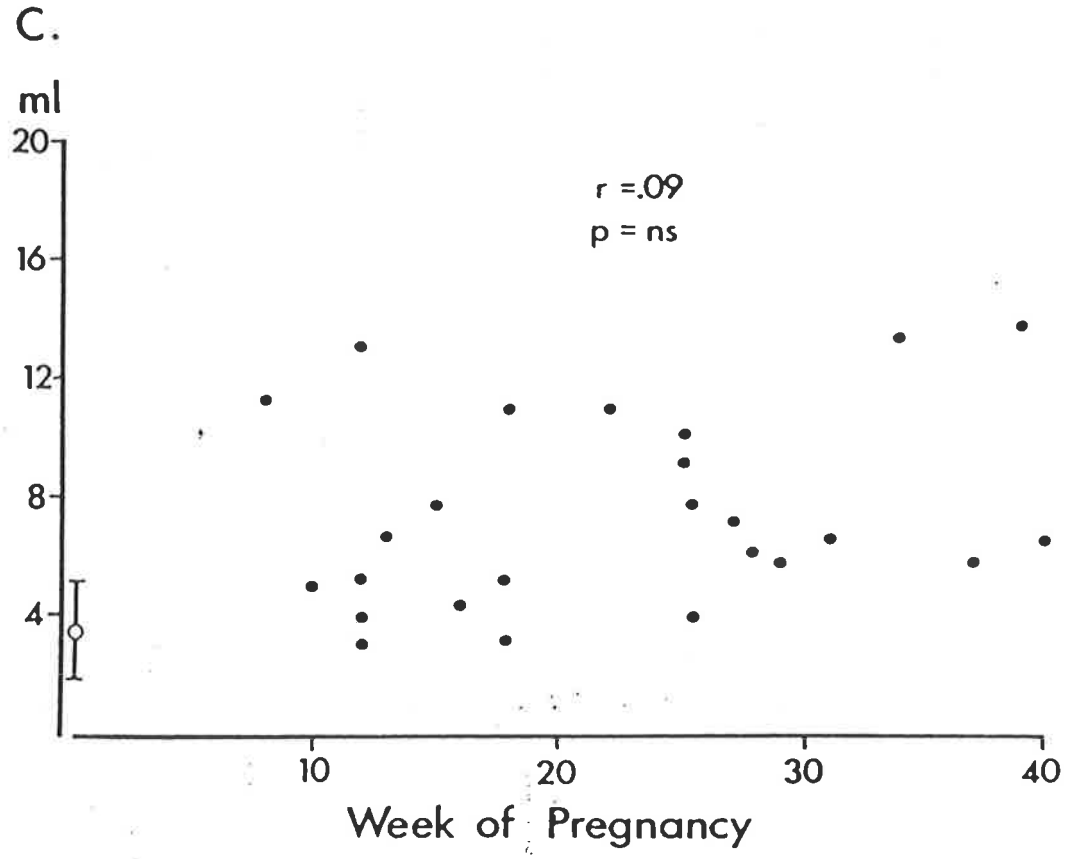

Figure 4C: Average hourly gallbladder volumes are plotted against week of pregnancy. Average hourly volumes (panel C) were not significantly correlated with week of pregnancy.

⊥○⊥ = mean ± standard deviation of controls

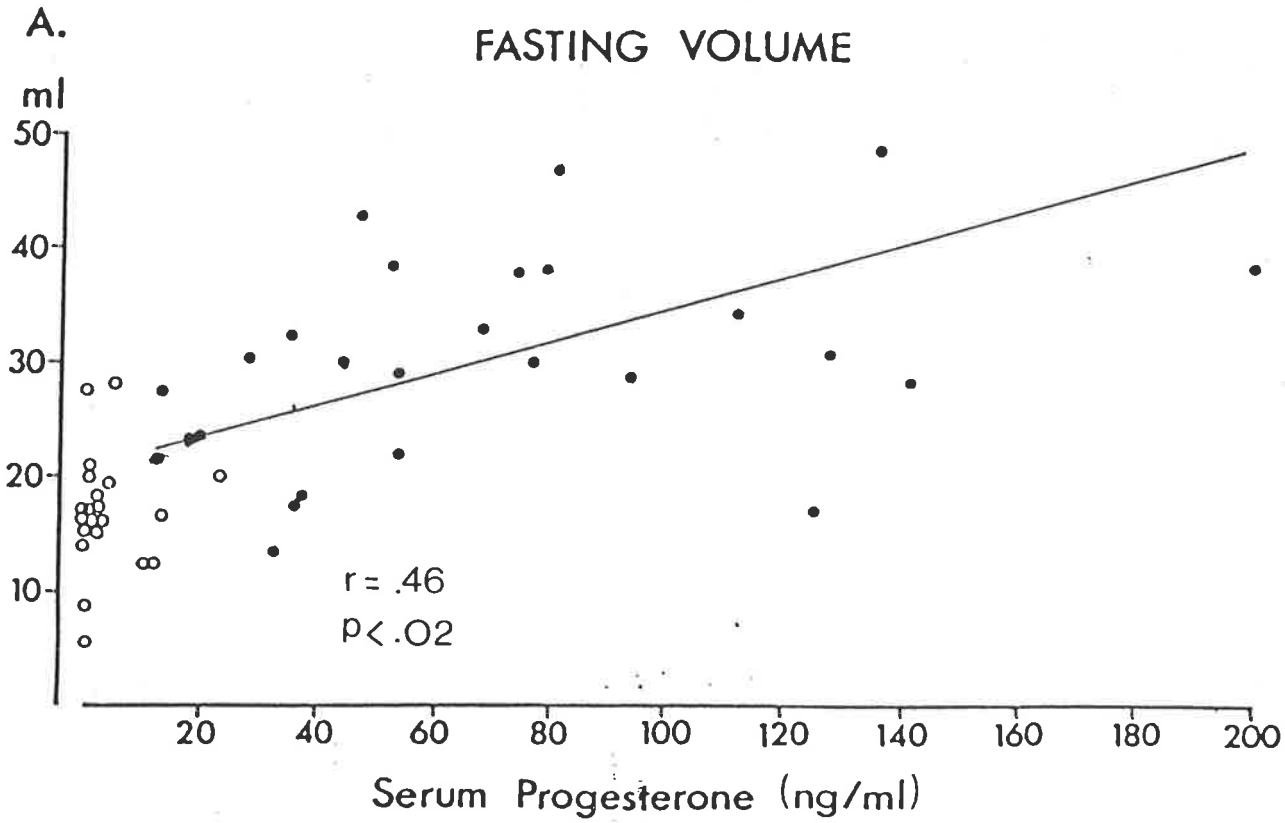

Figure 5A: Fasting gallbladder volumes of pregnant (●) and control (0) subjects are plotted against serum progesterone levels. Fasting volumes directly correlated with serum progesterone levels.

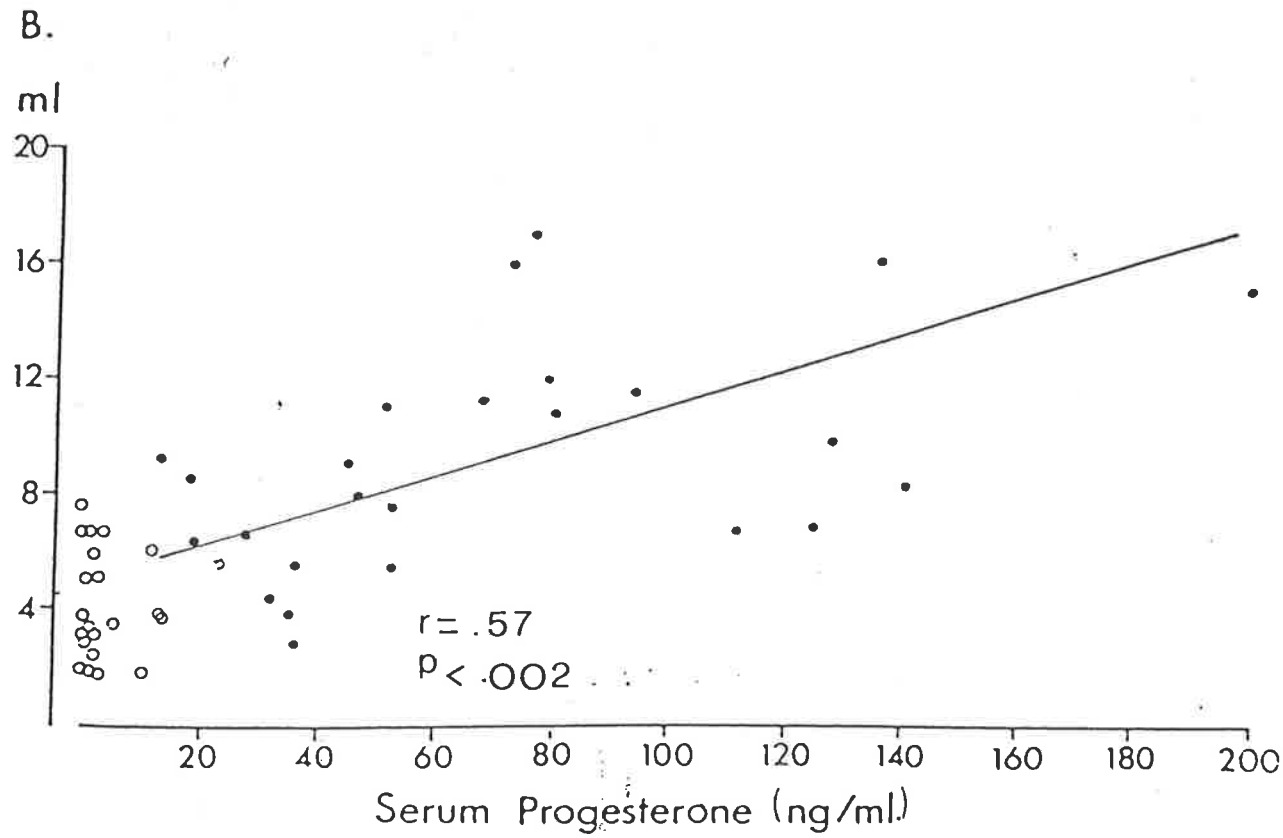

Figure 5B: Residual volumes of pregnant (●) and control (0) subjects are plotted against serum progesterone levels. Residual volumes directly correlated with serum progesterone levels.

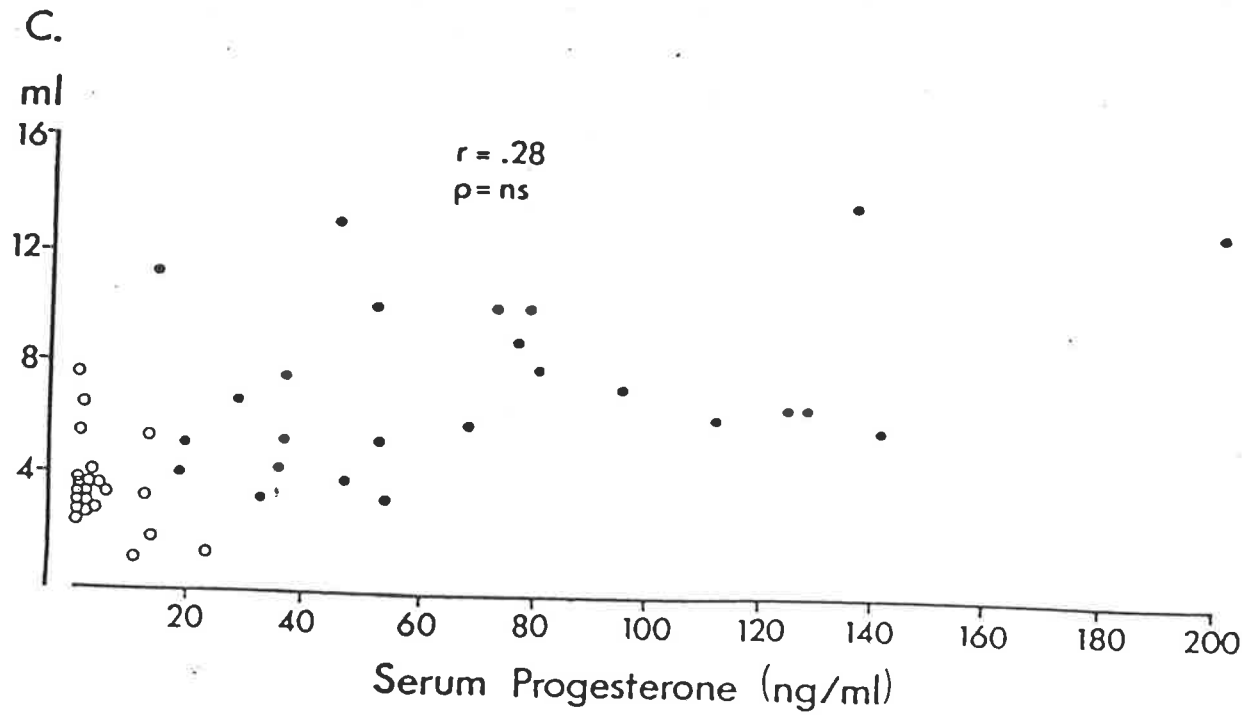

Figure 5C: Average hourly gallbladder volumes of pregnant (●) and control (O) subjects are plotted against serum progesterone levels. In pregnant women average hourly volumes did not directly correlate with serum progesterone levels.

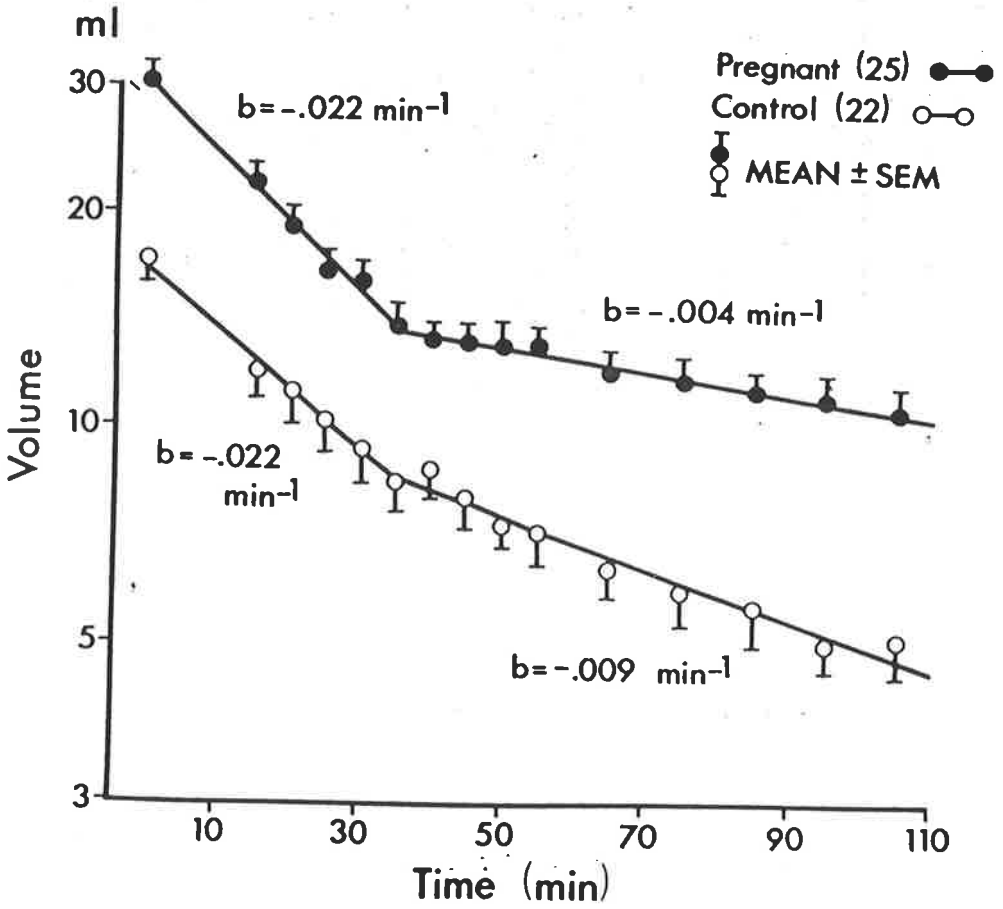

FIGURE 6: The gallbladder volumes of pregnant (●) and control (O) subjects before and after breakfast are plotted against time. The late rate of gallbladder emptying, calculated by ln/linear regression of gallbladder volume vs. time, is slower in pregnant subjects ($F_{(n1-2,n2-2)} = 8.513$; $p < 0.005$).

TABLE III

INDICES OF GALLBLADDER FUNCTION IN POSTPARTUM WOMEN

	Week of Study		Indices of gallbladder function							
			FV (ml)		RV (ml)		HV (ml)		$B(min^{-1})$	
Subject	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
AMil	34	1.8	38.0	15.8	15.1	4.0	13.5	7.8	0.021	0.013
DFit	28	9.0	34.7	9.9	6.8	2.7	6.3	3.0	0.020	0.020
MSco	37	8.0	28.3	26.1	8.2	5.1			0.012	0.016
SMac	39	4.0	48.7	20.6	16.1	5.0	13.9	5.1	0.014	0.023
KLic	18	0.3	22.0	51.2	5.5	26.3	3.3	26.3	0.025	0.006
		4.0		22.0		5.5		3.2		0.025

Abbreviations: See legend for Table 2; Pre = week of pregnancy, Post = week after delivery

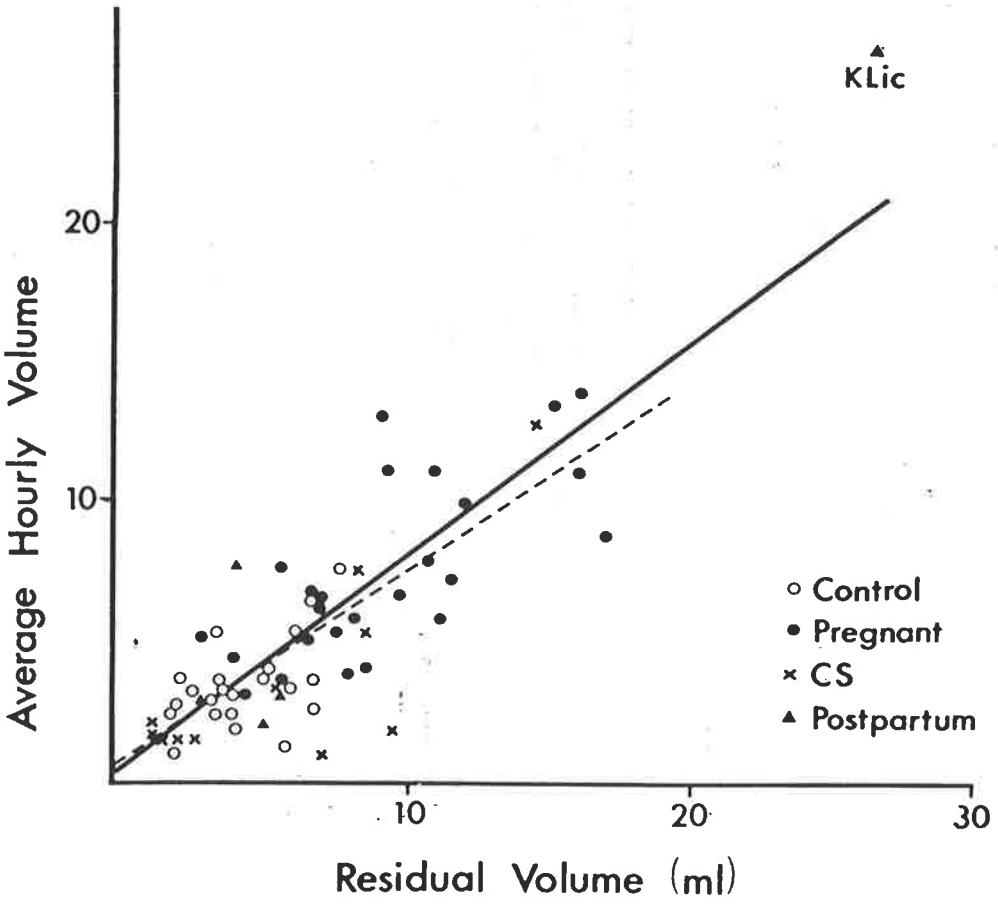

FIGURE 7: Average hourly volumes (HV) are plotted against residual volumes (RV) from all 63 studies (22 controls, 25 pregnant women, 5 postpartum women, and 11 contraceptive steroid users). The linear relationship with subject KLic (—) was HV = 0.78 RV + 0.3 ($r=0.88$, $p \ll 0.001$) and without KLic (--) it was HV = 0.69 RV + 0.6 ($r=0.72$, $p < 0.001$). CS = women taking contraceptive steroids.

with any index of gallbladder function.

In all 63 studies, hourly and residual volumes were similar (Figure 7) Thus, the residual volume after the morning meal approximated the hourly volume the remainder of the day.

B. GALLBLADDER AND SMALL INTESTINAL REGULATION OF BILIARY LIPID SECRETION DURING INTRADUODENAL INFUSION OF STANDARD AMINO ACID AND LIQUID FORMULA STIMULI

(i) Methods:

(a) Subjects and Procedures

Five healthy non-obese women, aged 21 to 28 were each studied twice. After they fasted overnight, a triple lumen polyvinyl tube was passed through the nose and positioned in the duodenum by fluoroscopy with genital shielding so that the proximal infusion orifice was adjacent to the ampulla of Vater, 12 cm from the distal collecting orifice. The third lumen was filled with mercury from proximal to distal orifice and sealed at both ends to aid in fluoroscopic guidance. After the tube was in position, either a mixed amino acid solution with 5mg BSP/100 ml as marker or liquid formula with beta-sitosterol as marker was continuously infused through the proximal lumen (See Appendix, Table 1a). The amino acid solution contained 5% (wt/vol) glucose and 4.3% wt/vol of mixed amino acids (Shaffer and Small 1977). Each litre of liquid formula contained 132 g of powdered skimmed milk, 810 ml of distilled water, 140 ml of polycose (Ross Laboratories, Columbus, OH) and 550 ml of corn oil. The mixture was sonicated with a sonifier cell disrupter (Branson Sonic Power, Plainview NY) for 30 min just before infusion. The resultant uniform emulsion was agitated every 30 min

throughout the study. The concentration of beta-sitosterol in the infusate was measured in aliquots taken every hour during the period of infusion and was constant. Liquid formula contains 40% of calories as fat. It has three times more carbohydrate, three times more calories and is slightly more hypertonic than the amino acid solution.

In earlier studies, 10-20% of subjects became nauseated and vomited during infusion of the amino acid solution, (Kern, Everson, De Mark et al 1981). To ensure paired data, each subject was first studied with amino acid so that only those completing the amino acid infusion without incident were studied with liquid formula. Two of seven subjects did not complete the amino acid study because of nausea and vomiting. The interval between the two infusions was 5-10h. Duodenal bile was continuously aspirated from the distal orifice at a rate of 0.5ml/min and each 30-min sample was treated separately. At the bedside, duplicate aliquots of each sample were extracted with 2:1 chloroform/methanol for phospholipid measurement while other aliquots were refrigerated at 4 degrees C for analysis of cholesterol, bile acid and markers. Real time sonographs of the gallbladder were obtained every 30 min by using an ADR model 2131 real-time scanner with a 33.5m Hz multiplexed linear array transducer, 13.5cm length(Advanced Diagnostic Research Corp, Tempe, AZ) (See Appendix). Blood samples for measurement of human pancreatic polypeptide were drawn when sonographs were obtained.

Small intestinal transit was measured by the lactulose breath test (Bond and Levitt 1975) (See Appendix). At the 5th h of study 10g of lactulose in 100ml of water was given as a bolus through the distal orifice. Breath samples for measurement of hydrogen were collected before and every 15 min for 4 h after the lactulose was given.

(b) Analytical Techniqes

Infusate and each 30 min sample were analysed for bile acid, phospholipid, cholesterol and marker. Bile acid concentration was measured spectrofluorometrically by the enzymatic method of Talalay (Hurlock and Talalay 1957), phospholipid colorimetrically by the method of Bartlett (Bartlett 1959) and BSP colorimetrically (Seligson, Marino, Dodson 1957). Cholesterol and beta-sitosterol were measured by gas-liquid chromatography by using coprostanol (5-beta-cholesterol-3-ol) as internal standard (Kern, Eriksson, Curstedt et al 1977). The amino acid solution contained no cholesterol or phospholipid. Liquid formula contained cholesterol (0.1umol/ml) and phospholipid (0.3umol/ml) The cholesterol and phospholipid concentration of each duodenal sample was corrected for infusate cholesterol and phospholipid (See Appendix).

Based on previous studies (Grundy and Metzger 1972, Grundy, Ahrens, Salen 1968) it was assumed that negligible cholesterol and beta-sitosterol absorption occurred over the 12cm perfused segment of bowel.

Gallbladder volume was measured from each gallbladder sonograph (see appendix). Serum human pancreatic polypeptide (HPP) level was determined by radioimmunoassay (see appendix).

Small bowel transit time was defined as the time of rise in breath hydrogen concentration from base line (see appendix). In each experiment, this was subjectively evaluated by eight observers blinded to the results of gallbladder emptying and biliary lipid secretion. Transit time was determined from the appearance of the plot of hydrogen concentration (parts per million) vs, time, and reported as the mean (+/- SD) of these eight

estimations.

(c) Analysis of data

Differences in gallbladder emptying, small bowel transit time serum levels of human pancreatic polypeptide, mean biliary lipid secretion rates and mean molar percent cholesterol during the two infusions were analysed by paired t tests. Data from all five subjects were included in all statistical analyses. Biliary lipid secretory relationships during each infusion were evaluated by linear and non-linear regression analysis; linear; $y=ax + b$; non-linear; $y = x/(b + ax)$, where y and x are hourly lipid secretory rates and a and b are constants.

Investigators have usually ignored biliary lipid output during the first 4 h of study, because of its fluctuations and have calculated secretion rates only after this period. Accordingly the results of this study were divided into the first 4 and last 6 h.

RESULTS

(a) Biliary lipid Secretion

With both infusions, secretion rates were lower in the last 6 h than in the first 4 h due to the high secretion rates in the first 2 h after initiating gallbladder contraction. During both the first 4 and last 6 h of study, the mean hourly secretion rates of bile acid, phospholipid and cholesterol showed considerable variability but were greater during the liquid formula than during the amino acid infusion (Table 4).

TABLE IV

BILIARY LIPID SECRETION RATES

	BA		PL		Ch	
	AA	LF	AA	LF	AA	LF
	μmol/h		μmol/h		μmol/h	
° First 4h						
1	1,815±363	2,761±2720	491± 95	771±917	107±23	157±217
2	1,895±849	4,868±1979	349±218	1,003±356	152±78	282±131
3	2,353±821	2,951± 790	546±245	1,023±390	191±77	303±222
4	1,479±470	3,160±1391	246±119	663±357	115±55	122± 93
5	902±343	3,908±1035	148±105	697±171	55±21	198± 59
P	<0.025		<0.005		<0.05	
† Last 6h						
1	1,258± 382	2,084± 951	238± 93	516±231	57±23	64±21
2	1,258± 508	3,205± 1,151	193± 92	613±144	79±29	136±38
3	1,265± 399	2,690± 740	208±105	801±182	97±34	205±41
4	1,382± 861	3,888± 1,042	207±189	661±176	95±72	128±31
5	986±1,020	4,527± 1,745	157±154	654±206	45±17	182±68
P	<0.02		< 0.001		<0.05	

BA, total bile acid; PL, phospholipid; CH, cholesterol; AA, amino acid infusion; LF, liquid formula infusion.

°Secretion rates measured during the first 4h of infusion of stimulus.

Secretion rates measured during the last 6h of the infusion of stimulus. The higher secretion rates during the first 4h of stimulus infusion are due to the high secretion rates that occur in the first 2h after initiation of gallbladder contraction.

The rates of bile acid and phospholipid secretion were increased more than that of cholesterol during liquid formula infusion, resulting in a lower molar percent cholesterol than during the amino acid infusion in four of five subjects during the first 4h ($p < 0.05$) and in all subjects during the last 6h ($p < 0.05$) (Figure 8). The increments in cholesterol secretion coincident with increments in either bile acid or phospholipid secretion were far smaller during liquid formula infusion (Figure 9 A-C). For these reasons, biliary lipid secretory relationships were quite different with the two infusions. More cholesterol was secreted per micromole bile acid or phospholipid during the amino acid infusion. The best fit of the combined data (both amino acid and liquid formula for cholesterol vs. bile acid, cholesterol vs. phospholipid or phospholipid vs bile acid) was the equation for a rectangular hyperbola, $y=x/(b + ax)$ (Table 5). However as bile acid secretion rate increased there was considerable divergence of cholesterol and phospholipid secretion, CH Sec max = 298 and PL Sec = 5555 (Table 5). As bile acid secretion increased there was little change in the ratio of phospholipid to bile acid but the ratio of cholesterol to bile acid decreased considerably.

(b) Gallbladder Emptying

In each subject gallbladder emptying was more complete with liquid formula infusion during both the first 4 ($p<0.05$) and last 6h ($p<0.02$) (Figure 10). Gallbladder volumes during amino acid infusion fluctuated considerably and there were periods of apparent refilling. On the other hand, during liquid formula infusion gallbladder emptying was prompt and more complete, and showed little fluctuation (Figure 11)

In all studies including both amino acid and liquid formula infusions, there were 23 periods, excluding the initial hour in which gallbladder

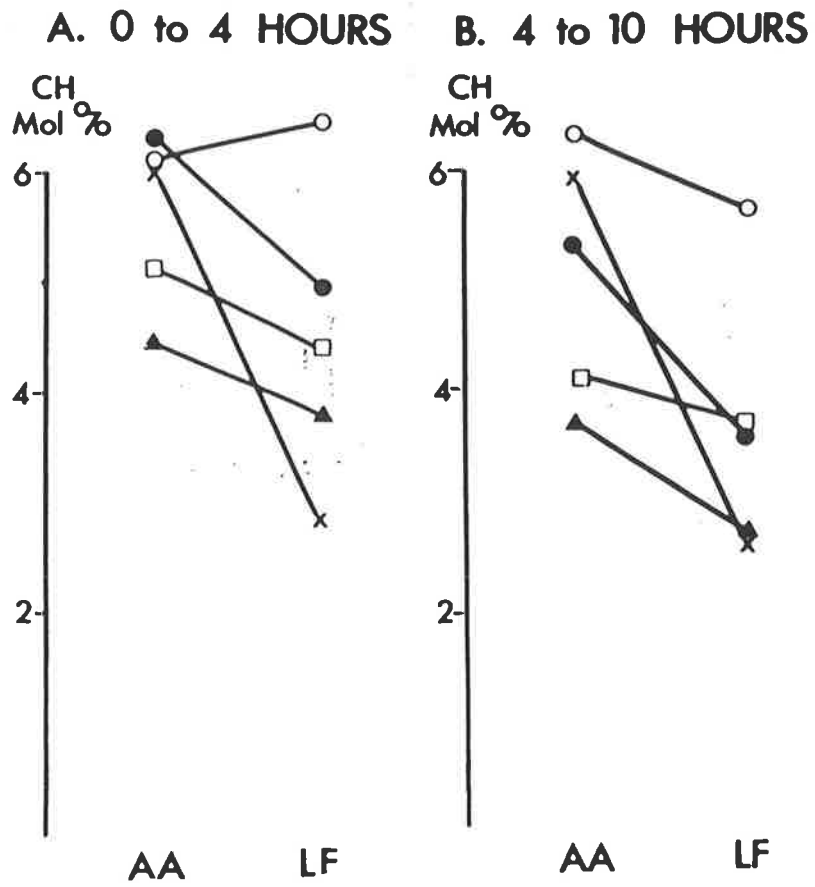

FIGURE 8: The mean molar percent cholesterol (CH mol %) was lower with liquid formula during the first 4 (panel A) and last 6h (panel B) of infusion. AA, amino acid infusion; LF, liquid formula infusion. Different symbols represent different subjects.

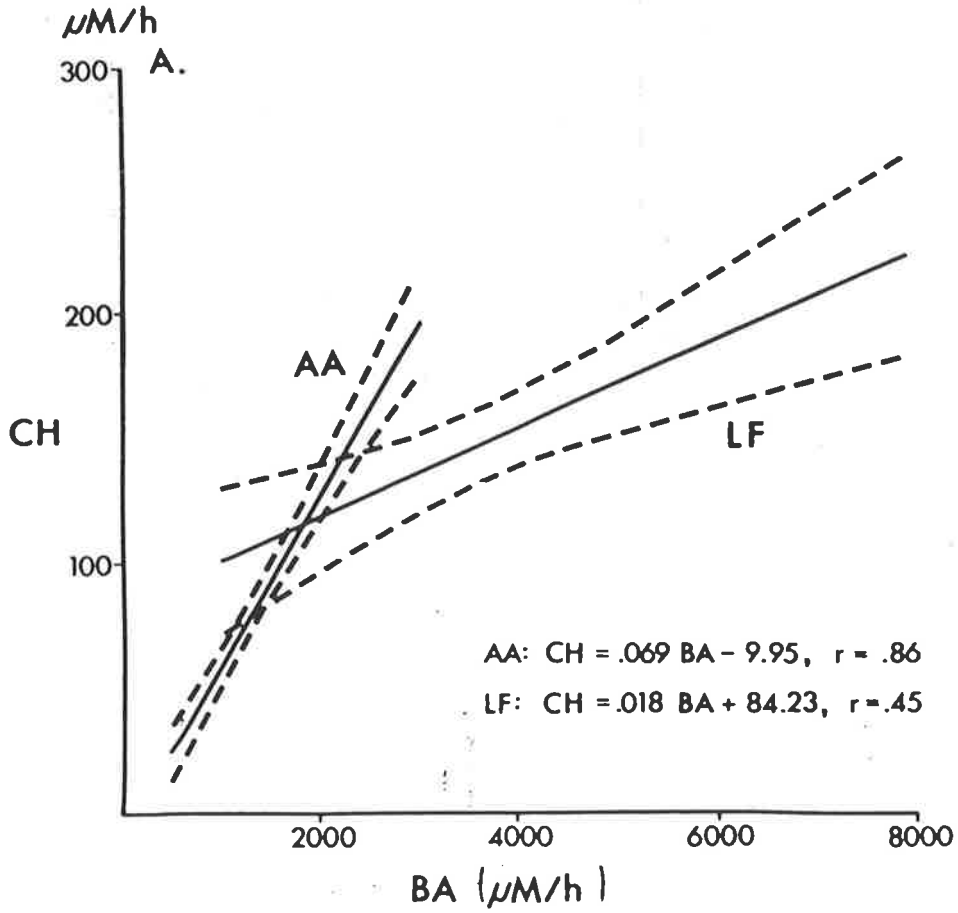

Figure 9A: Hourly secretion rates of cholesterol (CH) bile acid (BA) are plotted against each other. The solid regression lines represent the fit of the data to the equation y=ax + b, where y and x are secretion and a and b are constants. The 95% confidence interval for the slope of each regression is given by the dotted lines. Increments in BA secretion were associated with greater increments of CH secretion during AA infusion (p=52) than during LF infusion (n=57).

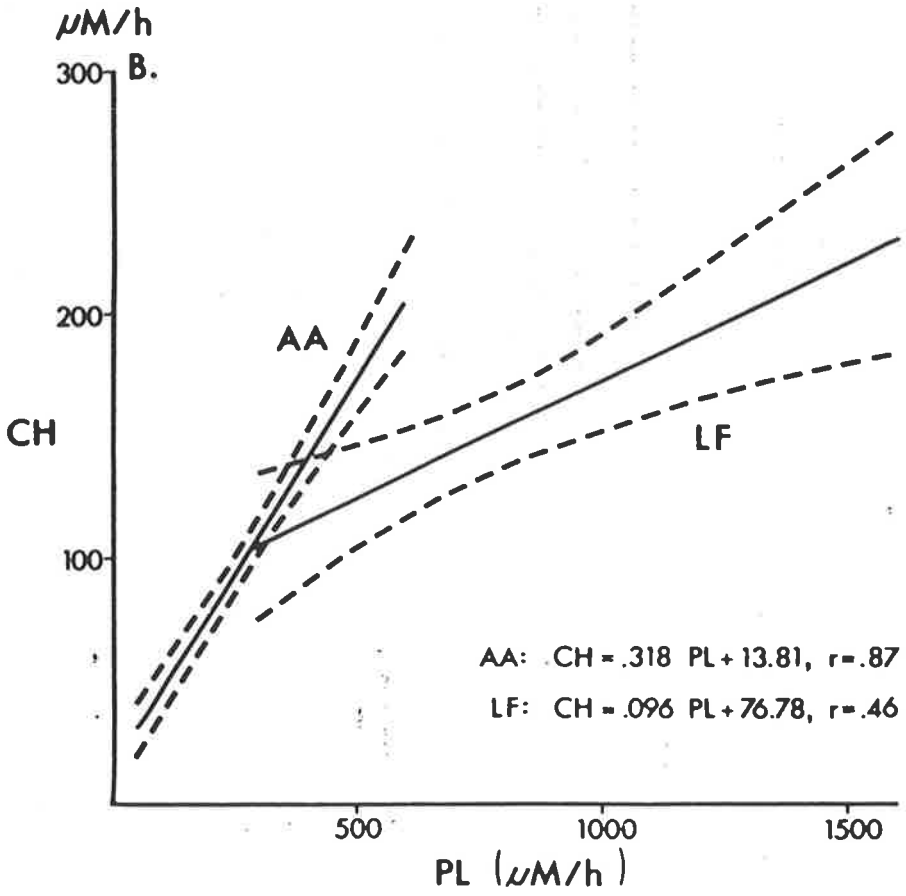

Figure 9B: Hourly secretion rates of cholesterol (CH) and phospholipid (PL) are plotted against each other. Increments in PL secretion were associated with greater increments of CH secretion during AA infusion (n=47) than during LF infusion (n=58).

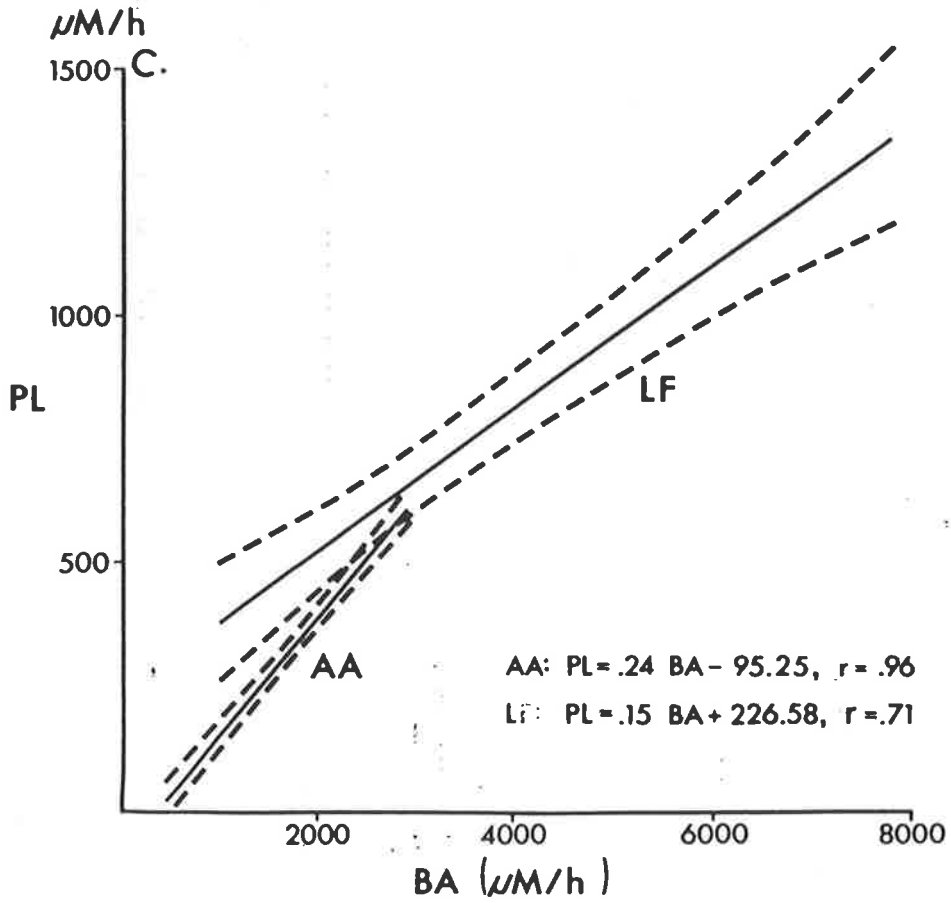

Figure 9C: Hourly secretion rates of bile acid (BA) and phospholipid (PL) are plotted against each other. Approximately the same relationship was observed between PL and BA secretion as for PL and CH during infusion of either AA (n=47) or LF (n=58).

TABLE V

REGRESSION ANALYSIS OF SECRETORY RELATIONSHIPS OF COMBINED DATA FROM AMINO ACID AND LIQUID FORMULA INFUSIONS

		Equations°	
		$y=x/(b+ax)$	$y=ax+b$
CH-BA ‡	RSS §	2.19	2.55
	Ch Sec_{max} ‖	298	-
CH-PL	RSS	2.10	2.38
	Ch Sec_{max}	269	-
PL-BA	RSS	4.11	4.26
	PL Sec_{max}	5,555	-

CH, cholesterol; BA, bile acid; PL, phospholipid.

°$y=ax + b$ describes a linear relationship between x and y.

$y = \frac{x}{b+ax}$ describes a hyperbolic relationship between x and y.

‡ The secretion rate (micromoles /hour) of the lipid to the left of the hyphen is always plotted on the y=axis while that of the lipid to the right of the hyphen is plotted on the x-axis.

§RSS, residual sum of sqaures x 10^{-5}. The equation that better fits the data is the one with the lower RSS.

‖ Sec_{max} is the maximum secretion rate (micromolars/hour) of y-axis lipid as the secretion rate of the x-axis lipid approaches infinity.

Sec_{max} equals 1 ÷ a in the equation, $y = x/(b + ax)$.

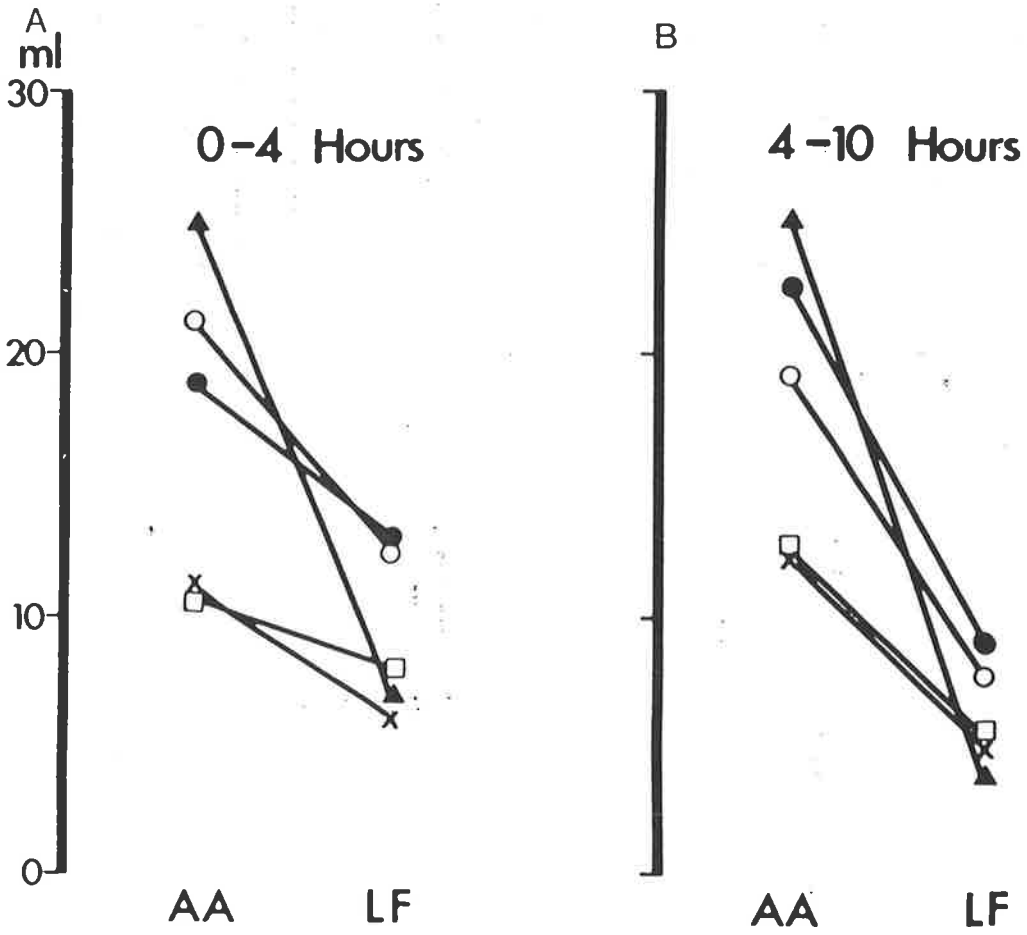

FIGURE 10: Gallbladder volume (millitres) during both the first 4 (panel A) and last 6h (panel B).

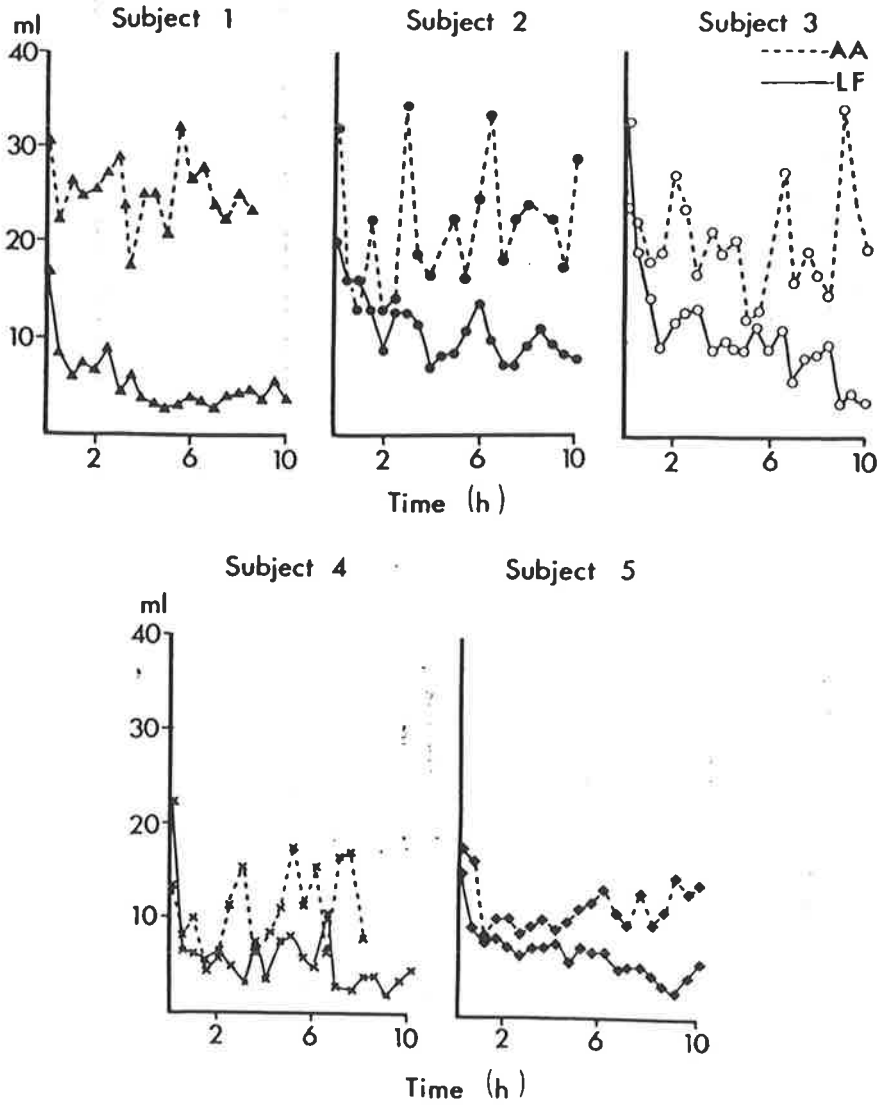

FIGURE 11: Gallbladder volume is plotted against time. Volumes during both amino acid (dotted line) and liquid formula (solid line) infusion are shown for each subject.

emptying was 5ml or greater. During 22 of these periods there was a concomitant increase in bile acid output.

(c) Small Bowel Transit Time

In four of five subjects, small bowel transit time was slower during amino acid infusion (Table 6).

(d) Human Serum Pancreatic Polypeptide

In four of five subjets levels of pancreatic polypeptide were two to threefold higher during both the first 4 and last 6 h with liquid formula infusion (Table 7). Subject 5 had no change in pancreatic polypeptide levels from baseline with either infusion. Nonetheless, this subject had findings which were similar to the rest of the group: increased gallbladder emptying, faster intestinal transit and increased bile acid secretion in response to liquid formula. In the other subjects, the pattern of the pancreatic polypeptide response was that of a sustained elevation from base line with minimal fluctuations.

C. THE EFFECTS OF CHRONIC OESTROGEN ADMINISTRATION ON BILIARY LIPIDS,BILE ACIDS AND GALLBLADDER FUNCTION IN POST-MENOPAUSAL WOMEN

(i) Methods:

(a) Subjects

TABLE VI

SMALL INTESTINAL TRANSIT TIMES DURING INFUSION OF STIMULI

Subjects	Transit time, min	
	AA	LF
	Mean±SD	
1	39±14	47±15
2	90± 0	45± 0
3	99± 8	21± 8
4°	240± 0	68± 8
5	45± 0	30± 0
P	<0.15	

Mean ± standard deviation of the transit time as determined by eight observers.

°No hydrogen rise from base line was detected after 240 min. Since this subject was capable of producing hydrogen, as observed during the liquid formula infusion, the lack of hydrogen rise during amino acid infusion indicates that her transit time was >240 min.

TABLE VII

HUMAN PANCREATIC POLYPEPTIDE RESPONSE TO INFUSION OF STIMULI

	HPP, pg/ml			
	First 4h		Last 6h	
Subject	AA	LF	AA	LF
1	91±21	319±161	93±16	424±94
2	150±60	264±104	108±22	198±59
3	91±36	221±262	107±38	213±86
4	43±16	187± 80	56±21	263±97
5	28± 7	26± 3	36±13	38±11
P	<0.025		<0.005	

HPP, human pancreatic polypeptide; AA, amino acid infusion; LF, liquid formula infusion. (mean±SD)

Nine post menopausal women, aged 28-47 were studied off and on Premarin(Ayerst) They had been taking conjugated equine oestrogens for at least 4 weeks (mean dose 1.39 mg daily) but no other drugs. To ensure paired data each subject was first studied at the end of four weeks oestrogen abstinence and again after six weeks of oestrogen intake.

(b) Gallbladder concentration and emptying after IV CCK

On day 1 of the study a triple lumen tube was positioned by fluoroscopy after an overnight fast. The proximal infusion orifice was adjacent to the ampulla of vater, 12 cm from the distal orifice. After the tube was positioned, distilled water with the nondiffusable marker BSP (5 mg/ml) was infused at a rate of 3-4 ml/min. Duodenal bile drained continously by gravity from the distal orifice. The gallbladder was stimulated to contract by continuous IV CCK-8 infusion (.02 ug/kg/h) over 90 min and gallbladder bile was aspirated for cholesterol saturation index, bile acid distribution by GLC and cumulative bilirubin output. Simultaneously, the amount of bile expelled and the rate of gallbladder emptying was estimated by real time ultrasound. Five min samples of bile were collected for 90 min. Aliquots were taken from vortexed samples and the residual bile was returned to the duodenum.

(c) Analytical Techniques

Each 5 min sample was analysed for bilirubin by the method of Rand and Di Pasqua (1962).

The concentration of bilirubin in gallbladder bile was determined as follows.

The 5 min collections of duodenal bile are analysed for bilirubin output by:

$$\text{bili (mg/5ml)} = \frac{(B)D \times (M)I \times R}{(M)D}$$

where (M) D= duodenal marker concentration (B) D = duodenal bilirubin concentration, (M)I = infused marker concentration R= mls markers solutions infused over 5 min. Since gallbladder emptying follows a first order decay function,the cumulative exponential function Bt = C(1-e-b(t-tL)) describes the output of gallbladder bilirubin into the duodenum, where B=cumulative milligrams of bilirubin put out at time t, TL time lag in billirubin output, C=total milligrams of bilirubin emptied, and b=the rate constant at which gallbladder contents enter the duodenum (when gallbladder emptying is complete b= the rate constant of gallbladder emptying). The linear parameter, C and the two non-linear parameters, b and TL are estimated from computer analysis of the plot of cumulative bilirubin output versus time. Since the amount of bilirubin ejected from the gallbladder, C and the volume (VE) of bile ejected from the gallbladder can be calculated one can estimate the concentration of gallbladder bilirubin (B) GB by calculating

(B) GB = C/VE

(d) Biliary Lipid Secretion

Liquid formula (see appendix) was infused following 90 min of the IV CCK-8 stimulus. The formula was infused over 8 h through the proximal port with beta-sitosterol as a marker. Duodenal bile was continuously aspirated from the distal orifice at a rate of 0.5 ml/min and each 30 min sample was analysed for biliary lipids. (see appendix) Real time sonograhs of the gallbladder were obtained every 30 min and volumes measured (see

appendix).

On day 3 another sample of gallbladder bile was collected for lithogenic index determined by the method of Hegardt and Dam(1971) and Holzbach, Marsh, Oleszewski et al(1973).

(e) Bile Acids

A sample of bile obtained after gallbladder stimulation by CCK-8 was added to methanol and 4N NaOH with 5-beta cholanic acid as an internal standard and hydrolyzed. The bile acids were then extracted, methylated with diazomethane (Back, Sjovall, Sjovall 1974) or with dimethoxypropane (Ali and Javitt 1970) and trimethylsilyl or acetate derivatives (Makita and Wells 1963, Roovers, Evrard, Vanderhaeghe 1968) were prepared. GLC was performed on 6ft glass columns at 220 degrees C with 1% HiEff 8BP (Applied Science Laboratories, Inc., State College, Pa.) on 100/120 mesh gas chrom Q with helium as the carrier gas at 30 ml/min (Kern, Eriksson, Curstedt et al 1977).

(f) Gallbladder volume and emptying with standard meals

On day 5 of the study gallbladder storage and emptying throughout the day and night was measured sonographically during ingestion of 3 standard meals (as described previously).

(g) Analysis of data;

Data are expressed as mean +/- SD. Differences between control and treatment periods were evaluated by the Wilcoxon signed rank test.

(ii) RESULTS:

(a) Gallbladder emptying

Table 8 shows the measurements of gallbladder volume and emptying. There was rapid gallbladder emptying over the first 30 min of the CCK-8 infusion but no significant differences occurred between the on and off oestrogen periods. Slower changes in gallbladder volume were seen after a mixed solid-liquid meal but there were no significant changes in emptying rates, fasting, residual or hourly volumes with chronic oestrogen ingestion.

(b) Gallbladder concentration

There was no apparent effect of oestrogens on bilirubin in gallbladder bile (Figure. 12).

(c) Biliary lipid secretion

The mean lithogenic indices of gallbladder bile in the on and off oestrogen periods were similar (Figure 13). There were no significant effects of oestrogen on the mean hourly secretion rate of any biliary lipid (Figure 14).

(d) Bile acids were assessed by GLC for distribution of cholic acid, chenodeoxycholic acid and deoxycholic acid. The small amounts of secondary bile acids (5 to 12%) were disregarded in calculating percentages of the major bile acids. There were no changes in the bile acid distribution associated with the use of oestrogens.

TABLE VIII

GALLBLADDER EMPTYING KINETICS IN POST-MENOPAUSAL WOMEN ON AND OFF PREMARIN

	FV(ml)		RV(ml)		$b(min^{-1})$	
MEAL						
Off	19 ± 9		6 ± 6		.034 ± .030	
On	16 ± 5	NS	5 ± 5	NS	.025 ± .016	NS
IV-CCK 8						
Off	18 ± 7		3 ± 2		.055 ± .029	
On	17 ± 7	NS	2 ± 2		.059 ± .049	NS

FV = Fasting Volume
RV = Residual Volume
b = Rate Constant of Emptying
NS = Not Significant (mean±SD)

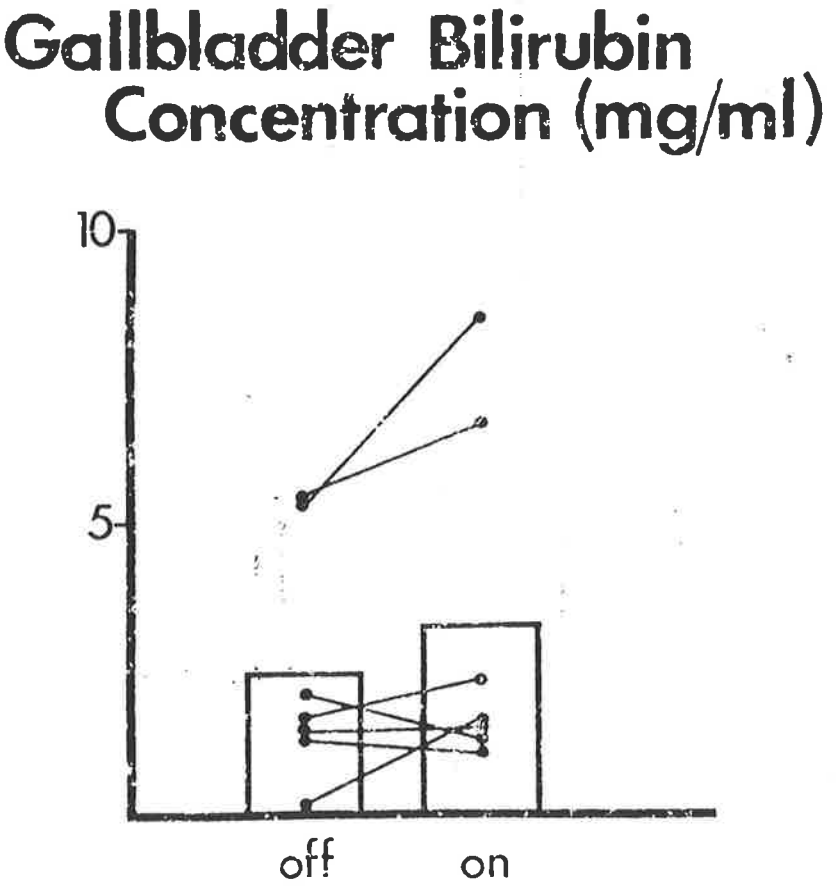

FIGURE 12

Gallbladder bilirubin concentrations on and off premarin.

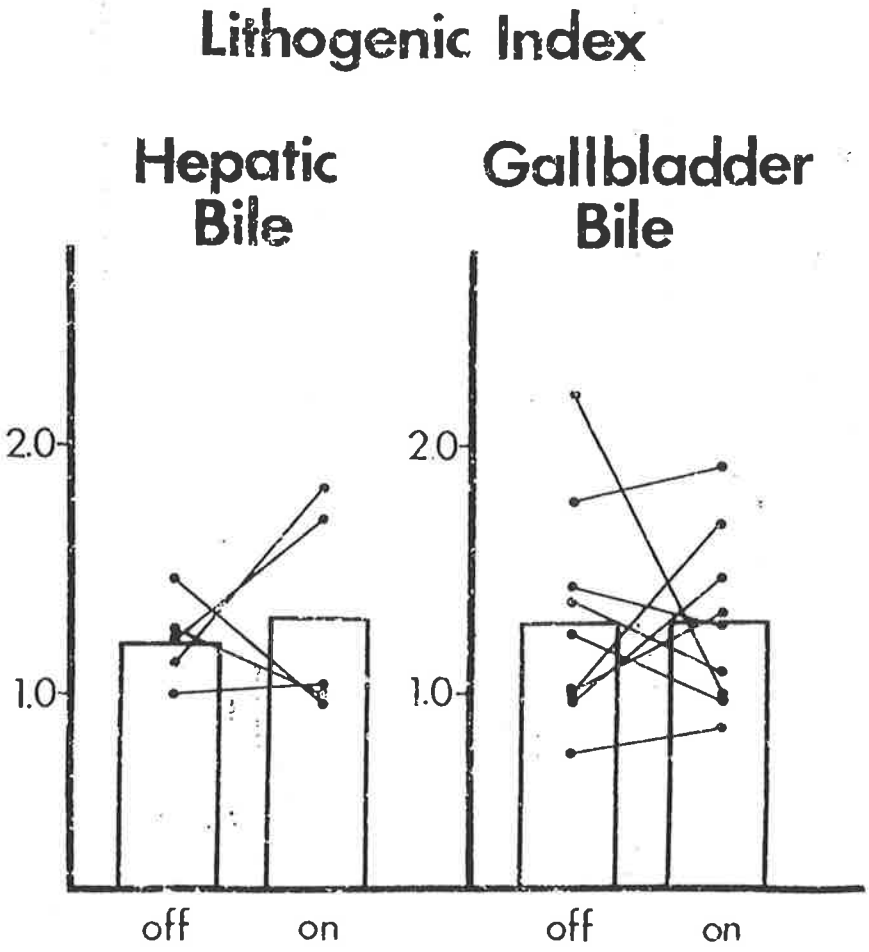

FIGURE 13

Lithogenic index of fasting hepatic and gallbladder bile on and off Premarin.

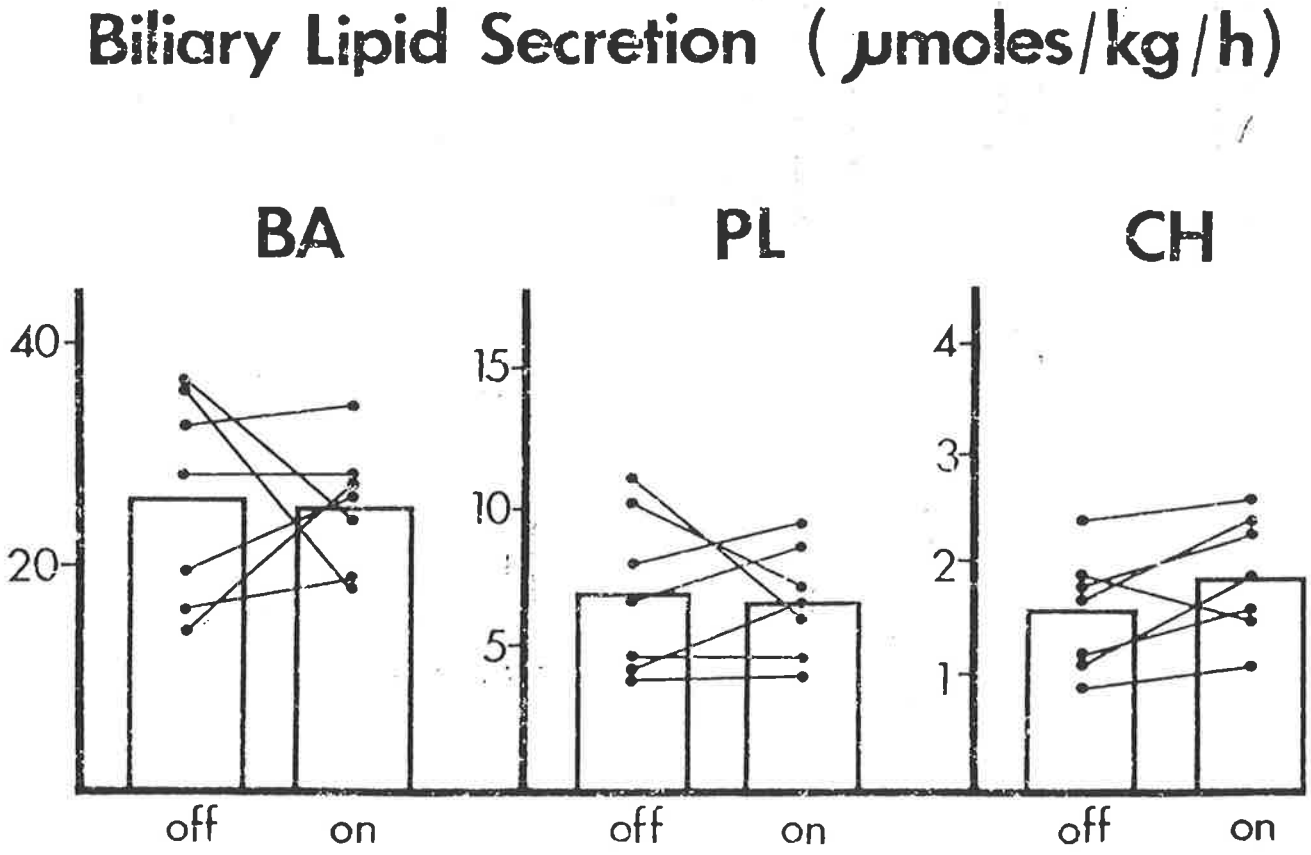

FIGURE 14

Biliary lipid secretion on and off Premarin. BA: Bile acid; PL: Phospholipid; CH: Cholesterol.

D. OROCAECAL TRANSIT TIME IN HUMAN PREGNANCY

(i) METHODS:

(a) Subjects

Fifty nine studies were performed (Table 9) in 27 healthy non-obese white women whose ages ranged from 16-34. Of 8 subjects initially studied in the first trimester, 4 were subsequently studied in the second trimester, 5 in the third trimester and 5 postpartum. Of 8 subjects initially studied in the second trimester 6 were studied in the third trimester and 5 postpartum. Seven of 11 were initially studied in the third trimester and postpartum.

(b) Gastrointestinal transit time

This was measured by the lactulose hydrogen breath test (Bond and Levitt 1975; La Brooy, Male, Beavis et al 1983; Solomons, Viteri, Hamilton 1977) (see Appendix).

Fasting serum progesterone levels were detemined by radioimmunoassay at Endocrine Sciences, Tarzana, California.

(c) Analysis of data

Group data were evaluated by Student's t-test for unpaired data,and serial studies by paired t-test.

(ii) RESULTS:

Mean gastrointestinal transit time was 99 min in the first trimester, 125

TABLE IX

DISTRIBUTION OF OROCAECAL TRANSIT TIME

STUDIES

Initial Study	Serial Studies			
	TM1	TM2	TM3	PP
TM1	(8)	4	5	5
TM2	-	(8)	6	5
TM3	-	-	11	7
TOTALS	8	12	22	17

ABBREVIATIONS: TM1 = first trimester, 7-13 weeks; TM2 = second trimester, 15-26 weeks; TM3 = third trimester, 27-39 weeks; PP = postpartum, 2-12 weeks. ( ) = period of initial study.

min in the second trimester, 137 min in the third trimester and 75 min postpartum (Table 10). There was considerable variation within each study group but variation was similar between study groups, the range of the percent coefficient of variation being 39 to 44%. The significance of differences in transit time between study groups was evaluated by both Student's t-test for unpaired data (S-test) and paired t-test for serial studies (p-test), (Table 11). The results of these 2 statistical analyses were identical. There was a significant increase in transit time from first to second trimester, a slight but insignificant increase from second to third trimester, and a marked decline postpartum. There was no significant difference between first trimester and postpartum transit time. An example of serial studies of breath hydrogen curves for one subject is shown in Figure 15. There was a sharp sustained rise in breath hydrogen at 45 minutes in the first trimester, 90 minutes in the third trimester and 30 minutes postpartum.

Serial transit time studies of women who were initially studied in the first trimester is shown in Figure 16. Transit time is expressed as the percent of maximum transit time observed for each individual. This value is plotted against the week of pregnancy or week postpartum. In each case, the maximum transit time was achieved in either the second or third trimester from 15 to 40 weeks. In 4 of the 5 subjects transit time decreased postpartum.

The relationship of transit time to serum progesterone is demonstrated in Table 12. The increment in transit time is greatest as the progesterone increases from 1 to 80 ng/ml. There is no further increment in transit time as progesterone levels increase from 80 to 230ng/ml. However, the wide variation of transit time within each range of serum progesterone precludes accurate determination of a "threshold" level of progesterone, above which

no further increase in transit time occurs.

When mouth to caecum transit time was plotted against serum progesterone for all trimesters and post partum, there was no significant correlation (figure 17). Therefore there is only a trend for transit time to increase with serum progesterone with any significant relationship possibly masked by biological variation.

TABLE X

MEAN AND VARIATION OF OROCAECAL TRANSIT TIME FOR EACH TRIMESTER OF PREGNANCY AND THE POSTPARTUM PERIOD

Study Periods	OROCAECAL TRANSIT TIME			
	N	$\overline{X}$	SD	CV (%)
		(min)		
TM1	8	99	39	39
TM2	12	125	48	38
TM3	22	137	58	42
PP	17	75	33	44

ABBREVIATIONS: N = Total number of subjects studied, $\overline{X}$ = mean, SD = Standard deviation, CV = coefficient of variation (SD $\div$ $\overline{X}$ x 100%), TM1 = first trimester, TM2 = second trimester, TM3 = third trimester, and PP = postpartum.

TABLE XI

STATISTICAL ANALYSIS OF DIFFERENCES IN OROCAECAL TRANSIT TIME BETWEEN STUDY PERIODS

	Study Period B					
	TM2		TM3		PP	
Study Period A	df	P	df	P	df	P
TM1						
S-Test	18	< .025	28	< .005	23	NS
P-Test	3	< .05	4	< .05	4	NS
TM2						
S-Test		-	32	NS	27	< .001
P-Test		-	9	NS	8	< .001
TM3						
S-Test		-	-		37	< .005
P-Test		-	-		15	< .001

ABBREVIATIONS: df = degrees of freedom, P = probability value, S - test = Student's t-test of the difference between study periods A and B, and P-test=paired t-test of the difference in serial studies between study periods A and B.

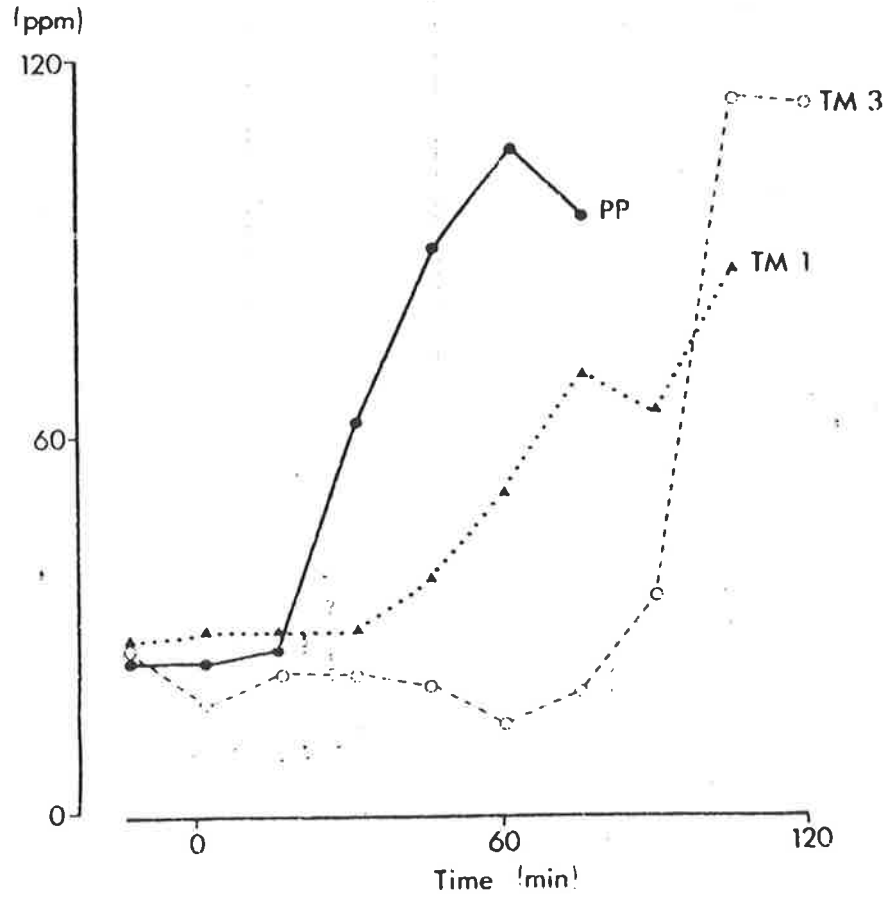

FIGURE 15

Example of serial lactulose - hydrogen breath tests for one subject. ppm is the concentration of H_2 in exhaled breath. Lactulose (10gm) was administered at time = 0.

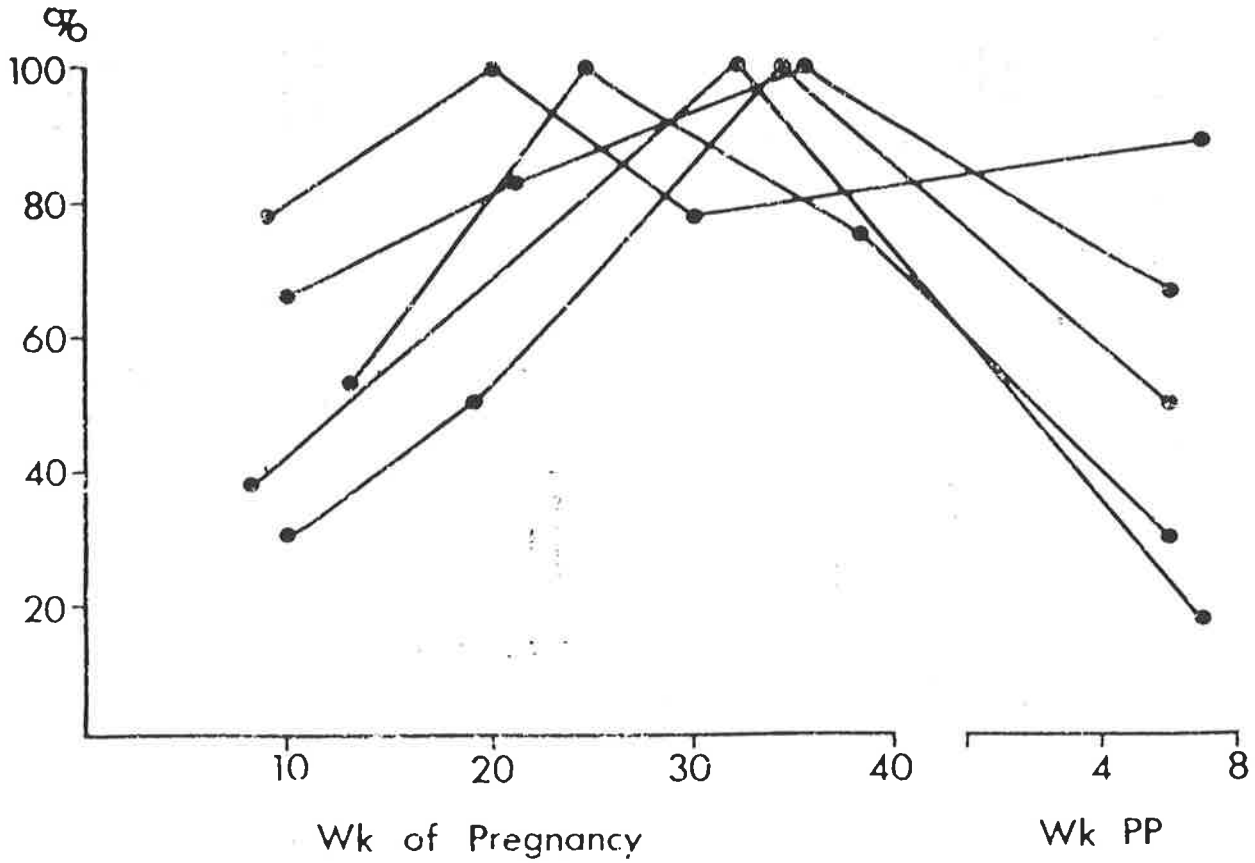

FIGURE 16

Serial measurements of orocaecal transit in women who were initially studied in the first trimester. % of maximum transit time is plotted on the ordinate. In each case maximum transit time occurred in late pregnancy. First trimester and postpartum transit times were similar.

TABLE XII

CHANGES IN MEAN OROCAECAL TRANSIT TIME WITH INCREASES IN SERUM PROGESTERONE LEVELS

Subjects[1]	N	Progesterone (ng/ml)	Transit Time (min)
Postpartum	15	1	72 ± 33
Pregnant	3	20 - 40	113 ± 35
	15	40 - 80	132 ± 57
	13	81 - 230	122 ± 50

[1]Serum for measurement of progesterone level was obtained in only 46 or the 59 studies. Serum was not obtained in two TM1, three TM2, six TM3 and two PP studies.

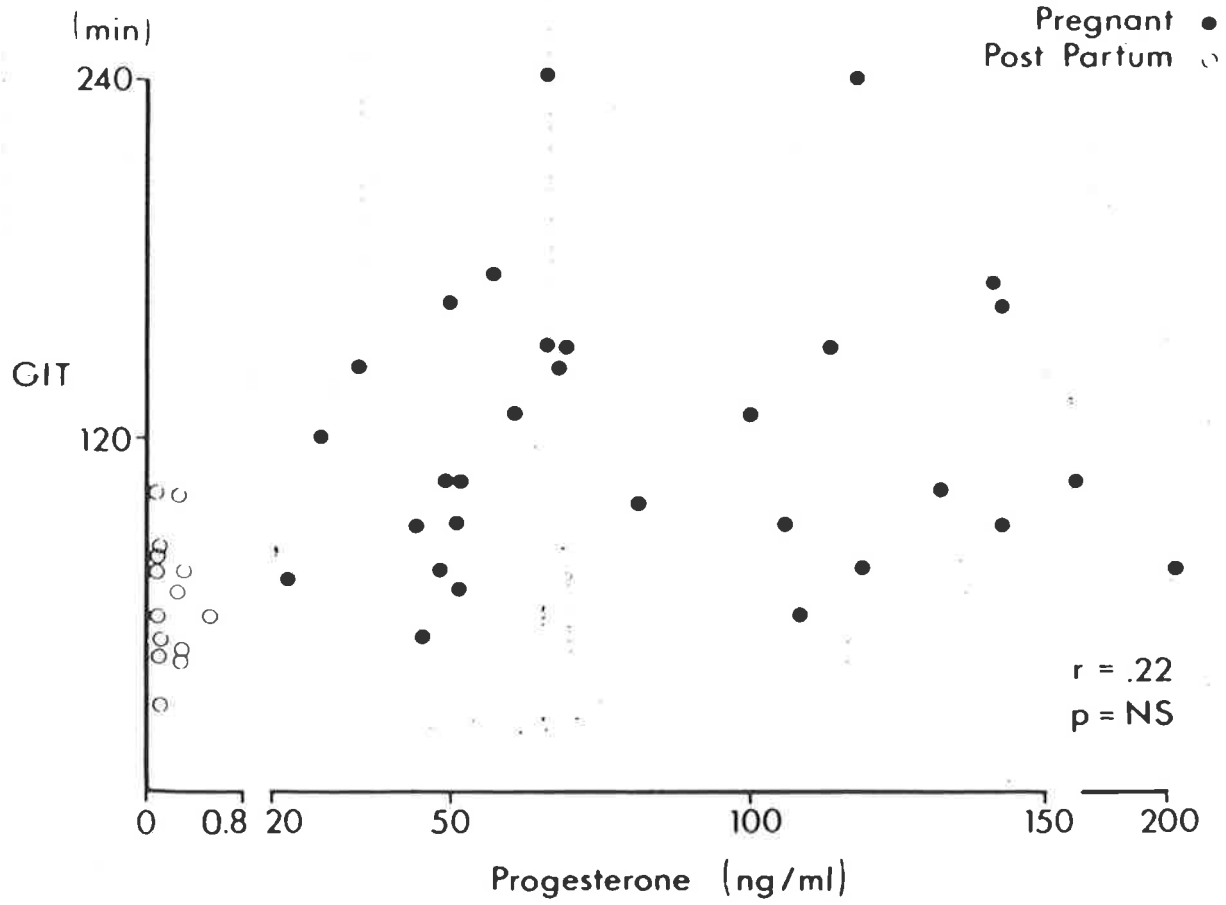

FIGURE 17 : Mouth to caecum transit time (GIT) is plotted against serum progesterone for all subjects.

E.CO-ORDINATION OF GASTRIC AND GALLBLADDER EMPTYING AFTER INGESTION OF A REGULAR MEAL

(i) METHODS:

(a) Subjects

Twelve healthy non-obese volunteers, 6 men aged 23.8 +/- 2.9 yr (x +/- SD) and 6 women, aged 27.0 +/- 2.5 yr were studied. Ultrasound examination of the gallbladder excluded gallstones in all subjects. Since a small amount of radioactive material was used for measuring gastric emptying, women were studied only during menstruation.

(b) Gastric emptying

After an overnight fast subjects ingested a standard breakfast of one egg labelled with 200uCi of 99m Tc-sulphur colloid, 2 slices of bacon, 2 pieces of toast with butter, 240ml of 2% fat (wt/vol) milk, and 10g of lactulose in 100ml of water. The meal contained 610 cal. 44% fat, and 12% protein. Most of the fat is associated with solid components of the meal and even if partially emulsified, would empty from the stomach with the solid phase (Jian, Vigneron, Najea et al 1982). The labelled egg was prepared by mixing raw egg with 200uCi of 99m Tc-sulphur colloid in an aluminium foil tray; the labelled egg was scrambled in and subsequently eaten from the foil tray. To determine binding of 99m Tc-sulphur colloid to the egg, it was mixed vigorously with fresh gastric juice obtained at endoscopy from a separate subject. The mixture was incubated at 37 degrees C for 6 h, mixed,

centrifuged, and radioactivity in the supernatant liquid and in the solid phase was assayed. More than 95% was in the solid phase which is similar to the findings of Kroop, Long, Alavi et al(1979).

(c)Gallbladder emptying

Subjects fasted overnight and real-time gallbladder sonographs were obtained, fasting every 5-10 min for the first 90 min after ingestion of the meal (15 min allowed for eating), and then every 30 min until the gallbladder refilled to at least 70% of its fasting volume. Gastric scintiscans were taken at the end of ingestion of the meal and every 5-10min for the next 2 h. Gallbladder sonographs and gastric scintiscans during this 2h period were obtained with the patient sitting upright. Subjects were then moved to a bed where they lay supine, with the head of the bed elevated 30 degrees for the remainder of the study.

(d) Gastrointestinal Transit time and serum HPP

Breath samples for hydrogen analysis were obtained fasting and every 15 min for 4 h after completing the meal. Venous blood for measurement of pancreatic polypeptide level was drawn through an indwelling venous catheter fasting and every 30 min throughout the study.

(e) Analytical techniques

Gallbladder volume was measured from gallbladder sonographs (see appendix). To compare results betwen individuals, volume was expressed either as percent of fasting volume or as percent of volume immediately after meal ingestion. The time of initiation of gallbladder refilling was determied from inspection of each plot of volume vs time.

Gastric emptying obeyed a zero-order function.(Heading, Tothill, McLoughlin et al, 1976).

$$\frac{DPMt \times 100}{DPMo} = kt + b$$

where DPM is disintegrations per min, DPMo is gastric DPM at completion of the meal, DMPt is DPM at time t, k is the constant of emptying, b is the intercept at t=o and 100 is the conversion factor for percent. k was calculated from linear regression of (DPMt/DPMO) x 100 vs time.

The plot of breath hydrogen vs time was evaluated by two gastroenterologists blinded to the protocol and results of the study. Estimates varied in only 2 cases and by only 15 min each.

(f) Analysis of data

Data are expressed as mean +/- SD. Differences between men and women were evaluated by Student's t-test. Correlations of various parameters were determined by bivariate regression analysis.

(ii) RESULTS:

There was no difference in gallbladder emptying, gallbladder refilling, gastric emptying, or HPP response between men and women (Table 13) Accordingly, data from all 12 studies were pooled for subsequent analysis. The mean orocaecal transit time of the women was twice that of the men, 73.6 +/- 20.2 and 37.5 +/- 22.7 min, respectively. The transit times in these women, who were studied early in the follicular phase of their ovulatory cycles, are similar to those previously reported by Wald et al, using a similar technique in healthy young women in the follicular phase of the ovulatory cycle, 97 +/- 31 min (Wald, Van Thiel, Hoechstetter et al 1981).

TABLE XIII

COMPARISON OF MEASUREMENTS MADE IN MEN AND WOMEN

	Men (6) $x \pm SD$	Women (6)	P
Gallbladder response			
FV (ml)	26.5±9.1	21.0±5.7	NS
b (min^{-1})			
fast	-0.015±0.003	-0.013±0.003	NS
slow	-0.007±0.002	-0.005±0.001	NS
Refilling time (min)			
initial	252±78	246±62	NS
70% FV	333±69	337±37	NS
Gastric emptying rate (% per min)	-0.35±0.10	-0.36±0.11	NS
HPP response			
integrated (pg/ml/min)	120±119	115±88	NS
time to basal (min)	325±52	338±71	NS
Gastrointestinal transit (min)	37.5±22.7	73.6±20.2	0.025

$x \pm SD$ = mean ± standard deviation; p= probability value; FV = fasting volume; b=rate constant of emptying; HPP=human pancreatic polypeptide; NS=not significant

(a) Gallbladder emptying

The time course of gallbladder emptying and refilling for all subjects is shown in Figure 18. A ln/linear plot of gallbladder volume vs time from 0 to 120 min revealed two phases of emptying, an initial fast phase from 0 to 30 min and a second slow phase from 30 to 120 min. The initial fast rate of emptying was 0.015 +/- 0.003 min -1, while the second slow rate was 0.006 +/- 0.001 min -1, the gallbladder remained tonically contracted until refilling began at 249 +/- 67 min (Figure 19). The time interval from initiation of gallbladder refilling to achieving 70% fasting volume ranged from 30 to 180 min (86.1 +/- 52.1; $\bar{x}$ +/- SD).

(b) Gastric emptying

The zero order rate constant of gastric emptying of solids after meal ingestion was 0.35 +/- 0.11% per min and the first order rate constant of the second slow phase of gallblader emptying was 0.006 +/- 0.001 per min (Table 13, Figure 20).Substituting the mean time of initiation of gallbladder refilling (249 min) into the equation defining the rate of gastric emptying, it can be seen that when gallbladder refilling begins, only 13% of solids remain in the stomach. In addition, using the zero-order rate constant, gastric emptying would be complete at 286 min, 1 h before the gallbladder refilled to 70% of original fasting volume 335 min.

Orocaecal transit time did not correlate with fast and slow rates of gallbladder emptying, rate of gastric emptying, time to initiate gallbladder refilling or time of gallbladder refilling to 70% of fasting volume.

The correlation between the percent of bile remaining in the gallbladder and percent of solids remaining in the stomach for each time point was highly significant (Figure 21).

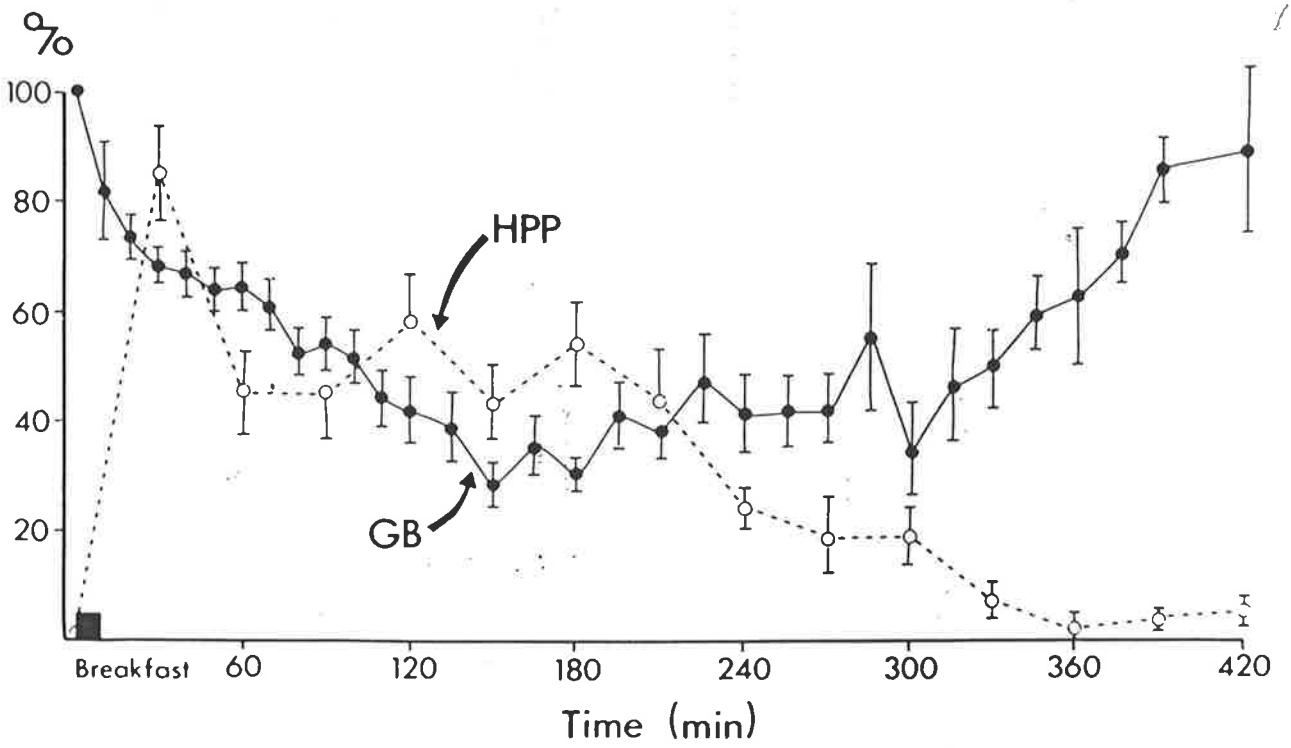

FIGURE 13 : Time-course of gallbladder volume (GB) and human pancreatic polypeptide response (HPP) in all subjects. Gallbladder volume is expressed as percent of fasting volume and HPP as percent of peak level. Vertical bar through symbol indicates mean ± SEM.

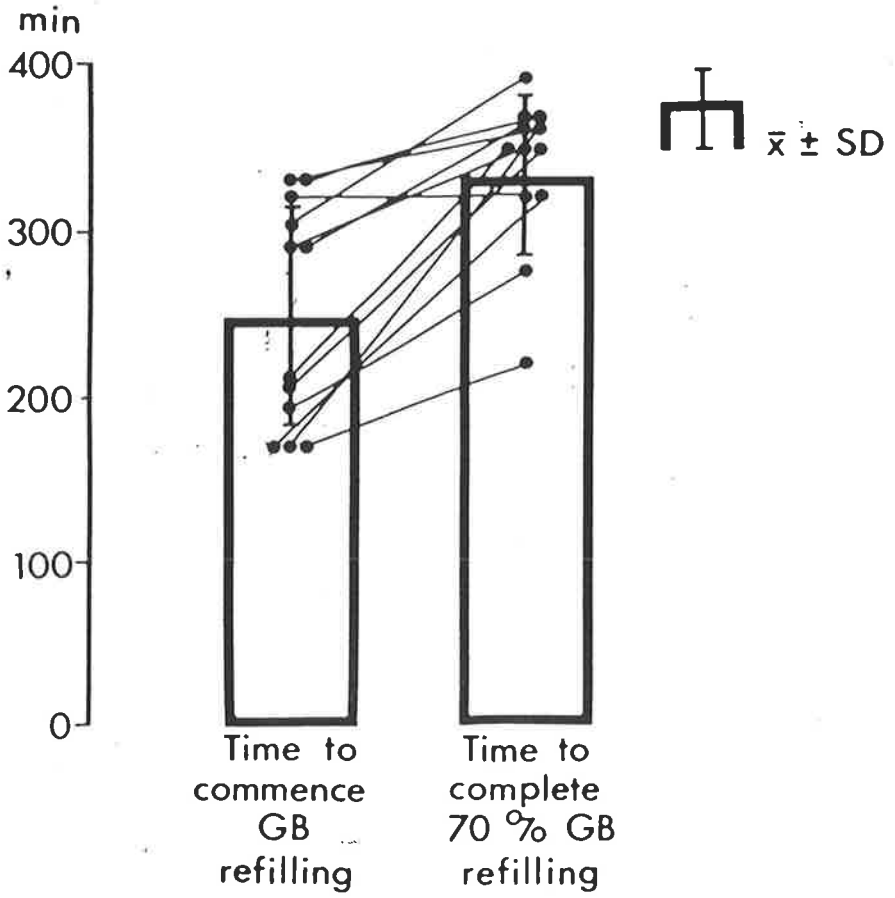

FIGURE 19: Time for refilling of gallbladder after breakfast

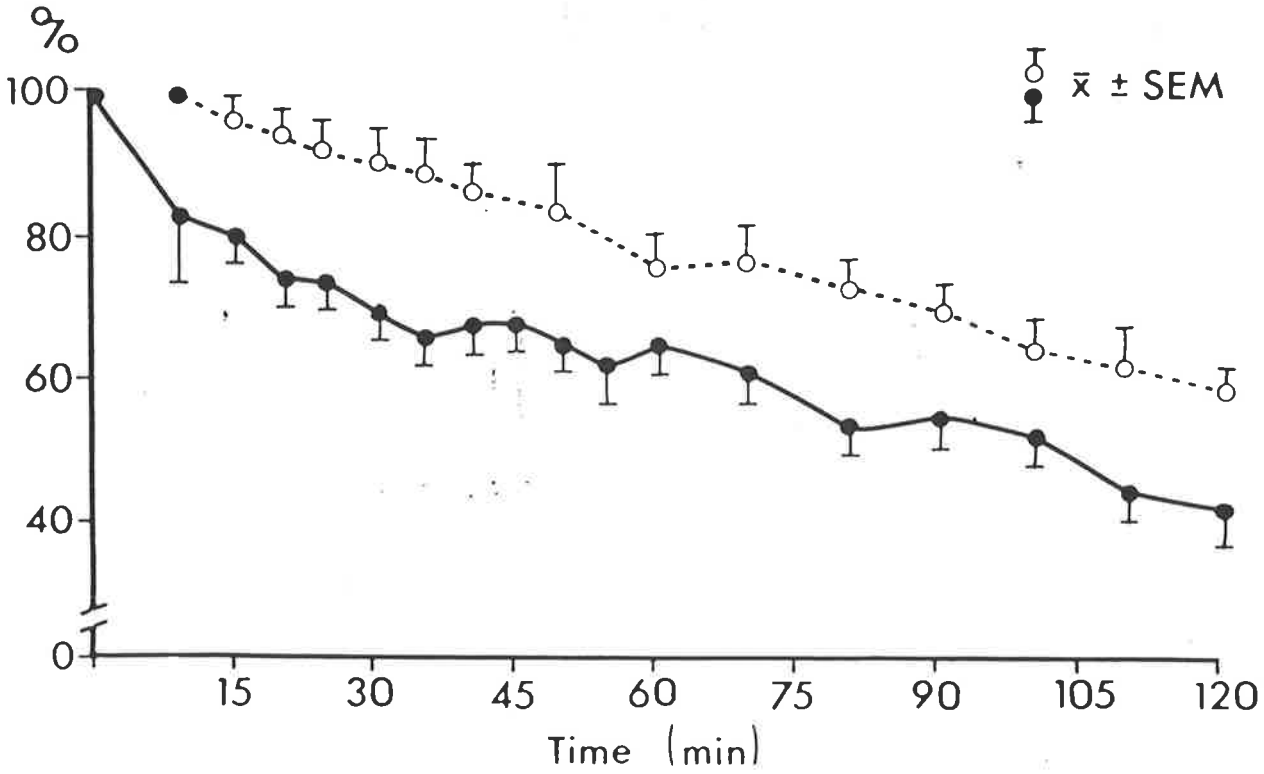

FIGURE 23: Time-course of gallbladder emptying and gastric emptying of solids for all subjects. Gallbladder emptying (closed circles) is expressed as percent of fasting gallbladder volume and gastric emptying (open circles) as percent of DPMs present in the stomach at completion of the meal. Gastric emptying obeyed zero-order kinetics and gallbladder emptying first-order kinetics.

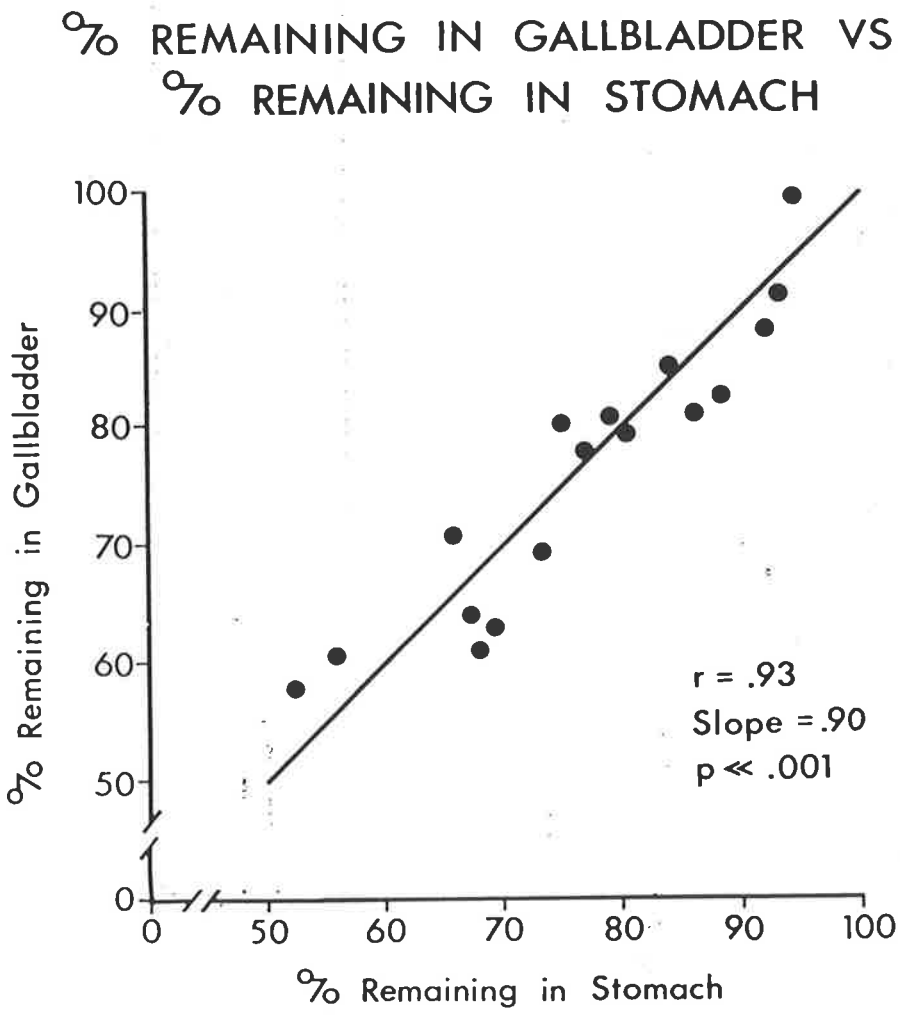

FIGURE 21: Correlation between the percent of bile remaining in the gallbladder and percent of original solids remaining in the stomach for the seventeen time points observed.

(c) Serum HPP

Plots of HPP level and gallbladder volume vs time are shown in Figure 17. The initial fast phase of gallbladder emptying is accompanied by a rapid rise and early peak in HPP response. The slow phase of gallbladder emptying and tonic gallbladder contraction is associated with a sustained elevation in HPP levels. The return of HPP levels to baseline roughly corresponds to the time of initiation of gallbladder refilling.

The integrated HPP response did not correlate with rates of gallbladder emptying, gastic emptying, or GIT. The initial peak HPP level did not correlate with the initial fast rate of gallbladder emptying. The integrated HPP level during the slow phase of gallbladder emptying and tonic gallbladder contraction did not correlate with the average gallbladder volume during the same interval of time.

CHAPTER IV: DISCUSSION

A. Pregnancy, Female Sex Steroids and Gallbladder Function

(i) Effects of the Ovulatory Cycle and Contraceptive Steroids

(ii) Effects of Pregnancy

(iii) Effects of Chronic Oestrogen Intake

B. The Effect of Gallbladder Emptying on Biliary Lipid Secretion

C. Orocaecal Transit Time in Pregnancy

D. Gastric and Gallbladder Emptying

DISCUSSION

A. PREGNANCY, FEMALE SEX STEROIDS AND GALLBLADDER FUNCTION.

Pregnancy causes greater than normal retention of bile in the gallbladder throughout the day and night. The retention is maximal at a time when bile is relatively supersaturated with cholesterol. Such changes could contribute to the increased incidence of cholesterol cholelithiasis associated with multiparity.

(i) Effects of the ovulatory cycle and contraceptive steroids

Fasting, residual and hourly volume were similar in the follicular and luteal phases of the ovulatory cycles. Rates of emptying after breakfast were the same in both phases of the ovulatory cycle. Contraceptive steroids increased fasting gallbladder volumes only.

Nilsson and Stattin (1967) used oral cholecystography to measure gallbladder emptying after bolus injection of cholecystokinin in both phases of the cycle in each of 10 subjects, and they found slower emptying in the luteal phase in 8 of them. However, the phase of the cycle was not documented by serum progesterone level, as in the present study, and 5 of these 8 had a 10% decrease in emptying in the luteal phase. Nonetheless this data indicates that the elevated progesterone levels associated with the luteal phase do not significantly alter either gallbladder volumes or emptying during ingestion of regular meals.

(ii) Effects of pregnancy

Assessment of the data in the way that has been performed in this section of the thesis requires further explanation. In general, the regression of data is performed to study the relationship between a predictor variable and a response variable. The regression analysis summarises the relation between the response and predictor variables and measures the correlation between variables. Before regressing data it is important to plot and examine data to check for outliers and to define nonlinear patterns in the data. It is also important to look for patterns suggesting that the data should be transformed to a different scale, such as logarithmic, to produce a better fit, to the data. The simple linear-regression model assumes that the relationship between x and y can be summarised as a straight-line graph. However, if the data do not seem to lie on a line or if the residuals show a clear pattern, the linear model may not be consistent with the data. One way to deal with this problem is to attempt to fit the data to a particular curve, such as a logarithmic, sinusoidal, or exponential curve, nonlinear regression, as discussed by Bliss (1970) and Snedecor and Cochran (1980). According to Godfrey (1985) if part of the data seems to lie along one line and part along another line, the data can be stratified. Using this technique, a different line is fitted to each segment of the data, In this case it is assumed that a linear relationship holds within each stratum, although the exact relationship may not be the same from stratum to stratum. Such a model is called a piecewise linear model. If a simple linear regression is used to study the relationship between initial gallbladder emptying and pregnancy, there is little difference between controls and pregnant subjects. However, it is appropriate and valid to use a logarithmic scale for gallbladder volume and to apply a piecewise regression. The result is that a significant difference is demonstrated between pregnant subjects and controls in the second slower phase of gallbladder emptying (Figure 6). Again this relationship would be missed if an alternative to a simple linear regression was not used.

Fasting and residual volumes were larger than control values in every trimester of pregnancy, and they increased linearly during pregnancy.

For fasting volume a correlation of r = .48 (P = .016) is obtained and the line y = 20.17 + 0.450 x is the line of best fit for simple linear regression. Given the relative large variation between individuals, this indicates an increase in volume over the pregnancy. The quadratic relationship does not significantly improve the fit over the linear (R2 changes from 0.226 to 0.257) and the equation y = 10.89 + 1.36x - 0.019x2 gives the best quadratic fit with a maximum volume at 36 weeks, This does not support the hypothesis of no increase in volume in the third trimester. An exponential growth model would behave similarly.

For the residual volume a correlation of r = .54 (P = .006) is obtained and the line y = 3.78 + .232 x is the line of best fit for simple linear regression. Given the relative large variation between individuals this indicates an increase in volume over the pregnancy.

The quadratic relationship again does not significantly improve the fit over the linear (R2 changes from .290 to .308) and the equation y = .48 + .55x - .0065x2 gives the best quadratic fit with a maximum volume at 42 weeks. This does not support the hypothesis of no increase in volume in the third trimester. An exponential growth model would behave similarly.

When the volumes in the three trimesters are compared the means are (23.7, 33.3 and 32.1) for fasting volumes and (6.4, 9.5, 10.5) for residual volumes. Statistically significant differences are shown by one way analysis of variance to occur at the levels of P = .064 (F= 3.12) for fasting volumes and P = 0.116 (F= 2.38) for residual volumes neither of

which quite reaches the commonly used value of .05 for statistical significance.

Hourly volumes from 11 a.m. until midnight were larger than those of controls throughout pregnancy, without progressive change (Figure 4C). The failure to find a progressive increase was due to large hourly volumes in 2 first-trimester subjects (Figure 4C). Because the progesterone level of these two subjects was appropriate for the week of pregnancy, the variation in hourly volumes is probably due to individual differences in gallbladder smooth-muscle response to increases in progesterone.

As fasting, residual and hourly volumes return toward normal in the postpartum period, it is probable that most of the changes in gallbladder volume during pregnancy are transient. Whether volumes return to prepartum values in a given individual cannot be determined from this study as no subject was studied before pregnancy.

Only the last phase of gallbladder emptying after breakfast was slower in pregnant women. The slower late phase of emptying may be due to impaired gallbladder contractility or slower gastric emptying. The late rate of gallbladder emptying correlates with gastric emptying of solids. Thus it is possible that the slower rate of gallbladder emptying after breakfast in pregnant subjects is partially due to slow gastric emptying of solids.

An overall summary of the gallbladder emptying curves would be less likely to contrast the differences in gallbladder emptying between control and pregnant subjects. The early rapid phase of gallbladder emptying is a reproducible finding as can be seen in Chapter III, Section E when a different control group of volunteers were studied including males and females. This early response is most likely vagally innervated. Fisher,

Rock and Malmud (1986) have recently shown in human volunteers that intact vagus nerves and cholinergic pathways are required in order for the gallbladder to respond to sham feeding. They found that the maximal cumulative gallbladder emptying response to sham feeding was 44%, which was not significantly different from the respoonse to bethanechol and to ingestion of a test meal of steak and potato. Cholinergic blockage with atropine eliminated the emptying response to sham feeding and sham feeding did not stimulate gallbladder emptying in patients with vagotomy.

A biphasic response has been reported for gastric emptying of digestible solid meals when measurements are taken during meal ingestion with a 'lag' phase preceding a linear emptying phase (Collins, Horowitz, Cook et al 1983). Therefore our early phase of gallbladder emptying is unlikely to be related to rapid initial solid gastric emptying.

Because the hormonal changes of pregnancy are complex (Kloppert and Fuchs 1977) it is not possible to identify with certainty the mediator or mediators of the alterations in gallbladder function. It is also not possible to exclude a direct or indirect neuronal influence produced by pregnancy. In addition neuronal changes could modify any hormonal effects

on motility. Nevertheless, progesterone, a known inhibitor of smooth muscle contraction (Cohen 1980; Somylo and Somylo 1970; Schultze and Christensen 1980; Fisher, Roberts, Grabowski et al 1978) is a likely candidate. The data shows a direct correlation of fasting and residual volume with serum progesterone concentrations. This is consistent with, but not proof of progesterone mediation. As there was a highly significant direct correlation between serum progesterone and week of pregnancy, and the correlation of each of these variables with fasting and residual volume were identical, it cannot be concluded that progesterone caused the effects. The changes in indices of gallbladder function could be due to other, unmeasured factors in pregnant women.

In summary, the phase of the ovulatory cycle did not affect gallbladder function, but pregnancy caused retention of bile in the gallbladder throughout day and night. Prolonged contraceptive steroid use induced an increase in fasting (overnight) but not daytime bile retention.

The results of this study indicate that in pregnant women, gallbladder bile retention is increased throughout the day and night. Bile retention is greatest in the second and third trimeters, a time when gallbladder bile is most lithogenic (Kern, Everson, De Mark et al 1981). Thus, gallbladder bile retention could contribute to the pathogenesis of cholesterol gallstones by allowing more time for nucleation and pecipitation of cholesterol crystals.

(iii) Effects of chronic oestrogen intake

This study has shown that oestrogen is unlikely to be responsible for the increase in bile cholesterol saturation which accompanies the use of oral contraceptives.

The results of the effects of chronic oestrogen intake on cholesterol secretion, cholesterol saturation index and gallbladder concentrating ability reported show no significant change with the doses used. One possible reason for the lack of response is failure of absorption of the conjugated oestrogens. Premarin contains equilin sulphate (25%)and as well as other conjugated compounds, includes sulphate triester of estrone (45%) 17 alpha tihydroequilin (15%), 17 alpha dihydroequilenin and 17 beta dihydroequilenin (15%). After oral administration of Premarin to postmenopausal women equilin sulphate and oestrone sulphate appear in the serum as unconjugated equilin and oestrone (Bhavrani, Sarda, Woolever 1981). These findings indicate absorption of oral Premarin.

In the study reported here the gallbladder contractile response to intravenous CCK, a mixed meal and intraduodenal liquid fomula was unaffected by chronic oestrogen administration. Oestrogen pretreatment in animals has previously been reported to enhance the in vivo contractility of the oesophagus, antrum and colon (Bruce and Behsudi 1979). Oestrogen pretreatment has not been shown to increase contractile responses in the i vitro guinea pig gallbladder, but it significantly decreases the threshold acetylcholine dose requirements (Ryan and Pellecchia 1982). It is possible that the gallbladder is relatively less sensitive than other tissues to th excitatory effects of oestrogen.

B. THE EFFECT OF GALLBLADDER EMPTYING ON BILIARY LIPID SECRETION

The observation in pregnancy of supersaturated bile and delayed gallbladde emptying raised the question of the role of the gallbladder in controlling biliary cholesterol secretion. Secretion studies using different strength stimuli were designed to study the effect of incomplete and almost complet

gallbladder emptying on biliary cholesterol secretion.

When previous secretion studies are compared it can be shown that bile acid secretion rates during continuous intraduodenal amino acid infusion (Shaffer and Small 1977; Kern, Everson, De Mark et al 1981) are less than those measured during continuous intraduodenal liquid formula infusion (Grundy and Metzger 1972; Mok, Von Bergmann, Grundy 1979; Bennion and Grundy 1975; Mabee, Meyer, Den Besten et al 1977; Valdivieso, Palma, Nervi et l 1979; Mok, Von Bergmann, Grundy 1978) and similar to that measured during intermittent liquid formula feedings (Mok, Von Bergmann, Grundy 1979, Northfield and Hofmann 1975; La Russo, Szczepanik, Hofmann 1977; Mok, Von Bergmann, Grundy 1980; La Russo, Hoffmann, Hofmann et al 1975) and in post cholecystectomy subjects (Shaffer and Small 1972; Key, Bonorris, Marks et al 1980) (see Table 3a÷ Appendix).

When similar groups of subjects are compared bile acid secretion rates during liquid formula infusion are more than twice those during amino acid infusion. In the present study during liquid formula infusion the rate of biliary lipid secretion was higher, gallbladder emptying was prompt and more complete, and small intestinal transit was faster. It can be concluded that the higher rate of biliary lipid secretion achieved during liquid formula in this and other studies is due to increased enterohepatic cycling of bile acid caused by more complete gallbladder emptying and faster small intestinal transit. Biliary lipid secretion rate during amino acid infusion is submaximal, due to incomplete gallbladder emptying and slower intestinal transit.

Biliary lipid composition is dependent on the rate of biliary bile acid secretion. In patients with bile fistulae the molar percent cholesterol of

bile increases as the bile acid secretion rate drops (Lindblad, Lundholm, Schersten 1977; Wagner, Trotman, Soloway 1976; Nilsson and Schersten 1969). In subjects with gallbladders, fasting sequesters part of the bile acid pool in the gallbladder, induces a lower bile acid secretion rate (Northfield and Hofmann 1975; Mok, Von Bergmann, Grundy 1980; Metzger, Adler, Heysmfield et al 1973) and increases cholesterol saturation of bile (Northfield and Hofmann 1975; Metzger, Adler, Heymsfield et al 1973).

When similar groups of subjects from different studies are compared, the molar percent cholesterol during continuous liquid formula infusion (2.9 +/- .9% Schwartz, Almond, Vlahcevic et al 1979; 3.4 /- .4% Bennion, Mott, Howard 1980) is less than that during continuous amino acid infusion (4.3 +/- .5% Kern, Everson, De Mark et al 1981). In the present study a similar difference has been shown between the two infusates in the same individual. It can be concluded that the decreased molar percent cholesterol during liquid formula infusion is the result of a higher rate of bile acid secretion due to more complete gallbladder emptying and more rapid small intestinal transit.

Since the molar percent cholesterol of bile varies with bile acid secretion rate the relationship of cholesterol secretion to bile acid secretion must necessarily be curvilinear over the full range of bile acid secretion rates. However, if only a portion of the range of bile acid secretion rates is examined, the relationship between bile acids or phospholipid and cholesterol may appear linear. When the data in this study from amino acid and liquid formula are combined the resulting relationship for bile acids and cholesterol is curvilinear (Figure 9). If the data from each infusion is examined separately then the relationships appear linear as only a small portion of the range of bile salt secretion rates is being represented (Figure 9). This may explain discrepancies shown in other studies where

some authors found linear relationships (Kern, Everson, De Mark et al 1981; La Russo, Szczepanik, Hofmann 1977; La Russo, Hoffmann, Hofmann et al 1975; Einarson, Grundy, Hardison 1979)while others showed them to be curvilinear (Lindblad, Lundholm, Schersten 1977; Wagner, Trotman, Soloway 1976).

Gallbladder volume fluctuated during infusion of both amino acids and liquid formula but large fluctuations (10-20ml) were seen only with amino acid infusion. During the study bile from the gallbladder was often expelled into the duodenum to join the stream of hepatic bile. When this occurs the measured secretional rate may not accurately determine the hepatic secretion rate.

Faster small intestinal transit increases the frequency of bile acid recycling and increases bile acid secretion rates. In this study faster intestinal transit occurred with liquid formula which resulted in greater biliary lipid secretion rates.

Since subject 4 produced hydrogen in response to lactulose during liquid formula infusion, the lack of increase in breath hydrogen during the amino acid infusion is most consistent with a markedly prolonged transit time. However, there may be other explanations for the lack of hydrogen response. The nasoduodenal tube may have slipped back into the stomach, the subject may have been colonised with colonic bacteria incapable of making hydrogen or this may represent day to day variation. The first seems unlikely as bile was continuously aspirated during the study and there was no drop in secretion rate in the 4 hours after lactulose was given. The second is also unlikely as a dramatic change in colonic flora would have had to occur within 1 week without the administration of drugs or antibiotics. The third is extremely unlikely as the day to day variations in transit time measured

by this technique have been defined and are minimal (La Brooy, Male, Beavis et at 1983), especially with intraduodenal infusion of lactulose (Bond and Levitt, 1977). Subject 1, who had the biggest difference in gallbladder emptying with the infusions, had no change in transit time. Interestingly, she had a smaller increase in bile acid secretion compared with the remaining subjects.

The more complete gallbladder emptying and more rapid intestinal transit during liquid formula infusion are probably related to greater stimulus for release of humoral mediators of gallbladder contraction and intestinal peristalsis. However it has been shown that CCK can enhance cholinergic innervation of the gallbladder (Foesel and Sewing 1978) and therefore some of the observed changes could be mediated by neurogenic pathways. Pancreatic polypeptide release from the pancreas parallels that of cholecystokinin and motilin (Floyd 1980; Keane, DiMagno, Dozois et al 1980), two hormones thought to be responsible for gallbladder emptying and small intestinal peristalsis. Reliable assays for the latter two hormones were not available but the two to threefold greater rise in serum pancreatic polypeptide in four of five subjects during liquid formula infusion indicates greater humoral stimulation.

These secretion studies have shown that incomplete gallbladder emptying and slower intestinal transit decrease the enterohepatic cycling of bile acids and increases the molar percent cholesterol of bile.

C. OROCAECAL TRANSIT TIME IN PREGNANCY

From the secretion study reported here and from work by others (Einarrson, Grundy, Hardison 1979; Mok, Von Bergmann, Grundy 1977; Valdivieso, Palma, Nervi et al 1979) it is apparent that small intestinal transit time is an important determinant of the rate of cycling of biliary lipids and thus

biliary lipid secretion rates.

In the first trimester of pregnancy, an increase in bile acid pool size without an increase in gallbladder volume has been previously reported (Kern, Everson, De Mark et al 1981). This prompted the sequential study of gastrointestinal transit in pregnancy to determine if prolonged transit time could explain the expanded bile salt pool of the first trimester of pregnancy.

The data in this thesis has shown that gastrointestinal transit is not significantly prolonged in the first trimester of pregnancy. It is prolonged in the second and third trimester and decreased postpartum, approximating first trimester values. The postpartum levels of progesterone measured in this study are comparable to those occurring in the follicular phase of the ovulatory cycle. Likewise, the transit times of women studied in the first trimester or postpartum period in this study are similar to those observed in the follicular phase of the ovulatory cycle or postpartum by other groups using similar techniques (Wald, Van Thiel, Hoechstetter et al 1982; Wald, Van Thiel, Hoechstetter et al 1981).

There is a threshold level of serum progesterone above which no further prolongation of gastrointestinal transit occurs. The concordance between studies of the relationship of small intestinal transit and gallbladder volume to progesterone levels suggests, but does not prove, that progesterone may be the mediator of the alterations in motility observed in pregnancy. Obviously, other hormones, such as oestrogens, prolactin or other hormones that increase during pregnancy, may correlate with the observed changes in transit time and gallbladder volume. In addition alterations in neuronal input may play a role in some of the observed changes.

More recently Caride, Prokop, Troncale et al (1984) studied small intestinal transit scintigraphically in healthy volunteers with 99m-technetium-diethylenetriamine penta acetic acid. Subjects ingested the scintigraphic solution with isosmotic lactulose. The arrival of the radionuclide label at the caecum correlated well with the rise in breath hydrogen (r=.77) and the transit times were similar for the scintigraphic method (73 $\pm$ 6.5 min; $\bar{x}$ $\pm$ SEM) and the lactulose breath test (75.1 $\pm$ 8.3 min). Individual variations in small intestinal transit time in Caride et al's study were significantly correlated with individual variations in gastric emptying. Bond and Levitt (1975) showed that individual variation in small intestinal transit time, as measured by the lactulose hydrogen breath test, could be significantly reduced by infusing lactulose directly into the duodenum via a transpyloric tube. These findings suggest that small intestinal transit time as measured by the lactulose breath test is significantly affected by the rate of gastric emptying. However, caution is indicated before assuming that transpyloric intubation is necessary for accurate measurement of small intestinal transit time. Differences in gastric emptying are unlikely to significantly affect the orocaecal transit time results in the first and second trimester of pregnancy as liquid emptying is not affected by the luteal phase of the menstrual cycle or pregnancy up to the second trimester (Horowitz, Maddern, Chatterton et al 1984; Schade, Pelekanos, Tauxe et al 1984). However as input of a meal into the small bowel is variable and depends on the gastric emptying rate, it is an advantage to quantitate both gastric emptying and orocaecal transit time simultaneously. Delayed gastric emptying is an unlikely cause of the prolonged gastrointestinal transit time occurring in late pregnancy. The delayed transit time is probably a result of prolonged small bowel transit time due to inhibition of small intestinal smooth muscle

contraction by female steroid hormones, most likely progesterone. However a neuronal effect of pregnancy on orocaecal transit cannot be excluded.

La Brooy, Male, Beavis et al (1983) have shown that the lactulose breath test can be made more reproducible by including a liquid meal. They showed that variation in transit times between individuals was considerable with all doses of lactulose ranging from 10 to 20gm (mean coefficient of variation of 18.5 to 28.3% respectively). The addition of lactulose to a liquid meal containing carbohydrate, fat and protein decreased the coefficient of variation to 10% in four subjects studied. It is therefore possible that I could have improved the variability in pregnant subjects by including a liquid meal with the lactulose dose.

The ideal technique to investigate intestinal transit should monitor the movement of the liquid or chymous contents through the lumen without disturbing the physiological control of gastrointestinal motility. Most techniques used to quantitate transit time to the caecum have inadequacies. The use of barium as an artificial meal to study small bowel transit has limitations (in particular in pregnancy) and the results cannot be extrapolated to other meals. Methods using non-absorbable markers like polyethylene glycol (PEG) require the use of an intraluminal tube to retrieve samples from a given location in the intestine. This produces stimuli that are not usually present and causes some patient discomfort. However, the determination of hydrogen in the breath after the ingestion of lactulose is non-invasive. The test does depend on the presence of bacteria in the large intestine to metabolise lactulose. At least 5% of the population, however cannot metabolise the sugar because they lack the proper bacterial strains in the colon. Hydrogen production may also be reduced because of low colonic pH (Perman, Modlers, Olson 1981). It is theoretically possible that a reduction in bicarbonate rich bile flow could

lead to a fall in caecal pH resulting in a decreased or even absent hydrogen peak following lactulose ingestion. Bile flow in pregnancy is probably reduced and the bicarbonate concentration of bile decreased by dilution. These pertubations would become more pronounced as pregnancy progresses and may explain why some pregnant women become non-hydrogen producers later in pregnancy.

LaBrooy, Male, Deavis et al (1983) have suggested that variations of small bowel motility in the fasted state probably account for the relatively poor reproducibility of the lactulose breath test in, or between individuals. However, their results cannot be extrapolated to pregnant women whose gastrointestinal smooth muscle is under the influence of complex neurohormonal changes. Such variation of orocaecal transit time in pregnancy has not been studied. Despite likely individual variability, the studies described in this thesis were still able to demonstrate significant second and third trimester differences. One problem in interpreting breath hydrogen derived orocaecal transit time measurements is that the results may not necessarily relate to the transit of food through the small intestine. Therefore it is important to know if the breath hydrogen peak correlates with the time that the head or the bulk of the meal reaches the caecum. If the initial hydrogen peak does not correspond with the head of the meal reaching the caecum then perhaps peak hydrogen excretion may be a more precise measure of mouth to caecum transit time. Read, Miles, Fisher et al (1980) compared the transit time of an isotopically labelled mixed meal with breath hydrogen excretion in 14 healthy volunteers by estimating radioactivity over the caecum. There was a highly significant correlation between the time of the increase in radioactivity ($r=.82$; $p < .001$) over the caecum and the time of the secondary increase in hydrogen excretion produced by lactulose fermentation. In 9 out of 14 subjects, the secondary increase in hydrogen excretion occurred at the same tine as the increase in

caecal radioactivity suggesting that measurement of breath hydrogen is a useful method to indicate when a meal enters the colon. In 10 subjects the maximum count over the caecum, corrected for decay of the isotope, attained the level of the initial gastric counts suggesting that all of the meal residue was present in the caecum after passage through the small intestine, There was a highly significant correlation ($r = .66$; $p < .01$) between the time that the maximum concentration of hydrogen gas was excreted in the breath in these subjects and the time that the maximum amount of radioactivity was recorded over the caecum. In 7 out of 10 subjects, peak hydrogen excretion occurred at the same time as the total radioactivity was recorded over the caecum and presumably indicated the time that all of the meal residue has entered the colon.

The close correspondence between caecal radioactivity and measurement of breath hydrogen suggests that the increase in hydrogen excretion may indicate the time when the head of the meal enters the caecum while in at least 50% of the subjects peak hydrogen excretion indicates the time when the bulk of the meal entered the colon. If this is the case, then the observation that these two times are closely correlated supports the idea that measurement of the increase in breath hydrogen excretion provides an index of the transit time of the head as well as the bulk of the meal. However, in view of the recent data of Malagelada, Robertson, Brown et al (1984) this observation is questionable. Arrival of chyme into the colon is influenced as much by the gastic emptying rate as it is by intestinal transit, and cannot be individualised by measuring the cummulative entry of marker into the caecum. Isotopic methods for intestinal transit time have several advantages over breath hydrogen estimations. The lactulose dose used for hydrogen breath testing can cause dose dependent changes in gastrointestinal transit (LaBrooy, Hale, Beavis et al 1983). The production of hydrogen in the intestine is dependent on the type of

bacterial flora present. The amount of non-absorbable carbohydrate ingested the day before the test plus oral bacteria can alter basal levels the following day (Read, Miles, Fisher et al 1980, Thompson, O'Brien, McCarthy et al 1978).

Digestible food labelled with a gamma emitting marker (eg 99m Tc-egg) cannot measure transit time of solids because the food carrier is hydrolysed. The development of 131 I labelled fibre by Malagelada, Robertson, Brown et al (1984) has provided a useful marker for study of transport of solids throughout the small and large bowel. The use of dual gamma labelled markers has demostrated that, unlike the stomach, the small bowel does not discriminate between the solid and liquid components of chyme. If both markers are ingested simultaneously the liquid component empties faster from the stomach and arrives at the caecum first but both solid and liquid markers travel along the small bowel at similar speeds. These results would suggest that contrary to the conclusions of Read, Miles, Fisher et al (1980), "first arrival" of chyme to the colon is not a reliable predictor of the subsequent transit spectrum of the bulk of chyme and therefore the hydrogen breath test cannot be used to quantify the transit time of the entire meal.

The findings in this study are consistent with other studies which show depression of smooth muscle contractility by female hormones. In general, progesterone reduced contractility while oestrogen has an excitatory effect on the same tissue. These findings suggest that the prolongation of gastrointestinal transit occurring in late pregnancy is a result of the increased physiological concentration of progesterone.

The apparent lack of production of hydrogen in 0 of 8 first, 1 of 12 second, and 4 of 22 third trimester subjects may be related to marked

prolongation of transit. Had samples been taken for 6 or 8 hours, it is probable that the hydrogen peak would have been detected. The findings that all studies with lack of hydrogen production occurred in late pregnancy supports this interpretation. However, LaBrooy, Hale, Beavis et al (1983) have demonstrated that low hydrogen producers on occasions excreted no hydrogen at all in expired air after lactulose. This finding indicates that some subjects are occasional non-producers of hydrogen. This could be because they harbour only small numbers of hydrogen - producing bacteria in the colon, which, with the normal shifts of bacterial flora, become depleted at times.

Although it is unlikely, lack of hydrogen production may also be due to pregnancy-induced changes in colonic bacteria. Breath hydrogen production is the net result of hydrogen production and hydrogen consumption by colonic bacteria. Since hydrogen consuming bacteria exist (Levitt, Hastings, Berggren 1974) it is possible that pregnancy induces a change in colonic bacteria favouring hydrogen consumption over hydrogen production. However, there are no experimental data to either refute or support this hypothesis. If changes in colonic flora occurred in pregnancy, then the absence of breath hydrogen at 240 min would be difficult to interpret.

This study and previous studies of gallbladder function in pregnant women suggest that changes in orocaecal transit or gallbladder volume cannot account for the observed increase in bile acid pool size that occurs in early pregnancy. Thus, the increase in bile acid pool may be related to a direct effect of female hormones upon the hepatic synthesis of bile acids.

D. GASTRIC AND GALLBLADDER EMPTYING

The finding of delayed and incomplete gallbladder emptying in pregnancy

after a mixed solid liquid meal could have resulted from impaired gastric emptying of solids resulting in diminished neurohumoral stimuli from the upper jejunum. Therefore it was important to study stomach and gallbladder simultaneously after a similar meal. In the present study it has been shown that, after ingestion of a standard meal containing 40% of calories as fat, the second slow phase of gallladder emptying was related to gastric emptying of solids. Gallbladder refilling began when 13% of solids remained in the stomach. This suggests that the concentration of fat and protein that enters the intestinal lumen after 50 min is probably insufficient to fully stimulate intestinal or pancreatic hormones, or both and maintain gallbladder contraction. Support for this is the drop to baseline serum HPP levels at the time when 13% of solids remained in the stomach. The initial rapid rate of gallbladder emptying may represent vagal innervation. Rock, Malmud, Fisher (1981) have shown that sham feeding can result in emptying of 50% of gallbladder volume. In addition atropine allows the gallbladder to accommodate larger infused volumes (Schoetz, La Morte, Wise et al 1981). Therefore continued vagal simulation could play a role in maintaining the gallbladder contracted post prandially.

Since commencing this study it has been shown that gastric emptying is biphasic with an early "lag" phase being demonstrated if counting continues throughout the meal. This lag phase is variable with a mean of around 20 min (Collins, Horowitz, Cook et al 1983). As 15 min was allowed for ingestion of the test meal this lag phase was not demonstrated. The gastric emptying studies in this thesis were perfonned with a posterior camera to allow abdominal ultrasound to be performed anteriorly. Akkermans, Jacobs, Smout et al (1984) using a similar sized meal showed that in emptying curves obtained from a camera posterior to the subject, a lag phase could not be distinguished. In their studies the geometric mean of both anteriorly and posteriorly obtained curves showed the lag phase clearly.

However, using a bran labelled meal Baxter,Grime, Critchley et al (1985) failed to show a lag phase using an anterior camera which according to the studies of Akkermans, Jacobs, Smout et al (1984) should show an exaggerated lag phase. Malagelada, Robertson, Brown et al (1984) initially showed a lag phase in only 2 out of 6 healthy patients using labelled bran and an anterior camera. Later studies more consistently demonstrated dual phases (Camilleri, Brown, Malagelada 1986). It does seem likely that by using/ only one camera an "early emptying" phase can be artificially introduced. However, Akkermans, Jacobs, Smout et al (1984) have shown by a dual-headed camera technique that after gastric emptying actually begins, gastric emptying rates measured by the two cameras are parallel. Therefore, the assessment of the slope of the post lag solid emptying phase is not invalid using one camera but where possible the geometric mean of gastric emptying should have been used.

There are obviously several potential inaccuracies that may reduce the sensitivity and specificity of gastric emptying tests using radionuclides. These problems may relate to the instruments, the types of labelled solids and the constituents of meals, the anatomy of the patients studied, analysis of gastric emptying data and the physical properties of the radionuclides themselves. For example, an important question of the study reported here is whether the radionuclide mixed with the scrambled egg remains bound with extracellular or intracellular fat. Meyer, Mayer, Jehn et al (1986) have recently shown that most of the intracellular fat empties with the solid food phase whereas most of the extracellular fat empties as an oil phase. However, both intracellular and extracellualr fat appear to leave the stomach at the same rate. It is also uncertain as to how much of the technetium label is bound to fat and how much is bound to protein and whether this is important in interpreting the gastric emptying results.

This study also shows that tonic gallbladder contraction is maintained for 4 hours after the standard breakfast. This explains the " flat " gallbladder volume curve observed in earlier studies where regular meals were eaten at 3.5 to 5.0 hourly intervals.

Unfortunately a reliable radioimmunoasay for HPP but not for CCK or motilin,was only available at the time of performance of these studies. The HPP response was biphasic, as reported. HPP levels dropped to basal values at approximately the time of initiation of gallbladder refilling and did not increase subsequently, suggesting that tonic gallbladder contraction is maintained by humoral stimulation in response to gastric emptying of solids. In addition, HPP, sometimes referred to as "anticholecystokinin hormone" (Sarles, Hage, Laugier et al 1979) is not responsible for gallbladder refilling after ingestion of a meal. Gallbladder refilling is most likely a passive process which occurs when emptying is no longer stimulated.

An interesting observation which deserves further investigation, is the twofold slower GIT in menstruating women compared to men. As women were menstruating at the time of the study it is possible that menstrual symptoms suppressed motility through a central mechanism.

Wald, Van Thiel, Hoechstetter et al 1981 reported that GIT in women is prolonged in the luteal phase of the ovulatory cycle presumably because of the increased level of progesterone. Had subjects in the luteal phase of the ovulatory cycle been studied it could be anticipated that the difference in GIT between men and women could have been even greater.

It is concluded that gastric emptying of the solid portion of a meal

containing fat induces and maintains tonic gallbladder contraction, probably through continued release of humoral mediators, from small bowel and pancreas. When gastric emptying is complete, humoral stimulation ceases and the gallbladder refills. This gastro-biliary integration helps maintain mixing of bile acids with ingested fat for optimal fat ingestion and absorption. Altered gallbladder emptying may be secondary to altered gastric emptying.

CHAPTER V

CLINICAL IMPLICATIONS OF ALTERED GALLBLADDER EMPTYING AND BILIARY LIPID SECRETION IN PREGNANCY

A. THE ROLE OF MORNING SICKNESS

(i) Clinical features of morning sickness

(ii) Diet and cholesterol gallstone formation

B. THE POTENTIAL RELATIONSHIP OF DISEASES OF THE ALIMENTARY SYSTEM TO ALTERATION IN BILIARY LIPIDS IN PREGNANCY

(i) Mucosal protective effects of biliary cholesterol

(ii) Cholecystitis in pregnancy

(iii) Reflux oesophagitis and peptic ulcer disease

CLINICAL IMPLICATIONS OF ALTERED GALLBLADDER EMPTYING AND BILIARY LIPID SECRETION IN PREGNANCY

A. THE ROLE OF MORNING SICKNESS

(i) Clinical features of morning sickness

Pregnancy with its attendant physical hormonal and physiologic alterations, significantly affects the functioning of the gut at many levels. It displaces stomach and small intestine and mobilises portions of the colon from their customary anatomic locations; it alters motility of oesophagus, stomach, small intestine, colon and biliary tree; it impairs appropriate functioning of the lower oesophageal sphincter; it decreases gastric acidity; it is associated with changes in the selective absorption mechanism of small intestine and colon and probably in pancreatic secretion as well and it attenuates the body's response to such crises as inflammation, perforation and obstruction.

Nausea, with or without vomiting is a common, suggestively diagnostic, self limiting symptom of early pregnancy. In its most innocous form it is "morning sickness" experienced as nausea by as many as 50% and as vomiting by approximately 30% of women during their first trimester (Fairweather 1968; Scher 1965). Both these conditions generally arise during the first 12-14 weeks of pregnancy. It is felt typically upon arising often before any food has been ingested. It may persist at 14 weeks in as many as 40%, by 16 weeks in less than 20% and by 20 weeks in less than 10% (Diggory and Tomkinson 1962).

In morning sickness, no specific gastrointestinal lesion has been demonstrated. Many theories have been advanced. The literature has centred on four general areas; allergy to putative placental or seminal toxins (none isolated); alteration in gastrointestinal motility (no good correlation demonstrated); hormonal excesses or deficiences acting directly on the gut or on the central nervous system (poor correlation between hormonal titres and clinical symptoms: Soules 1980); and psychosomatic factors (no correlation between vomiting and personality profiles: Palmer 1973). The changes in orocaecal transit time and gallbladder emptying occurred in late pregnancy and no changes in gastric empting have been demonstrated in the first trimester. Therefore, it is unlikely that motility disturbances induced by pregnancy can explain the symptoms of morning sickness. A central cause for morning sickness seems more likely and the decreased incidence of severe vomiting in recent years has been linked to a more relaxed attitude to pregnancy, availability of abortion and birth control, as well as less restrictive obstetrical attitudes and regimes (Jacobs and Janowitz 1965).

ii) Diet and cholesterol gallstone formation

Several studies have shown an increase in cholesterol saturation of human hepatic and gallbladder bile after an eight to 16 hour fast (Metzger, Adler, Heymsfield et al 1973; Williams, Morse, MacDonald et al 1977; Bloch, Thornton, Heaton 1980). Fasting is associated with the storage of a part of the bile-acid pool in the gallbladder, a decrease in the rate of biliary bile-acid secretion, without parallel diminution in the biliary secretion of cholesterol and the hepatic production and secretion of supersaturated bile. This effect might be accentuated by previous bile-acid deficiency or excess cholesterol secretion or both. While high cholesterol intake (Den Besten, Connor, Bell 1973) and overweight appear to play an important role

in gallstone formation, short term, low calorie diets don't appear to effect biliary cholesterol saturation (Schlierf, Schellenberg, Stehl et al 1981).In addition, prolonged fasting, greater than 16 hours significantly decrease bile lithogenicity. Therefore if morning sickness plays a role in gallstone formation it is unlikely to be through an effect on total caloric intake alone, reducing gallbladder emptying. A more likely gallstone risk factor from morning sickness is the effects of meal type and meal frequency on gallbladder emptying. The importance of gallbladder emptying in the formation of gallstones was demonstrated by Messing, Bories, Kunstlinger et al (1983). They showed that patients fed by total parenteral nutrition for 6 weeks developed biliary stasis and gallstones. While it is unlikely that women with morning sickness would undergo prolonged fasting, meal frequency is likely to be reduced, Capron, Delamere, Herve et al (1981) studied 115 patients with radiolucent gallstones. The duration of fasting was measured from the end of the last meal of the day until the beginning of the next meal (breakfast or lunch). In the youngest patients (20-35 years) the duration of overnight fasting was significantly longer in the patients with gallstones than in age and sex matched controls.

Therefore, in pregnancy prolonged overnight fasting in the first trimester due to morning sickness could contribute to other risk factors for gallstone formation by increasing cholesterol saturation of bile and reducing gallbladder emptying.

An alternative explanation for a possible role of morning sickness in cholesterol gallstone formation may be via a change in dietary preference which might increase biliary cholesterol saturation. A beneficial effect of wheat fibre, in the form of bran, on the cholesterol saturation of bile has been observed by most (Pomare, Heaton, Low-Beer et al 1976; McDougall, Yakymyshyn, Walker et al 1978; Watts, Jablonski, Toouli 1978) but not all

workers (Tarpila, Miettinen, Metsaranta 1978). This decrease in biliary cholesterol saturation with dietary fibre is observed only when the bile is initially supersaturated. Therefore lack of dietary fibre could be responsible for some of the biliary lipid changes in pregnancy.
On epidemiological and experimental grounds, Heaton (1973) postulated that the consumption of refined carbohydrates (better described as fibre depleted foods) favours the secretion of bile supersaturated with cholesterol and hence increases the risk of gallstones. This hypothesis has more recently been supported by the extensive epidemiological study of Scragg, McMichael and Seamark (1984). It has also been argued that the nature of refined foods, especially refined sugars, is such that they inevitably inflate the intake of energy. Thornton, Emmett and Heaton (1983) showed that a diet rich in refined carbohydrate resulted in gallbladder bile being more saturated with cholesterol compared with a diet excluding refined foods. However, the refined diet contained much less fibre. Werner, Emmett, and Heaton (1984) showed no effect of a six week ad libitum diet supplemented with refined sucrose, on the cholesterol saturation index of fasting gallbladder bile or biliarty lipid secretion rates. These recent results suggested that a refined diet is pathogenic only in as much as it is deficient in dietary fibre. However, as the high sucrose diet adversely affected plasma lipids, it is possible that in the long term, it might increase the risk of gallstones. As pregnancy progresses past the first trimester, women are more likely to seek high fibre foods to counteract impending constipation. However, in the first trimester nausea may make pregnant women avoid the bloating and abdominal discomfort of unrefined carbohydrates which could then lead to an increased risk of gallstone formation as a result of increased biliary lipid secretion.

The above results suggest that certain diets may predispose to biliary

cholesterol saturation. The mechanism may be via biliary cholesterol saturation of through impaired gallbladder emptying. Data in Chapter III has shown that amino acids are less potent than a mixed liquid formula in causing gallbladder emptying. Gallbladder emptying in human subjects has also been studied after meals of different composition (Rock, Malmud, Fisher 1981). Gallbladder emptying after a mixed solid meal is decreased significantly compared to a liquid meal for 75 mins after meal ingestion. By 90 min, the maximal gallbladder emptying responses are similar. The maximal gallbladder emptying responses to 5% and 50% glucose and to amino acids are similar but all are less than the maximal response to a mixed liquid meal. Therefore, gallbladder emptying is affected by the physical and chemical composition of ingested meals and any change in dietary preference could result in altered and sometimes incomplete gallbladder emptying. The subsequent biliary stasis could predispose to gallstone formation.

B. THE POTENTIAL RELATIONSHIP OF DISEASES OF THE ALIMENTARY SYSTEM TO ALTERATION IN BILIARY LIPIDS IN PREGNANCY

(i) Mucosal protective effects of biliary cholesterol

In experimental animals, bile salts have caused damage to the small and large intestine, stomach and gallbladder and in man they have been implicated in the pathogenesis of several gastrointestinal inflammatory states such as gastric ulcer and oesophagitis.

The mucosa of the gallbladder, oesophagus, stomach and duodenum are presented with the problem of a potentially injurious solution of harmful bile salts which are powerful detergents able to penetrate lipid bilayers and at high concentrations solubilise membrane phospholipids ultimately

causing membrane damage.

As well as representing an excretory substance, cholesterol may also have an important function in bile (together with phospholipids) of preventing the damaging effects of bile salts on the biliary and intestinal epithelium. Bile salts placed in the canine gallbladder cause haemorrhagic cholecystitis but the onset of inflammation can be delayed by adding cholesterol (Riegel, Ravdin, Johnston 1931). Further evidence for the protective effect of biliary cholesterol comes from a study on the effects of dihydroxy bile acids on the human jejunum (Broor, Slota, Ammon 1980). It was shown that bile salts induced secretion of water and electrolytes into the intestinal lumen (as opposed to the normal absorption of water and electrolytes) but that this effect could be blocked by adding cholesterol to the bile salt solutions. These observations suggest that biliary cholesterol has a role in preventing bile salt induced mucosal damage.

Biliary supersaturation by cholesterol in fasting and pregnancy may protect the biliary and intestinal epithelium from the toxic effects of bile salts.

(ii) Cholecystitis in pregnancy

Despite the prior evidence that pregnancy predisposes to gallstones, the incidence of cholecystitis is low, around .02- .07% (Sparkman 1957; Namlin, Bartlett, Smith 1951). This does not exceed the rate in non-pregnant women of the same age group and in fact, parity and age do not influence the occurence of symptoms.

It is possible again that cholesterol has a protective action and despite significant gallbladder stasis cholesterol crystals and epithelial damage

are not more likely to occur. There are several possible explanations for this apparent protective action. One possibility is that bile salts are bound to cholesterol and phospholipids by lipophilic association within mixed micelles and are therefore less able to react with the epithelium along which they are being transported. Another is that absorption of cholesterol by the gallbladder and incorporation into membranes, alters the cellular physiology and morphology of the biliary epithelium.

(iii) Reflux oesophagitis and peptic ulcer disease

Broadly defined, reflux oesophagitis is the constellation of symptoms and/or consequences to the oesophagus which result from contact of gastric or intestinal contacts with the oesophageal mucosa. Reflux oesophagitis leads to the substernal burning discomfort of heartburn. This symptom has been reported in 30-70% of pregnancies, occuring daily in as many as 25% (Atlay, Gillison, Horton 1973; Nebel, Fornes, Castell 1976). Onset is usually in the second trimester and frequency and intensity often escalates as the pregnancy proceeds, only to vanish after delivery. All pregnant women, irrespective of heartburn symptoms, tend to exhibit more non-propulsive motor activity of the oesophagus with decreased wave amplitude and slower peristaltic speed (Nagler and Spiro 1961).

Although a source of discomfort, heartburn is usually not a serious symptom. Peptic oesophageal stricture in pregnancy is rare (Swinhoe, Cochrane, Wishart 1981), as is haemorrhagic oesophagitis.

Patients with reflux and regurgitation often comment that the fluid tastes bitter rather than sour and is yellow or green in colour due to the presence of duodenal contents. It has been known for some time that bile salts in concentrations found in gastric contents will cause permeability

changes in the oesophageal mucosa (Safaie-Shirazi, DenBesten, Zike 1975).

The lack of severe complications of reflux oesophagitis in pregnancy could be due to increased binding of bile salts as a result of a relative excess of biliary cholesterol. In addition, bile salts are more likely to be diluted out in gallbladder bile and impaired gallbladder emptying may lead to less bile salts entering the duodenum at any one time point.

Similarly to oesophageal disease, the complications of peptic ulcer disease are rare in pregnancy. In fact, peptic ulcer disease itself is distinctly rare in pregnancy (Sandweiss, Podolsky, Saltzstein 1943) with the condition tending to improve symptomatically in at least 80% of cases as pregnancy progresses (Clark 1953). The rarity of the condition and its improvement during pregnancy have been linked to diminished gastric acidity and the healing effects of oestrogen. However, it is possible that the damaging efects of refluxed bile acids may be reduced by dilution and the presence of greater amounts of biliary cholesterol.

CHAPTER VI: FINAL DISCUSSION

FINAL DISCUSSION

Chapter III of this study has shown that biliary lipid secretion rates are dependent on stimulation of gallbladder emptying and small intestinal transit. Reported differences in mean secretion rates between groups of subjects from different studies or between different groups of subjects within the same study may be due to differences in stimulation of gallbladder emptying and intestinal transit. Monitoring gallbladder emptying and intestinal transit during biliary lipid secretion studies is important for the interpretation of results. In addition in healthy individuals gallbladder emptying and small intestinal transit affect biliary lipid composition.

In the control group of women studied by ultrasound after a mixed meal the initial rate of gallbladder emptying after breakfast was approximately 40% of that previously reported using a small volume liquid meal (Braverman, Johnson, Kern 1980). The liquid meal contained 520 cal (35% fat, 12% protein). Increasing the volume, fat and amount of solid in a meal slows the emptying of both liquids and solids from the stomach (Moore, Christian, Coleman 1981; Malagelada 1977, Moberg and Carlberger 1974, Kroop, Long, Alavi et al 1979, Cortot, Phillips, Malagelada 1981). The results of studying gastric and gallbladder emptying simultaneously suggest that the large volume high fat, mixed solid liquid meal described probably emptied slowly from the stomach, reducing the rate of release of neurohumoral mediators of gallbladder contraction from the upper small bowel and pancreas, and resulted in the slower rate of gallbladder emptying. Different rates of gastric emptying of liquids and solids may have caused the two phases of gallbladder emptying observed in this study, although

vagal innervation could also explain the initial rapid phase and contribute to prolonged gallbladder contraction.

This study has shown that gallbladder refilling does not occur until 6-7 hours after breakfast alone and that this long period of tonic gallbladder contraction (or lack of gallbladder refilling) is related to slow emptying of solids from the stomach. Because meals were ingested at 3-5 hour intervals by women in the control group the gallbladder did not refill. This also accounts for the close agreement of residual and hourly volumes i.e. the smallest gallbladder volume achieved after breakfast approximated the gallbladder volume for the rest of the day.
The lack of significant gallbladder refilling before and after the noon and evening meals in all groups was due to the composition and frequency of ingestion of the meals.

In addition to the changes observed in gallbladder emptying, gastrointestinal transit time is prolonged in late pregnancy. It decreases postpartum to the level previously reported for the follicular phase of the ovulatory cycle. Both orocaecal transit time and serum progesterone levels rise progressively between progesterone levels of 0 to 80ng/ml. This does not exclude, however, the possibility that other hormones or neuronal changes produced during pregnancy might mediate these effects. It appears that the increased bile acid pool size occurring in early pregnancy is not secondary to sequestration of bile acid either within gallbladder or small intestine. Instead the increased bile acid pool is probably a direct metabolic or hormonal effect on bile acid synthesis.

The effects on biliary lipid secretion and gallbladder function seen during pregnancy were not reproduced in post menopausal women taking chronic oestrogen therapy. However, the results are applicable only to the doses of oestrogen used in this study. The other studies reported here implicate progesterone as the most likely hormone to be causing disturbance of biliary lipid secretion and smooth muscle function in the gallbladder and gastrointestinal tract.

The studies described in this thesis strongly support a pathogenetic role for gallbladder stasis in gallstone formation.Recent observations from animal studies support this hypothesis. The prairie dog is a relatively good animal model for studying certain aspects of cholesterol gallstone formation. The prairie dog increases cholesterol absorption with increased dietary cholesterol intake and secretes this excess cholesterol into hepatic bile which is stored in the gallbladder (Meyer and DenBesten 1977). Within a few days cholesterol gallstones begin to form. Preceeding stone formation the gallbladder volume enlarges due to stasis (Meyer, DenBesten, Gurnell 1978, Hutton, Sievery, Vennes et al 1982).

Sphincterotomy (surgical removal of the specialised musculature that controls bile flow from the common duct) allows free drainage of the gallbladder and prevents gallstone formation, even when bile is supersaturated with cholesterol.

Following sphincterotomy gallbladder volumes remained significantly smaller than in sham operated animals. Atropine abolishes the protective effects of sphincterotomy and gallstones are allowed to form. (Hutton, Sievert, Vennes et al 1982).

Does stasis play a role in cholesterol cholelithiasis in man? Messing and his colleagues (Messing, Bories, Kunstlinger et al 1983) reported that

almost half of 23 patients on long term parenteral hyperalimentation in whom no gallbladder abnormalities were initially demonstrated developed biliary "sludge" which is a mixture of biliary pigment and cholesterol crystals. In all instances these observations were made a minimimum of three weeks after initiation of total parenteral nutrition (TPN). In six of their sludge positive patients who subsequently developed cholelithiasis, four had normal ultrasonography studies during the initial one to three weeks of TPN. In addition, Roslyn, Pitt, Mann et al (1983) reported a higher than expected incidence of gallbladder disease including acalculous cholecystitis during the course of prolonged TPN. These studies strongly suggest a pathogenetic role for gallbladder stasis.

Studies of gallbladder emptying in patients with gallstones give variable results. Northfield, Kupfer, Maudgal et al (1980) using scintigraphy found an increased sensitivity to intravenous CCK in many gallstone patients. Conversely Fisher, Stelzer, Rock et al (1982) showed a normal response to CCK but a diminished response to an ingested mixed meal. They showed that this diminished response was not due to slower gastric emptying. In other studies gallbladder emptying has been shown to be delayed (Shaffer, McOmond, Duggan 1980) or normal (Van Berge Henegouwen, Hofmann 1978, Northfield and Hofmann, 1975, Nok and Grundy, 1978). These discrepancies cannot be reconciled at this stage. They do not appear to relate to the age or sex of the patients studied. Whether these changes represent the cause or the result of gallstones has not been established.

Some clinical situations suggest that gallbladder stasis alone is not a sufficient prerequisite to gallbladder disease. Coeliac sprue is characterised frequently by gallbladder enlargement associated with sluggish contractility (Low-Beer, Heaton, Heaton et al 1971, Low-Beer, Harvey, Davies et al 1975). Yet no unusual susceptibility to cholesterol

cholelithiasis has been reported in these coeliac sprue patients. Possibly the reason is that the intestinal lesion produces an undersaturated bile. A similar situation exists for truncal vagotomy patients (Glanville and Duthie 1964, Inberg and Vuorio 1969, Nielsen 1964).

Future studies of gastrointestinal motility in pregnancy will need to concentrate on relatively non-invasive techniques. Further refinement of the innovative ultrasound technique of King, Adam, Pryde et al (1984) could yield valuable new data on changes in transpyloric fluid movement as pregnancy progresses. The scatter of data between trimesters shown in this thesis demonstrates the need for sequential studies on the same individuals to detect significant changes between trimesters. A reliable ultrasound technique for measuring gastric emptying would be an ideal method for non-invasive sequential studies in pregnancy.

Potential problems with hydrogen breath testing have been outlined. Further studies of transit time in pregnancy should include a mouth wash to eliminate oral bacterial hydrogen production. In addition, a liquid meal as recommended by La Brooy, Male Beavis et al (1983) could improve reproducibility. Because of the uncertainty as to the reason for the lack of breath hydrogen production in some third trimester subjects it may be more precise to consider an alternative form of transit time measurement. Most methods of measurement use radionuclides but more recently Kellow, Borody, Phillips et al (1986) have described an accurate measurement of orocaecal transit using the appearance in venous blood of the sulphur component of orally ingested sulphasalazine. This method may prove useful in the study of transit time in pregnant women, but the need for frequent blood sampling would be a significant drawback. It should also be noted that there is a close correlation between the appearance of breath hydrogen from oral lactulose and the appearance of sulphapyridine in the venous

blood after ingestion of sulphasalazine.

In future studies of the effects of pregnancy on orocaecal motility, gut hormones which change during pregnancy should be correlated with observed motility changes. The digestive capacity of the gut is increased during pregnancy and lactation,. Antral and plasma levels of gastrin have been shown to be elevated in lactating rats (Lichtenberger & Trier, 1979) and there is a significant suckling-related effect on somatostatin levels in pigs (Uvnas- Moberg, Eriksson, Blomquist et al 1984). Somatostatin levels would be of particular interest in pregnancy as infusion of somatostatin has been reported to inhibit the gallbladder emptying response to hypertonic glucose administered orally (Johansson, Kollberg, Efendic et al 1981).

In the future it would be worthwhile systematically studying alteration in gallbladder function in other special patient groups. As in other studies of the physiologic and metabolic alterations occurring during normal pregnancy, alterations in gallbladder motility function, if demonstrable in other high risk groups, would be unlikely to be an isolated abnormality but one possibly amenable to therapeutic manipulation and therefore worth knowing about.

APPENDICES

A. Real Time Ultrasound Method for Determining Gallbladder Volume

B. Characteristics of Infusates

C. Comparison of Methods for Measuring Gallbladder Emptying

D. Reported Studies of Biliary Bile Acid Secretion In Subjects with Intact Gallbladders and Subjects Status Post Cholecystectomy

E. Measurement of Small Bowel Transit Time by the Hydrogen Breath Test

F. Measurement of Serum Human Pancreatic Polypeptide

REAL TIME ULTRASOUND METHOD FOR DETERMINING GALLBLADDER VOLUME

Sum of cylinders method: This method was originally used but not validated, in cholecystographic studies of gallbladder volume. Volume is calculated from sonograms by dividing the gallbladder image into a series of cylinders of equal height (h). A transparent grid of parallel lines, with constant distance (h) separating lines, is placed over the gallbladder image. The longitudinal axis of the gallbladder image is positioned perpendicular to the grid of parallel lines. Utilising the formula for volume of a cylinder, the formula for gallbladder volume becomes:-

$$V = \sum_{i=1}^{n} \frac{\pi d_i^2 h_i}{4} \qquad (1)$$

where h_i and d_i are the respective height and diameter of the ith cylinder, and n represents the total number of cylinders in the series. But since h_i is the distance between parallel lines on the grid and is the same for each cylinder in the series, the formula for gallbladder volume is:-

$$V = 0.785\ h_i \left(\sum_{i=1}^{n} d_i^2 \right) \qquad (2)$$

When calculating volume with this method only sonographs of the longitudinal projection are used. When the gallbladder is curved, as it often is, the longitudinal projection will not pass through the central axis of the gallbladder (Figure 1a). Displacement of the

longitudinal projection from the central axis causes the diameter measured from the longitudinal projection to be smaller than the true gallbladder diameter (Figure 1b). To correct for this, a factor E is introduced:-

$$E = \frac{\text{AP diameter + width}}{2} \qquad (3)$$

$$\times \quad \frac{1}{\text{Greatest diameter on longitudinal projection}}$$

Since this correction factor applies only to the measurement of diameter, the formula for gallbladder volume becomes:-

$$V = 0.785hE^2 \left(\sum_{i=1}^{n} d_i^2\right) \qquad (4)$$

When one applies correction factor E_i one assumes that the displacement of the longitudinal projection from the central axis is uniform throughout the length of the gallbladder. This is not always true, especially in long, narrow tapering segments of the gallbladder, where volume is small. The volume in these areas would be overestimated slightly, but the effect on total volume is minimal. Also, in situations where the longitudinal axis is parallel to, but displaced from, the central axis, the calculated GB volume would tend to underestimate GB volume in areas of tapering. Again, the effect on total volume would be small.

No correction factor for magnification (as is used in cholecystographic volume determination) is necessary for ultrasound measurements. The measurement and calculation (using a hand calculator) of volume by the corrected sum of cylinders method requires approximately 5 minutes for each photograph.

APPENDIX

TABLE I[a]

CHARACTERISTICS OF INFUSATES

	Liquid formula	Amino Acid Solution
Composition, g/dl	Fat 6.4 CHO 14.1 Protein 4.7	Fat 0 Glucose 5.0 Amino Acid 4.3[1]
Calories. cal/ml	1.3	0.4
Osmolality, mosmol/liter	543	420
Marker	β-sitosterol	BSP
Infusion rate, ml/min	3.2 ± 0.2	3.7 ± 0.9

CHO, carbohydrate. Osmolality was measured by freezing point lowering.

Amino Acid	Gm/Litre
Lysine	5.5
Tryptophan	2.0
Phenylalanine	5.5
Methionine	8.5
Threonine	3.8
Leucine	7.9
Isoleucine	3.7
Valine	6.1

TABLE 2a

COMPARISON OF METHODS FOR MEASURING GALLBLADDER EMPTYING

AUTHOR	Sex M:F	Weight	Age	Stimulus	Technique	Mean b (min^{-1})
DUANE WE. & HANSON KC. 1978	11:0	100-160	21-62	test meal	ICG marker dilution	-.0241
ENGLERT E.& CHIU SW. 1966	13:2	114-180	26-68	"fatty"meal	I^{131}-Iopanic acid scintigraphy	-.0385
	8:0	131-201	23-65	90 min CCK-IV (11.7 Ivy U/Kg/min)	"	-.0347
	4:0	131-175	26-49	Total dose as bolus over 2 min	"	-.0115
SPELLMAN SJ, SHAFFER EA., ROSENTHALL L. 1979	10:9	?	20-68	CCK - .001 Crick Harper Raper	Tc-HIDA scintigraphy	-.010M, -.010F
				CCK-.002	"	-.030M, -.027F
				CCK-.004	"	-.040M, -.032F
BRAVERMAN D, JOHNSON M & KERN F. 1980	0:11	95-130	18-27	Liquid formula	Real Time US	-.0523
PALFRAMNA & MEIRE HB, 1979	14:0	?	23-41	"fatty" meal	Real Time US	-.02

TABLE 3a

REPORTED STUDIES OF BILIARY BILE ACID SECRETION IN SUBJECTS WITH INTACT GALLBLADDERS AND SUBJECTS STATUS POSTCHOLECYSTECTOMY

	Bile acid secretion, μmol/h	
	MEAN	RANGE
Subjects with gallbladders[o]		
Liquid formula, continuous	2,200	1,550-2,920
Liquid formula, intermittent	1,650	1,470-2,015
Amino acid, continuous	1,150	1,073-1,236
Subjects postcholecystectomy[1]	1,390	1,149-1,588

All data were converted to μmol/h by using the published weights of subjects and where applicable, by assuming an average molecular weight of conjugated bile acids of 500. Mean is the average of reported control means; numbers in parentheses are references.

[o]The only criterion for inclusion in this heading was that the group served as a control population i.e., they had no liver or gallbladder disease. Most "control" populations were quite heterogeneous; age ranges 18-87, sex ratios (male:female) from 0:1 to 1:0; percent of ideal body weight from 86 to 281%.

[1] Studies were done by duodenal perfusion technique after patients had completely recovered from surgery.

Measurement of Small Bowel Transit Time by the Hydrogen Breath Test

Orocaecal transit time was measured by the lactulose hydrogen breath test (Bond and Levitt, 1975; Bond and Levitt 1977; La Brooy, Male Beavis et al 1983; Solomons, Viteri, Hamilton 1977). Subjects were advised to avoid excess intake of starch polysaccharide the day before the test. After an overnight fast, subjects ingested 10 grams of lactulose in 100ml of water. Breath samples for measurement of hydrogen concentration were obtained fasting and every 15 minutes for 4 hours. Breath hydrogen concentration was measured with a Quintron model S gas chromatograph (Quintron Instruments Inc. Milwaukee, Wisconsin) using argon as carrier gas at 18ml per minutes, a molecular sieve column, and thermal conductivity detector. Orocaecal transit time was defined as the time of the first sustained rise in breath hydrogen concentration from baseline. If after 4 hours no rise in breath hydrogen occurred, the transit time was recorded as 240 minutes. In each study of pregnant women, transit time was estimated by 3 observers blinded to the study protocol and design. Each subject's transit time was reported as the mean of the 3 estimates. Transit time varied among observers in 24% of studies but in each case by only 15 minutes. In the secretion study less observations were available and eight observers were used. Bond and Levitt (1975) validated the H_2 breath test technique as a measure of small intestinal transit by simultaneously measuring the appearance of non-aborbed PEG at the ileum with a rise in breath H_2 produced from the intestinal bacterial metabolism of ingested lactulose.

Measurement of Serum Human Pancreatic Polypeptide

Serum human pancreatic polypeptide(HPP) level was determined by radio-immunoassay in the laboratory of Dr. Ian Taylor, at the Centre for Ulcer Research and Education, Veterans Administration Centre Wadsworth Hospital, Los Angeles, California. Mean intra and interassay precision

were 4% and 8% respectively. Recovery of HPP added to serum deviated by no more than 17% from the expected values over the range of 40-340 pg/ml serum. Human pancreatic polypeptide was plotted against time and the integrated response was measured from the area under the HPP curve.

Calculation of Biliary Cholesterol and Phospholipid in Secretion Studies

The cholesterol and phospholipid concentration of each duodenal sample was corrected for infusate cholesterol and phospholipid using the calculation:-

$$[CH]\ net = [CH]_D - \left(\frac{[CH]_I\ [Beta\ S]_D}{[Beta\ S]_I}\right)$$

[CH] net : Concentration of cholesterol in duodenal bile sample due to biliary secretion (micromoles/millilitre)

$[CH]_D$: Measured cholesterol in duodenal samples

$[CH]_I$: Infusate cholesterol concentration

$[Beta\ S]_I$: Infusate beta-sitosterol concentration

$[Beta\ S]_D$: Duodenal sample beta-sitosterol concentration

$[CH]_D$ net (or $[PL]_D$ net) is used in subsequent calculations of secretion rates (as $[X]_D$, see below) by the standard marker dilution equation:-

$$X\ sec = \frac{[X]_D}{[M]_D}\ [M]_I,\ R.\ 60\ min$$

Xsec : Secretion rate of a particular biliary lipid, X, (micromoles per hour).

$[X]_D$: Duodenal concentration of X (micromoles per millilitre)

$[M]_D$: Duodenal concentration of marker (micromoles per millilitre).

$[M]_I$: Infusate marker concentration

R : Rate of infusion (millilitres per min)

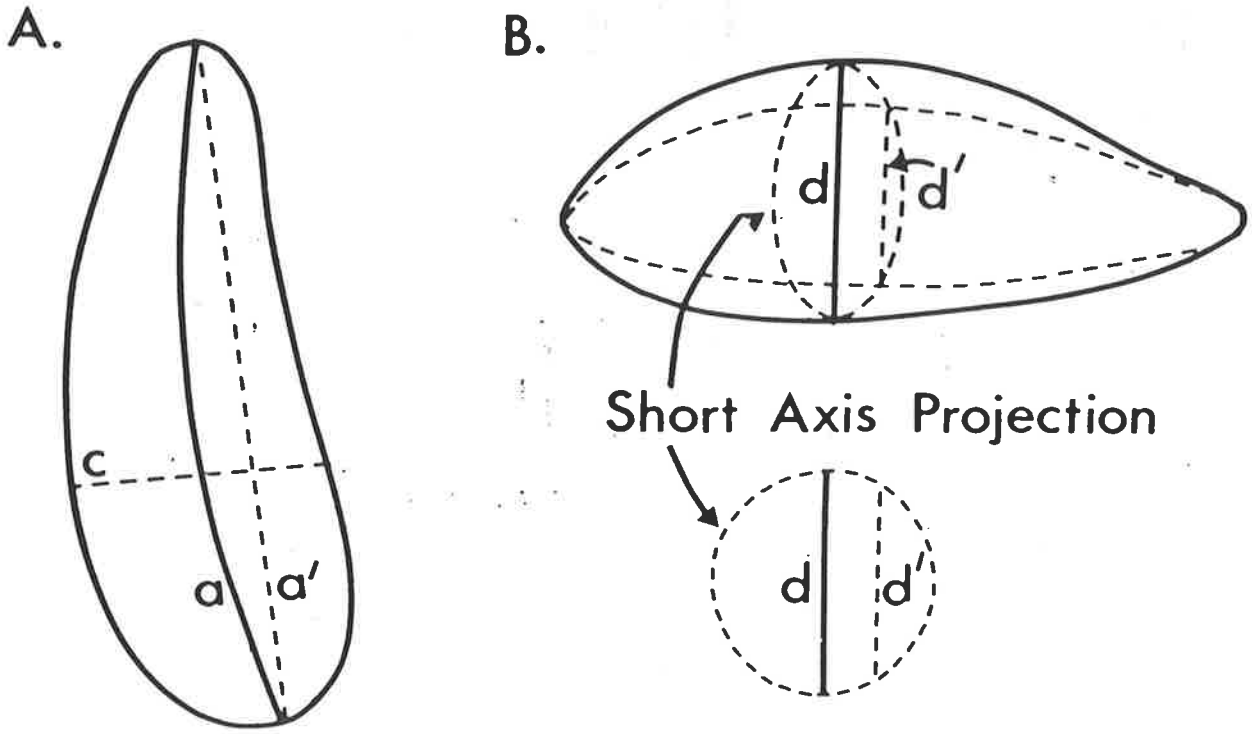

FIGURE 1a

Diagram showing relationships of "a" central axis of the gallbladder, "a'", longitudinal axis from sonogram and "c", short axis from sonogram. B. Sketch of ultrasound longitudinal projection (-----) superimposed upon actual size (—) of gallbladder. The short axis projection is also shown with diameter "d" (d=AP diameter + width)/2. "d" is the diameter which is measured from the longitudinal projection.

SUBJECT CONSENT FORM
FOR
PARTICIPATION IN CLINICAL INVESTIGATION PROJECT

Original Consent
29 Jan 82

UNIVERSITY OF COLORADO MEDICAL CENTER

ct Description: Estrogen Effects on Biliary Lipids and Bile Acid Kinetics. (A Bile Acid Method for Measuring Bile Acid Kinetics in Pregnant and non-Pregnant Women)

being asked to participate in a study of bile composition to be conducted under the sion of Dr. Fred Kern, Jr., and his colleagues at the University of Colorado Health s Center. The purpose of the study is to learn why certain types of people have a isk of developing gallstones. Gallstones are very common. They cause a great deal of and result in almost one million operations in the United States every year. If their s more clearly understood, there is hope of preventing them or treating them without ion.

agree we will hospitalize you in the Clinical Research Unit at Colorado General l for one day and three mornings of outpatient evaluation. There will be no expense. this hospitalization you will be asked to swallow a small, thin tube (ND tube) (one/ mes) which will be passed through your nose into your upper small intestine and will in place the morning(s) of study. It may cause mild discomfort, but experience with nds of patients has shown that it involves no major risks. Fluoroscopy will be needed. sitioning of the tube and you will consequently receive a small dose of radiation.

l ask you to take by mouth a small amount of a substance, bile acids, labeled with the (not radioactive) isotope, ^{13}C. The amount of ^{13}C is less than 1% of the amount lly present in your body and has absolutely no harmful effects.

your stay in the Clinical Research Unit, you will receive an infusion of a digested n solution through the ND tube which will be used to stimulate your gallbladder to ct, as it usually does after every meal. This injection will usually be given to you morning before breakfast and is necessary so that the doctors can collect a small of bile (5 ml, approximately 1 teaspoonful) through the tube. The bile will be ed for bile acids, cholesterol and other fats secreted by your liver. Six blood samples) will be taken for serum bile acid analysis. Blood hormones, lipids and routine tries will be determined.

dard diet containing ordinary food will be provided by the Clinical Research Unit hout the entire study period.

ll receive no direct benefit from these studies. Please ask questions. The research ng supported by the National Institutes of Health.

event your participation in this research supported by the National Institutes of directly results in physical injury to you, medical treatment will be available, but this time there is no compensation available for any such injury. Further information this treatment may be obtained by calling Dr. Fred Kern, Jr., at 394-7131.

HORIZATION: I have read the above and understand the discomforts, onveniences, and risks of this study. I agree to the participation of _____. I understand that if I (he) refuse(s) participate or withdraw(s) at any time, my (his) treatment will not affected in any way.

Signed: _____

nesses: _____ (Physician) _____ (Registered Nurse)

Date _____

5

SUBJECT CONSENT FORM 9/2/80
FOR
PARTICIPATION IN CLINICAL INVESTIGATION PROJECT

UNIVERSITY OF COLORADO MEDICAL CENTER

Project Description:

You are being asked to participate in the study of the effect of pregnancy and sex hormones on gallbladder size and contraction. This study is conducted under the supervision of Dr. F. Kern, Jr., and Dr. M.L. Johnson and their colleagues at the University of Colorado Medical Center. The purpose of the study is to learn whether gallbladder size and contraction is abnormal under the influence of pregnancy and sex hormones.

The test will require fasting overnight, then taking by mouth a liquid meal. Ultrasound measurements of gallbladder size will be done before the meal, and at 5 minute intervals afterwards for one hour.

Ultrasound technique is a widely used diagnostic method in obstetrics, gynecology and all of medicine. It is a harmless and simple technique, free of any known adverse effects. It may be used safely in pregnancy. There is no special preparation required and no discomfort is experienced during the procedure.

An intravenous needle will be placed in your arm for the removal of blood samples. You can expect to experience some pain at the moment the needle goes into your arm. In addition to this momentary pain, there will be minor discomfort of having the needle taped in your arm. In about 10 percent of cases a small amount of bleeding under the skin will produce a bruise (hematoma). The risk of temporary clotting of the vein is about 1 percent, while the risk of infection of a hematoma or significant external blood loss is less than one in 1,000. A total of 80cc of blood will be removed over a 90 minute period through this needle.

To determine the transit time of the small intestine a small amount (10g) of a non-absorbable sugar (lactulose) will be administered orally. This sugar produces hydrogen gas in the expired breath which can be collected harmlessly. Lactulose may produce minor bowel looseness transiently with mild abdominal cramping.

This study will not benefit you personally, but the information obtained should be of value to others. The results of the study may be published in medical journals, but you will not be identified in any way.

The study is supported by a grant from the National Institutes of Health. This study will be conducted free of charge to you.

In the event your participation in this research directly results in physical injury to you, emergency medical treatment will be initiated and arrangements for subsequent medical treatment will be made. However, the costs of such medical treatment and compensation for such injury will not be provided by the Medical Center, the Investigator(s) or the Sponsoring Agency, and will have to be covered by resources available to you.

AUTHORIZATION: I have read the above and understand the discomforts, inconveniences, and risks of this study. I agree to the participation of _____. I understand that if I (he) refuse(s) to participate or withdraw(s) at any time, my (his) treatment will not be affected in any way.

Signed: _____

Witnesses: _____ (Physician) _____ (Registered Nurse)

Date _____

FOR PARTICIPATION IN CLINICAL INVESTIGATION PROJECT
UNIVERSITY OF COLORADO HEALTH SCIENCES CENTER

Project Description:

You are being asked to participate in the study of the relationship of gastric and gallbladder emptying. This study is conducted under the supervision of Dr. F. Kern, Jr., and Dr. William Klingensmith III, and their colleagues at the University of Colorado Medical Center. The purpose of the study is to learn the importance of the rate of emptying of the stomach in terms of gallbladder emptying.

The test will require fasting overnight, then taking by mouth a standard meal. Ultrasound measurements of gallbladder size will be done before the meal, and at 5 minute intervals afterwards for one hour.

Ultrasound technique is a widely used diagnostic method in obstetrics, gynecology and all of medicine. It is a harmless and simple technique, free of any known adverse effects. It may be used safely in pregnancy. There is no special preparation required and no discomfort is experienced during the procedure.

An intravenous needle will be placed in your arm for the removal of blood samples. You can expect to experience some pain at the moment the needle goes into your arm. In addition to this momentary pain, there will be minor discomfort of having the needle taped in your arm. In about 10 percent of cases a small amount of bleeding under the skin will produce a bruise (hematoma). The risk of temporary clotting of the vein is about 1 percent, while the risk of infection of a hematoma or significant external blood loss is less than one in 1,000. A total of approximately 150 cc of blood will be removed.

The amount of radiation you will receive is 1.26 rads (approximately the same amount you would receive from two standard gallbladder x-ray studies).

This study will not benefit you personally, but the information obtained should be of value to others. The results of the study may be published in medical journals, but you will not be identified in any way.

The study is supported by a grant from the National Institutes of Health. This study will be conducted free of charge to you.

In the event your participation in this research directly results in physical injury to you, emergency medical treatment will be initiated and arrangements for subsequent medical treatment will be made. However, the costs of such medical treatment and compensation for such injury will not be provided by the Medical Center, the investigator(s), nor the Sponsoring Agency, and will have to be covered by resources available to you.

AUTHORIZATION: I have read the above and understand the discomforts, inconveniences, and risks of this study. I agree to the participation of _____. I understand that if I (he) refuse(s) to participate or withdraw(s) at any time, my (his) treatment will not be affected in any way.

Signed: _____

Witnesses: _____
Physician

_____
Registered Nurse

Date: _____

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