NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

Canada

THE UNIVERSITY OF ALBERTA

THE EFFECT OF PROLONGED INTESTINAL TRANSIT TIME ON BILIARY LIPIDS IN THE DOMESTIC PIG

BY

© Norman D. Causton B.Sc., M.D.

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN EXPERIMENTAL SURGERY.

DEPARTMENT OF SURGERY

EDMONTON, ALBERTA

FALL 1987

ISBN 0-315-40902-9

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Norman D. Causton, M.D.

TITLE OF THESIS: THE EFFECT OF PROLONGED INTESTINAL TRANSIT TIME ON BILIARY LIPIDS IN THE DOMESTIC PIG.

DEGREE: Master of Science in Experimental Surgery.

YEAR THIS DEGREE GRANTED: 1987.

Norman D. Causton

Norman D. Causton
7711 - 157th. Street
Edmonton, Alberta
T5R 2A1

DATE: JUNE 30, 1987

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE EFFECT OF PROLONGED INTESTINAL TRANSIT TIME ON BILIARY LIPIDS IN THE DOMESTIC PIG, submitted by Norman D. Caust[redacted] in partial fulfillment of the requirements for the degree of Master of Science in Experimental Surgery.

Olin G. Thinston

(Supervisor)

Kenneth Baum

DATE: June 30, 1987

ABSTRACT

Previous studies have indicated that cholesterol saturation of bile is associated with prolonged intestinal transit time (so called colonic stasis) characteristic of populations eating a low-fiber/high-refined-carbohydrate diet. This study was undertaken to determine the effect of prolonged intestinal transit time on biliary lipids (cholesterol, phospholipids, total bile salts).

Young domestic pigs weighing 10 - 30 kilograms had an anti-peristaltic limb of colon fashioned to mechanically prolong intestinal transit time as measured by the passage of radiopaque markers. Gallbladder bile for determination of biliary lipids was obtained by needle aspiration at celiotomy before reversal of a 10 - 35 centimeter colonic segment, and at 4 and 8 weeks after reversal. The number of animals studied at 0, 4, and 8 weeks was 19, 13, and 9 respectively. The animals were kept on a cholesterol free diet throughout the study.

Pre-operative intestinal transit time in the animals was 53.5 $\pm$ 7.0 hours ($\bar{X}$ $\pm$ SEM) and was 175.2 $\pm$ 20.9 hours in animals after reversal of a colonic segment ($0.05 > p > 0.02$). Cholesterol saturation index of bile was 0.40 $\pm$ 0.03 in the control period, and 0.38 $\pm$ 0.03 and 0.39 $\pm$ 0.05 at 4 and 8 weeks after colonic reversal. None of the observed differences was statistically significant.

Under the conditions of this study a marked prolongation of intestinal transit time did not increase the

cholesterol saturation index of gallbladder bile. This result does not support the commonly held belief that prolonged intestinal transit time is a factor leading to cholesterol gallstone formation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE

INTRODUCTION

Gallstones have been found in an Egyptian mummy (circa 1500 B.C.)[1], and probably have always been present in man. The prevalence of gallstones is difficult to ascertain, as it changes with the age of the population and with the geographic locale. As well, most gallstones are asymptomatic. However, it has been estimated that 20 million Americans have gallstones[2].

There is a hard to prove clinical impression among physicians that the incidence of gallstones is increasing, and this is reinforced by the rising demand for and performance of cholecystectomy[3]. Each year approximately 80,000 Canadians undergo the procedure[4], and it is estimated that in America up to 10,000 persons per year die from gallstone disease or its complications[2].

The apparent rising incidence of gallstones in Western Society has resulted in great research interest in the subject. Several different theories have been formulated in an attempt to explain the high prevalence of gallstones. Africa provides the best documented examples of populations which are almost free of cholelithiasis[3]. In general gallstones seem to have a higher incidence in Western Society, the highest prevalence in the world being found in North American Indians[3]. Canadian Eskimos had a very low incidence until the 1960's when Westernization of their lifestyle began. Epidemiological studies have found that a high intake of refined carbohydrate and a lack of dietary

fiber are common to populations with a high incidence of gallstones[3,5].

Clearly gallstone disease is a very important factor in the health of the general population.

PATHOGENESIS OF GALLSTONES

Cholesterol gallstones (containing more that 70% cholesterol) account for the majority of stones in Western Society[1]. The most common associated conditions of gallstones are female gender, higher social class, high parity in females, obesity, diabetes, and coronary artery disease[1,3].

The solubility of cholesterol in bile depends upon the relative concentrations of the three major lipid components in bile: bile salts, phospholipids, and cholesterol. The original work on saturation used a triangular phase diagram to display the interactions between these components (Figure 1.). The authors believed it was possible to measure the individual components, plot them and from the location of the plot point know whether or not the person had gallstones[6]. Although accepted at the time, it has now been shown that the separation of bile into normal and abnormal groups based on cholesterol saturation is not possible, saturation and supersaturation being found in "normal" persons (those without cholesterol gallstones)[7,8]. The cause of this common occurrence of bile saturation with cholesterol is not known.

At least two conditions are required for the production of cholesterol gallstones: supersaturation of bile with cholesterol, and nucleation of the cholesterol monohydrate crystals[2]. Bile supersaturated with cholesterol commonly occurs in normal subjects (that is persons without

BILE COMPOSITION - TRIANGULAR COORDINATES

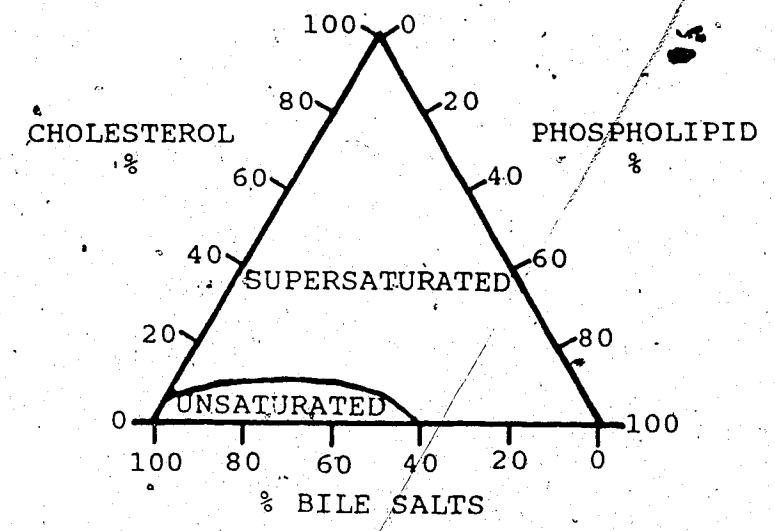


FIGURE 1.

gallstones) and there is considerable overlap in cholesterol saturation levels between patients with and without gallstones[7,8]. Why supersaturation results in gallstone formation in some people and not others is unknown. Clearly supersaturation is necessary, but other factors must be operating.

The presence of nucleating agents in bile may influence whether cholesterol will crystallize from micellar solution[8,9,10,11]. There are studies which suggest that the gallbladder of patients with cholesterol gallstones may add a nucleating factor to the bile thus facilitating cholesterol crystal formation[9,12,13]. Other studies, however, seem to implicate an anti-nucleating factor in preventing crystal nucleation[14,15,16]. The concepts of pronucleating factor excess and/or anti-nucleating factor deficiency resulting in gallstone formation are not mutually exclusive, and current literature states that both of the factors (inhibiting and promoting) do exist and that both of their effects can be demonstrated under appropriate conditions[17]. However, at this time little is known about the physical characteristics of the factors, or about the changes in biliary levels that occur and appear to be essential in gallstone formation.

Mucin, which is elucidated from the wall of the gallbladder, also may be an important pronucleating factor[18,19]. Glycoprotein is often found at the center of stones, and mucin hypersecretion is apparently triggered by lithogenic bile[18] and is known to precede gallstone

formation in hamsters[2]. Gallstones have been shown to have mucin incorporated into their matrix[20]. Cholesterol crystals are frequently seen in the mucus gel layer adherent to the gallbladder mucosa[21], and the cholesterol concentration in the mucus gel layer is almost twice as high as that in the liquid gallbladder bile[22]. Similar findings are seen in animal models of cholesterol cholelithiasis[23,24]. Mucin is known to be a large component of gallbladder sludge[25], and sludge is known to coexist with and to precede gallstone formation. Human gallbladder mucin accelerates the nucleation of cholesterol crystals in bile in a dose dependent fashion[9], but removal of all the mucin from bile does not affect the rapid nucleation time of lithogenic gallbladder bile[9]. It would therefore appear that mucin is not absolutely essential to gallstone formation, but it may play a role in early gallstone nucleation in some cases. The evidence linking mucin in the pathogenesis of gallstones, however, remains indirect[2].

Some have proposed that cholesterol gallstones form around a central nidus of pigment. The binding of bilirubin and cholesterol by mucin may explain the presence of both at the center of many cholesterol gallstones[25]. Recent literature indicates that the center of cholesterol gallstones contains very little calcium bilirubinate[26]. Thus the theory that cholesterol gallstones form around a central nidus of a small pigment stone appears to be untenable[25,26].

In summary, cholesterol nucleation has been described as an interaction between the absolute degree of cholesterol supersaturation and the outcome of a balance between the activity of nucleation inhibitors and promotors[27]. Once the crystal has formed it grows by crystallization on its surface. Although an important concept in the pathogenesis of gallstones, this thesis will not comment on nucleation any further.

CHOLESTEROL METABOLISM

i) INTRODUCTION

Cholesterol is used in all cellular membranes and is a component of plasma lipoproteins. It is found in all body tissues[28], and sufficient cholesterol is essential for good health. The plasma cholesterol is only a small fraction of the total body pool, yet it has been the subject of much interest since it seems to correlate with the degree of atherosclerosis present in the body. Much of our knowledge of cholesterol metabolism has been derived from studies dealing with atherosclerosis, but is directly relevant to biliary cholesterol metabolism. As will be explained later, the metabolism of cholesterol, bile salts, and biliary cholesterol excretion are all intimately linked.

ii) SOURCES OF CHOLESTEROL

Sources of cholesterol are endogenous and exogenous (dietary).

Human beings appear to absorb cholesterol in the range of 20-50% of that ingested regardless of the concentration in the diet[29]. The average dietary intake of cholesterol in the North American diet is 500 mg. per day, which represents about 0.1% of the total daily dietary intake of dry solid nutrients. Cholesterol is present in high concentrations in red meats, seafood, and animal fats. Plant foods are almost devoid of cholesterol. Bile acids are essential for efficient cholesterol absorption, and the absorption of

dietary fat and fat soluble vitamins would be markedly impaired without them[30]. The intestinal cholesterol consists of exogenous cholesterol, biliary cholesterol, and cholesterol derived from desquamated cells. It is absorbed almost exclusively by way of intestinal lymph as chylomicrons.

Other sources of cholesterol are endogenous synthesis in the liver, intestine, and peripheral tissues. These tissues produce cholesterol for their own use and for export.

iii) SYNTHESIS

Every animal tissue is capable of some degree of cholesterol synthesis[28]. The two major sources of endogenous cholesterol are the liver and the ileum[30]. The rate of cholesterol biosynthesis appears to be under at least two controls, that is the availability of cholesterol and the actual bile acid levels. Different tissues vary in response to these mechanisms.

Liver cholesterol synthesis is largely inversely proportional to the cholesterol content of the diet[31]. The liver responds to changes in plasma levels of cholesterol containing chylomicra rather than levels of high-density (HDL) or low-density lipoproteins (LDL)[28,32]. In man liver synthesis is not a major source of plasma cholesterol[28,33].

The intestine is very important quantitatively as a source of endogenous cholesterol, it may contribute more by biosynthesis than the liver[31]. Intestinal biosynthesis is

regulated by the intestinal concentration of bile salts[28,31], and does not appear to respond to plasma cholesterol levels.

There are several fates possible for the synthesized cholesterol. It can be incorporated into cellular membranes or plasma lipoproteins, secreted into the bile as cholesterol, transformed into bile salts, or stored as cholesterol esters.

iv) CHOLESTEROL HANDLING IN THE BODY

Virtually all plasma cholesterol is loosely bound to lipoproteins which have evolved to facilitate cholesterol transport. Lipoproteins are specialized systems which serve to solubilize and transport cholesterol, triglycerides, and phospholipids which are otherwise water insoluble, through plasma from sites of lipid absorption and synthesis to sites of storage and utilization[30].

There are 4 major classes based on electrophoresis characteristics and centrifugation weights[30],

Chylomicrons.....non-migrating lipoprotein

VLDL.....pre-β-lipoprotein

LDL.....β-lipoprotein

HDL.....α-lipoproteins.

HDL, LDL, and VLDL (very low-density) lipoproteins are spherical particles with a neutral lipid core (triglycerides and cholesterol ester) and a surface made up of the α or β apoprotein, cholesterol and phospholipid[30,32]. Some species variation in the lipoproteins does occur[32]. They are

synthesized mainly in the liver, although the intestine does contribute some VLDL and HDL. LDL is largely if not totally derived from catabolism of VLDL[30,32].

Chylomicrons are formed at the intestine and carry dietary triglycerides to the plasma and tissues. VLDL are similar to chylomicrons[30].

The plasma HDL and LDL appear to be the lipoproteins most involved in cholesterol metabolism related to gallstone disease. LDL appears to be used in transporting native and esterified cholesterol to peripheral tissues. Studies have shown that fibroblasts possess specific LDL receptors, and the bulk of plasma cholesterol has been shown to be delivered to peripheral tissues via LDL[30].

The complete metabolic functions of HDL are unknown, but it may play a role in triglyceride clearance, and cholesterol esterification in the plasma. HDL also appears to play a role in removal of cholesterol from the peripheral tissues, the HDL acting as a reverse transport vehicle[30]. It acts to bring cholesterol to the liver, the major site for cholesterol breakdown and the major site for HDL and LDL removal from the plasma. HDL also appears to block LDL deposition by competitively binding the LDL receptors[30].

v) EXCRETION

The concentration of cholesterol in tissues in mammals is ultimately controlled by the rate of its elimination from the metabolic pools. It can be eliminated by urine and fecal routes, epithelial sloughing, and sebaceous

secretions. In humans urinary losses are negligible, skin losses amount to about 83 mg/day, and epithelial sloughing is negligible. Fecal excretion is in the form of free cholesterol and as bile acids[28,30]. When fecally excreted as bile acids or cholesterol it is subject to reabsorption in the small intestine[30].

Bile acid synthesis plays an important role in maintaining cholesterol homeostasis because cholesterol breakdown to bile acids and their excretion account for the majority of cholesterol which is removed from the body[30,34,35,36]. At least 60% of radioactive cholesterol injected into rats is converted and excreted as bile acids within 15 days[30].

There is some disagreement as to whether the rate of cholesterol secretion is correlated with the rate of newly synthesized cholesterol[37]. As well, in man increasing dietary intake results in an increased loss of neutral fecal steroids, but the fecal excretion of bile acids does not appear to increase[38]. This is different from animals which do appear to increase their bile acid excretion[28].

Not all liver cholesterol has equal access to the enzymes of bile acid synthesis[39]. It has been suggested that newly synthesized cholesterol is the preferred precursor for hepatic bile synthesis[40,41,42,43]. There appear to be definitive hepatic cholesterol precursor sites associated with the synthesis of bile acids and the secretion of biliary cholesterol[44,45]. These sites derive a substantial proportion (70%) of their cholesterol from the

plasma, and 60% of this cholesterol is unesterified. In studies with a patient with a bile fistula, the free labelled cholesterol from HDL was more rapidly incorporated into biliary cholesterol than free cholesterol from LDL. These findings indicate that the liver in man selectively utilizes and secretes free cholesterol from HDL[46] rather than LDL[47], supporting the concept that HDL plays a role as a reverse cholesterol transport vehicle from the peripheral tissues to the liver[30,45,46].

The other major route of biliary excretion is as native cholesterol. Approximately 80% of biliary cholesterol is derived from the plasma cholesterol[44]. It has been shown that HDL-cholesterol is more rapidly converted into biliary cholesterol than LDL-cholesterol[45]. The bile saturation index has been shown to be negatively correlated with the HDL-cholesterol level, and this has been taken as proof of the clinical correlation of the risk of gallstones and atherosclerosis coexisting in patients[48]. When hepatic cholesterol synthesis increases in response to a need for more sterol in the body, a greater proportion of biliary cholesterol is derived directly from newly synthesized sterol, but total biliary cholesterol output is unchanged. In contrast, when more cholesterol is synthesized than is needed to maintain cholesterol balance, biliary cholesterol output may increase. Such excess biliary sterol is derived primarily from the transport of newly synthesized sterol directly across the canalicular membrane[49].

In summary, cholesterol metabolism is closely linked to bile salt metabolism. Cholesterol from plasma and newly synthesized liver cholesterol both can be used for bile salt synthesis and excretion of native cholesterol into the bile, and these account for the vast majority of cholesterol lost from the body.

BILE SALT METABOLISM

i) INTRODUCTION

The functions of bile acids in the gastrointestinal tract are:

1. Detergent function of dispersing and solubilizing dietary fats for hydrolysis and absorption,
2. Activation of some pancreatic lipolytic enzymes,
3. Stimulation of secretion of water and salt from the small and large intestine,
4. Effect on the motility of the intestine and the secretion of intestinal hormones[50].

Within the biliary tract bile salts are essential for solubilizing cholesterol. They do this by formation of micelles along with phospholipids[51].

Bile acids form the major catabolic pathway of cholesterol excretion, and they are necessary to allow native cholesterol to be solubilized and excreted in the bile.

ii) SYNTHESIS

Endogenous versus exogenous[39], and newly synthesized versus equilibrated[40] cholesterol have been examined as the preferred substrates for bile salt synthesis, and all are possible substrates[30]. In the intact rat newly synthesized liver cholesterol has been shown to contribute 25% of the bile acid precursor, 75% of the bile acid is from plasma and other cholesterol[52]. In man 31% of bile acid precursor

cholesterol was estimated to be newly synthesized in a study where bile fistula prevented recycling[44]. Therefore it would appear that bile acids are synthesized from a mixture of dietary and endogenously synthesized cholesterol. However, there are no studies of animals on a cholesterol free diet[53].

The primary bile acids (cholic and chenodeoxycholic acids) are synthesized in the liver. The rate of cholic acid synthesis is normally about twice that of chenodeoxycholic acid[54], and it is formed via a separate metabolic pathway from chenodeoxycholic acid in man[55].

Bile acid synthesis can be inhibited by oral or intravenously infused bile acids[55]. The enzyme cholesterol 7α-hydroxylase is the first and the rate limiting step[34,57] in the metabolic pathway.

The short term regulation of cholesterol 7α-hydroxylase activity is thought to be by an interconversion between a phosphorylated (active) and a dephosphorylated (inactive) form[58]. The interconversion appears to be mediated by cytosolic factors[59]. Acyl-CoA: cholesterol -O-acyltransferase (ACATase), another key enzyme in cholesterol utilization is also activated by phosphorylation. HMG CoA reductase (the major enzyme in cholesterol biosynthesis) activity is directly linked to the activity of cholesterol 7α-hydroxylase[34,60]. It may also be regulated by phosphorylation and dephosphorylation, the phosphorylated form being inactive[61]. Thus the hepatic concentration of cholesterol may be controlled by the

coordinate control of phosphorylation of the three enzymes[61,62].

In man the major controlling factor over the rate of bile salt synthesis appears to be the amount of bile salt returning to the liver from the gut via the portal vein. When bile salts are prevented from returning to the liver the rate of bile salt synthesis in the liver increases manyfold. Bile acids (with the exception of lithocholic acid) are choleretic agents, that is they will increase the bile flow when infused intravenously[62], and there is a close relationship between bile flow and the hepatic excretion rate of bile acids[63]. Acute interruption of the enterohepatic circulation of bile acids in man by diversion of bile flow causes the rate of bile secretion to decrease by 50%[64]. From these observations has evolved the concept that bile acids regulate their own synthesis by a negative feedback mechanism involving the rate limiting enzyme of bile acid synthesis cholesterol 7α-hydroxylase. Cholic acid feeding has been shown to suppress the activity of cholesterol 7α-hydroxylase, while cholestyramine increases the activity and cholesterol feeding has no effect[34]. Feeding chenodeoxycholic acid suppresses cholesterol 7α-hydroxylase activity even more than cholic acid feeding[65]. Conjugated bile salts appear to be about twice as potent in suppressing bile salt synthesis as unconjugated salts, and therefore colonic deconjugation may be a determinant of overall bile salt synthesis[65], since up to 30% of the bile

salt pool is deconjugated during each cycle of the enterohepatic circulation.

Another mechanism for regulating bile acid synthesis is cholesterol availability. As has been previously said, newly synthesized cholesterol is believed to be the preferred substrate for bile acid synthesis[41,44], but dietary cholesterol is converted as well[44,46,47]. Bile acid synthesis and secretion is increased when the hepatocyte cholesterol pool is increased by feeding a cholesterol rich diet[66].

iii) ENTEROHEPATIC CIRCULATION

In the enterohepatic circulation of bile acids, the acids are secreted by the liver into the bile, reabsorbed from the intestine, returned to the liver by the portal vein, and then resecreted by the liver. The enterohepatic circulation is the body's method for conserving bile acids.

The primary bile acids that enter the colon are bacterially transformed into secondary bile acids. The secondary bile acids in man are deoxycholic, lithocholic, and ursodeoxycholic acids, and they have their own enterohepatic circulation[54].

The enterohepatic circulation is relevant to the chemistry of bile acids because it influences the biliary bile acid composition. It has been found that chenodeoxycholic acid conjugates are conserved to a greater degree than those of cholic acid, and therefore the bile

becomes preferentially enriched in chenodeoxycholic acid conjugates.

Most of the mass of the bile acid pool is in the small intestine (97%)[67] and the rest is in the liver. One to 5% of the pool is excreted daily, and in the steady state this amount is replaced by new synthesis[30].

The ileal reabsorptive process is competitive for the different bile acids[49,68], and is sodium dependent[69,70]. Ileal active transport facilitates rapid absorption of bile acid conjugates in the ileum[71].

Bacterial modification begins in the distal small intestine, but most of the bile acids are reabsorbed from the small intestine before bacterial modification can occur. The small fraction of bile acids which escapes active or passive absorption in the small intestine passes into the colon where it is essentially totally biotransformed by bacteria[72].

The fate of secondary bile acids depends upon their intrinsic physical properties, their interaction with luminal contents such as food residues and bacteria, the passive permeability of the large intestine, and probably their residence time in the colon. A fraction varying from 20-50% is reabsorbed[54]. Since non-ionic diffusion is rapid, at normal bowel pH free bile salts are absorbed better than glycine conjugates, and taurine conjugates are almost totally dependent upon active ileal reabsorption[30]. Reabsorption of dihydroxy acids (deoxycholic and chenodeoxycholic acids) appear to be similar to and perhaps

even better than cholic acid[77]. Taurine conjugated and conjugated trihydroxy bile salts are transported more readily than glycine conjugated and conjugated dihydroxy bile salts, respectively[68].

Lithocholic acid residues are sulfated and therefore are not reabsorbed to any great extent[62,72,78]. This has survival advantages as lithocholic acid is hepatotoxic when fed to animals[54].

About one-half of the deoxycholic acid formed is reabsorbed and passes to the liver. The enterohepatic circulation of deoxycholic acid may have some relevance to cholelithiasis, since several studies suggest that deoxycholic acid induces the formation of a supersaturated bile[79], and there is a high negative correlation between the proportions of deoxycholic acid in bile and the degree of bile saturation with cholesterol[80]. An increase in the recirculation of deoxycholic acid reduces the amount of chenodeoxycholic acid in the bile by selectively suppressing its synthesis[59,81]. Deoxycholic acid might therefore be an important determinant of cholesterol saturation in bile since it suppresses synthesis of the one bile salt known to decrease the secretion of cholesterol[82].

Ursodeoxycholic acid is formed from chenodeoxycholic acid via intermediates. Colonic bacteria are responsible[83].

In humans the passive ionic and non-ionic diffusion recovery of bile acids in the colon has been estimated at about 200 mg/day[84], but is not considered to be a significant phenomenon quantitatively[30].

Secretion and metabolism of bile salts depend upon their hepatic uptake from the portal blood. Interruption of the enterohepatic circulation results in accelerated synthesis of bile acids[85] and restoration or enhancement of bile acid flux inhibits synthesis[56]. The mechanism of the effect is unknown[30], but it is known that the nature of the bile acids returning to the liver can influence the enzymes regulating their metabolism[86].

The feeding of any bile acid results in enrichment of it and its secondary bile acid in the enterohepatic circulation[54]. Bile salt fed animals secrete more bile than controls and the given bile salt predominates in the bile[31].

Feeding small amounts of deoxycholic acid raises the bile cholesterol saturati leading to the suggestion that increased return of deoxycholic acid to the liver alters its metabolism to favor the secretion of bile supersaturated with cholesterol[82]. Since deoxycholic acid is formed in the colon it is suggested that populations with high colonic absorption of metabolized cholic acid (that is deoxycholic acid) have an increased disposition to form gallstones[82]. The effect disappears when larger amounts of deoxycholic acid are given[87,88,89]. The effect is possibly due to an enlarged deoxycholic acid pool suppressing hepatic chenodeoxycholic acid synthesis[81].

Giving chenodeoxycholic or ursodeoxycholic acid in humans diminishes the output of cholesterol in the bile[90,91], and reduces biliary cholesterol saturation. This results in the conversion of saturated bile to the

unsaturated state[86,92,93,94]. The exact mechanism is unknown[95]. When cholic acid is fed the bile is composed predominantly of cholic and deoxycholic acids and the chenodeoxycholic acid synthesis rate decreases by 50%[91]. This is believed to occur because chenodeoxycholic acid suppresses hepatic cholesterol, but not bile acid secretion[90,96,97]; a property not shared by cholic acid[86,90,94,96]. Taurocholate suppresses the activity of both HMG CoA reductase and cholesterol 7α-hydroxylase, but taurochenodeoxycholate (and ursodeoxycholate) apparently affect only HMG CoA reductase[98,99], implying that taurocholate suppresses both cholesterol and bile acid synthesis, while taurochenodeoxycholate and ursodeoxycholate suppress only cholesterol synthesis and permit a normal rate of bile acid production[86]. These effects have allowed chenodeoxycholic and ursodeoxycholic acid to be used as pharmacological agents for the treatment of gallstone disease[100,101]. There have been many trials that all show bile desaturation results from administering adequate oral dosages of chenodeoxycholic or ursodeoxycholic acid. This occurs in almost all patients, and after 1 to 2 years will result in dissolution of radiolucent gallstones in the majority of patients who ingest optimal dosages[102,103]. The use of chenodeoxycholic acid and development of other desaturating agents has been hampered by the lack of an animal model of cholesterol gallstone disease[99].

Most patients with cholesterol gallstones that are not obese have a reduced bile acid pool size within the

enterohepatic circulation[104]. A small pool size could lead to decreased bile acid secretion and oversaturation of bile with cholesterol. There are three possible reasons for a small pool: increased losses, decreased synthesis, or increased cycling frequency. There is evidence to suggest that decreased cholic acid synthesis[104] and a reduced level of hepatic cholesterol 7α-hydroxylase activity[105] occur in patients with gallstones.

iv) COLONIC METABOLISM

Under normal conditions the deconjugation of bile acids occurs in the terminal ileum, cecum, and colon with consequent precipitation or adsorption of the bile acids onto fibrous material[51,73,74]. It has been shown that deconjugation by splitting the peptide bond occurs in the cecum[74,75]. The bile acids are then converted into secondary bile acids[76]. The fecal end products are so varied in structure that their qualitative and quantitative assay pose a difficult task[30].

Most if not all fecal bile acids are unconjugated[31], and as has been previously stated conjugated bile acids have a greater ability to decrease bile acid synthesis. Deconjugation and dehydroxylation are mediated by the bacteria of the colon, and the effects of manipulating the colonic flora are interesting.

Human studies show that antibiotics have a profound effect on bile. When bile acids are metabolized by gut flora the end products are toxic with lithocholic acid being

the most toxic[106]. In rats, gut sterilization with antibiotics increases the biological half-life of cholic acid. Antibiotic administration in pigs resulted in a significant reduction in the half-life of chenodeoxycholic acid, from 6.4 to 5.7 days[106]. Changes in bacterial flora affect bile acid excretion. When germ free rats are fed cholic acid, only taurocholate is found in their feces. Bile acid excretion is lower than normal in germ free rats[30].

Metronidazole (which is active against many colonic bacteria) when given orally reduced the proportion of deoxycholic acid in the bile, raised that of chenodeoxycholic acid, and lowered the bile cholesterol saturation[107].

The addition of lactulose to the diet reduced deoxycholic acid and increased chenodeoxycholic acid levels in the bile significantly. Bile was initially saturated with cholesterol and it became less saturated in all but one patient[108]. Lactulose is metabolized in the colon and reduces the pH to about 5.0. In vitro, 7α-dehydroxylation of bile acids is inhibited at a pH of 6 - 6.5[109]. Thus the expected result from lactulose is an increase in primary bile acid metabolism with a decrease in deoxycholic acid and an increase in chenodeoxycholic acid, resulting in a reduction in bile saturation. This is what was observed[109].

The colon affects many aspects of biliary homeostasis. Because of the frequent coincidence of a high proportion of deoxycholic acid in bile and cholesterol gallstone disease

it has been suggested that there could be a causal relationship between them[82,110]. This hypothesis has been formulated based on circumstantial evidence that deoxycholic acid absorbed from the colon may also in normal subjects without gallstones inhibit selectively hepatic synthesis of chenodeoxycholic acid, thereby enhancing secretion of supersaturated bile and subsequent gallstone formation[81,111]. However, other studies have not supported this hypothesis. In one, an association of high deoxycholic acid absorption and low proportion of chenodeoxycholic acid in the bile was found only in a group of 'older' patients. There was found to be some degree of inhibition of cholic acid synthesis in the older group. Overall, the data seemed to suggest that obesity is a stronger determinant of cholesterol supersaturation in bile than deoxycholic acid absorption from the colon[112].

v) EXCRETION

Diet is known to influence bile acid excretion. Dietary cellulose[113], pectin[114], lignin[115], and soybean meal[116] have all been shown to increase bile acid excretion rates. Ingestion of polyunsaturated fats also appears to increase bile acid excretion[117,118]. The interest in this type of work lies in the possibility that in spite of the many metabolic checks and balances, enhanced fecal excretion of lipid will drain away the body's excess cholesterol[79].

Cholestyramine, which binds bile acids and dramatically increases fecal losses, has a very marked effect in causing

a large increase in both cholesterol and bile acid synthesis, the bile acid increase being almost twice that of cholesterol[94].

In summary, bile acids appear to regulate their own synthesis through negative feedback control on the liver. This feedback also alters the spectrum of bile acids present in the bile. The enterohepatic circulation and colonic metabolism of biles acids may be important in gallstone disease since increased levels of deoxycholic acid apparently inhibit synthesis of chenodeoxycholic acid but not of cholesterol, and result in an increase in the biliary cholesterol saturation.

DIETARY CONSIDERATIONS

The incidence of gallstones has been related to the dietary intake of fiber[3,5]. Other authors have pointed to the intake of refined carbohydrates as the culprit[3,5,119,120]. Consumption of saturated animal fat is not implicated in the causation of gallstones, as unwesternized Eskimos and Massai tribesmen who ingest large amounts of animal fat rarely get gallstones. Stones are induced in dogs and hamsters by feeding high starch and sugar diets[3], not by the addition of saturated animal fats. The effects of these diets can be reversed by the inclusion of fiber[121].

i) CARBOHYDRATES

The proponents of a linkage between the intake of refined carbohydrates and gallstones point to three circumstantial proofs:

1. Since the incidence of gallstones is higher in all of Western Society there must be a common factor. Carbohydrates in Western Society are almost all refined, whereas Africans eat almost no refined carbohydrates. Eskimos have only recently been exposed to refined carbohydrates, but quickly introduced large amounts into their diets and are now suffering from increased rates of cholelithiasis, as well as dental caries and obesity[122].

2. Refined carbohydrates cause obesity because they have a high ratio of energy intake to satiety[123], and obesity is a risk factor for gallstones.

3. To induce cholesterol rich stones in experimental animals it is necessary to give them a diet rich in refined carbohydrates. Such diets cause suppression of bile salt synthesis by the liver and hence a small bile salt pool results[3].

ii) FIBER

Far from being the inert substance it was once thought to be, fiber can be shown to have a variety of physiological and biochemical effects on the digestive system[79]. Most experiments with fiber test it in an artificial form stripped from the nutrients which it normally encloses, and therefore the results of such studies must be questioned.

Bulk fiber in the diet displaces nutrients, slows the intake of sugars and starches (because the fiber increases food bulk and requires chewing), and it reduces the absorptive efficiency of the gut[123]. The source as well as the size of the fiber particles may be important[124]. Coarse bran flakes produce greater effects than fine bran flakes on colonic transit time in patients with constipation[125].

Fiber enriched diets have been observed to change cholesterol saturated bile to an unsaturated state[126,127,128]. However, when fiber is given to healthy volunteers with unsaturated bile there is no change in the

cholesterol saturation index or the mean percentages of cholesterol, bile acids, or phospholipids in the bile[129]. In saturated bile the effects of bran show an intriguing similarity to the more potent effects of feeding chenodeoxycholic acid, and are opposite those of feeding deoxycholic acid[79]. The mechanism by which bran influences cholesterol secretion is unknown. In one study subjects with probable cholesterol gallstones ate refined and unrefined diets for 6 weeks. As expected the cholesterol saturation index was significantly lower on the unrefined diet. On the refined diet the bile contained relatively less cholic and slightly more deoxycholic acid. There were no significant differences in the total bile acid pool sizes, or in the rate of primary bile acid synthesis or fractional turnover. The conclusion was that the consumption of refined carbohydrates increases bile cholesterol saturation[128]. Similarly, in rats fiber ameliorates the effects of adding cholesterol and fat to the diet[121]. When added to the diet, wheat bran can reduce the intestinal uptake of cholesterol, thus affecting the levels of circulating steroids[130]. When pigs were fed a cholesterol free mash diet versus a semi-purified diet fecal bile acids increased about 3 1/2 times on the mash. Hepatic HMG CoA activity levels also increased. It was inferred that the changes were secondary to loss of steroids in the feces[131].

Possible effects of fiber on bile salt metabolism include:

1. Alteration in gastric emptying and small bowel transit rate, which would be expected to affect the frequency of enterohepatic cycling. This has not yet been studied.

2. Fiber might act like an ion-exchange resin and by binding bile acids might influence their absorption by the small bowel, colonic metabolism and/or reabsorption by the colon.

3. By altering the physical, chemical, and perhaps bacteriological environment of the colon, dietary fiber might influence the colonic metabolism and absorption of bile acids[79].

The consequence is alteration of the quantity and type of bile acid returning to the liver via the portal vein. This is known to play a regulatory role in the new synthesis of cholesterol and bile acid[79].

BINDING EFFECTS

Since small bile salt pools are found in subjects with supersaturated bile[126], and since the pool can be decreased by feeding animals fiber depleted sugar or starch diets, it is possible that adding fiber to the diet might expand the bile salt pool and increase the amount of detergent available to solubilize cholesterol in the gallbladder[132].

Raw wheat bran when fed to volunteers causes chenodeoxycholic acid synthesis and pool size to increase significantly. Deoxycholic acid pool size decreases, cholic acid synthesis and pool size are unchanged. When the same

bran was fed to patients with presumed cholesterol stones their bile became significantly less saturated. The conclusion was that bran probably acts in the colon to reduce the formation or absorption of deoxycholic acid, a substance which impairs chenodeoxycholic acid synthesis[132].

COLONIC EFFECTS

The slow passage of material through the colon allows for prolific bacterial growth and increased digestion of fiber[133].

Wheat bran can alter intestinal and consequently hepatic bile acid metabolism and in some cases can decrease bile cholesterol saturation. Such studies confirm that what takes place in the colon is of some importance systemically[54,82]. Possible colonic effects of fiber are:

1. Some fiber (e.g. pectin) is almost totally fermented in the colon. Fermentation results in a much larger total bacterial cell mass in the colon and this may result in more 7 α-dehydroxylation.

2. With fermentation the pH of the bowel will fall and this will result in less 7 α-dehydroxylation.

3. Less primary bile acid may be available for degradation because of strong binding to fecal residues, decreased transit time (and therefore less time for degradation and reabsorption)[134,135], and/or a larger fecal mass

(and therefore a decreased concentration of degraded bile salts available for reabsorption)[129].

4. Increased transit rate means that colonic bacteria have less time to act on the bile acids.

5. The bulk effect of the bran may prevent physical contact between the bile salts and colonic bacteria, and therefore decrease deconjugation and dehydroxylation of cholic and chenodeoxycholic acids.

The effects of bran are probably limited to the colon where it decreases cholic acid metabolism or deoxycholic acid metabolism, or both[79]. It has been shown that feeding bran results in a decreased deoxycholic (secondary bile acid) acid level in the bile[128,136]. These effects have been observed to be independent of the changes in transit time and imply that bran reduces the colonic bile salt degradation[136].

In summary, a lack of fiber may be very important in the pathogenesis of gallstones since fiber can unsaturate bile as well as altering the bile salt profile. The effects of bran appear to be twofold. First it decreases the amount of carbohydrate intake, and secondly in the colon it affects the bile salt metabolism.

ANIMAL MODELS OF GALLSTONE DISEASE

A genetically susceptible, spontaneous and naturally occurring animal model for human cholesterol gallstone disease is yet to be identified. The induction of stones in an animal model essentially depends upon the achievement of bile supersaturation in the given species, and there are only a limited number of ways to produce this[137]. Some primates can be induced to have cholesterol cholelithiasis only with prolonged feeding of cholesterol rich diets. In work with hamsters, stones can be reliably induced with a diet that induces essential fatty acid deficiency and a consequent marked increase in total body (and especially hepatic) cholesterol synthesis. The work to the present time has been aimed at characterizing the effects of altered lipid metabolism. There has never been any serious suggestion of direct relevance to human gallstone disease. The use of prairie dogs is difficult since they are somewhat difficult to handle and are seasonally unavailable. The use of a 1.2% cholesterol diet does induce gallstones, but also induces a strikingly unphysiological endogenous overload to this normally herbivorous species[137]. In animals the major portion of ingested lipid is in the form of triglycerides, but small amounts of cholesterol are also present[32]. Mice can be induced to stone formation on a cholic acid cholesterol diet[137].

THE PIG AS A MODEL OF GALLSTONE DISEASE

Swine have long been used in studies of cholesterol metabolism because they are more like humans in response to high-fat, high-cholesterol diets than are most other experimental animals including subhuman primates; they eat virtually all of any reasonable diet; and they develop atherosclerotic lesions that are similar in many ways to those in man[138,139]. Swine are also similar to man being omnivorous, and having a similar digestive and cardiovascular system[140]. Excretion of bile acids is 249-317 mg./day on various diets, which is almost identical to that in man. The pig like man has little ability to sequester large amounts to excess cholesterol in tissues other than arte[illegible] and plasma, and the liver can store only moderate amounts[138]. In swine (specifically miniature pigs) cholesterol kinetics and fate seem similar to those in man[30]. Bile acid synthesis from administered cholesterol begins within 15 minutes, reaches a maximum at 6 hours, and then maintains an equilibrium for several days[141].

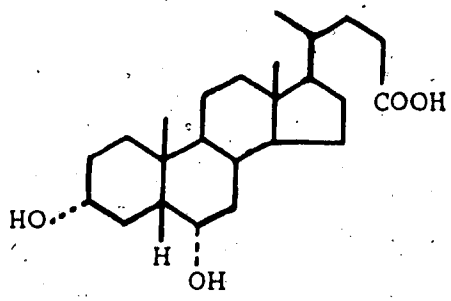
Gallstones are occasionally found in pigs, and usually are composed of calcium salts of lithocholic acid as well as 3-beta,6-α, dihydroxy-5-β-cholanic acid[142]. Pigment stones are seen in swine, mixed stones are less common but are seen as well[143,144].

Presumably the pig has a vegetarian as opposed to a carnivorous bile[145]. The pig has seven bile acids present in its bile (Figure 2.). Hyodeoxycholic, hyocholic

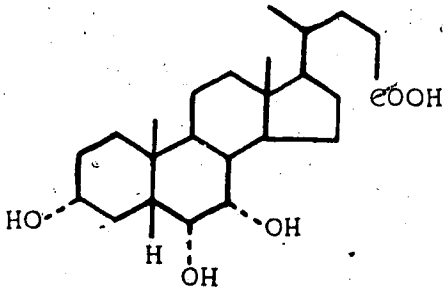
THE BILE ACID SPECTRUM OF THE PIG

(modified from Ref. 147)

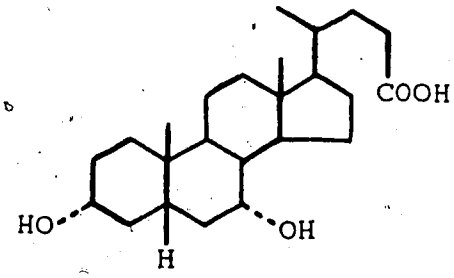
MAJOR CONSTITUENTS



HYODEOXYCHOLIC ACID

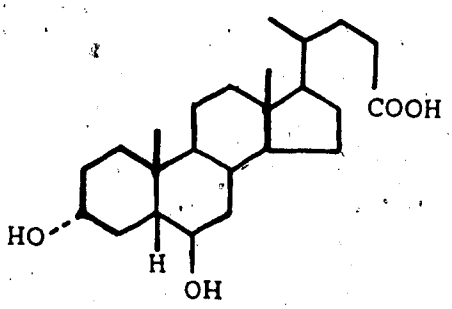


HYOCHOLIC ACID

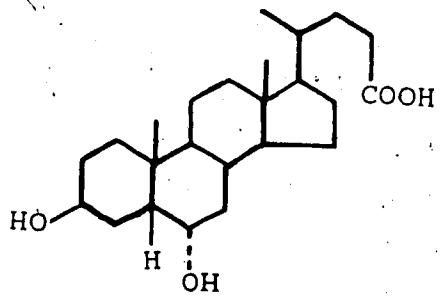


CHENODEOXYCHOLIC ACID

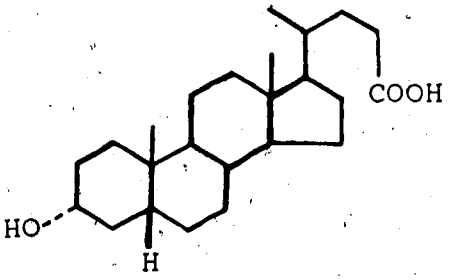
MINOR CONSTITUENTS



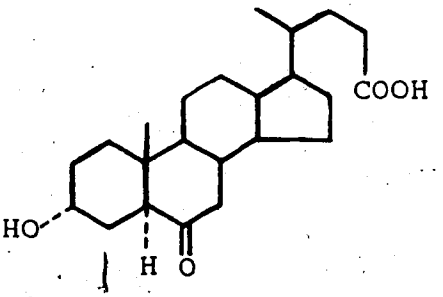
3-ALPHA,6-BETA
-DIHYDROXYCHOLANIC ACID



3-BETA,6-ALPHA
-DIHYDROXYCHOLANIC ACID



LITHOCHOLIC ACID



3-ALPHA-HYDROXY-6-KETO
-ALLOCHOLANIC (6,10,11) ACID

FIGURE 2.

(3-α,6-α,7-α- trihydroxycholanic acid) which is unique to pigs[142], and chenodeoxycholic acids are the main constituents[146]. Cholic acid is present in the pig in minute amounts[31].

As in man, bile flow in pigs increases in response to bile acid infusion in blood[146].

In pigs, fistula bile contains glycine and taurine conjugates of hyocholic and chenodeoxycholic acids only. These are agreed to be the primary bile acids in the pig. The gallbladder bile contains in addition hyodeoxycholic, 3-α,6-β and 3-β,6-α-dihydroxycholanic acid, 3-α-hydroxy-6-oxocholanic, and lithocholic acids. These additional alterations occur in the intestine[142].

The significant bile acids in the pig are chenodeoxycholic and hyodeoxycholic acids[31]. It has been reported that the pig does not have the ability to form hyodeoxycholic acid from hyocholic acid in the liver[106]. However, others[31] have reported that hyodeoxycholic acid is present in germ free pigs, and it is therefore a primary bile acid. Hyocholic acid is formed in the liver from chenodeoxycholic acid[147] (Figure 3.).

The half-life of chenodeoxycholic acid in the pig is 6.4 days, similar to the 6 days in man.

A high-cholesterol, high-fat diet given to pigs results in formation of arterial plaques and lesions absent from control animals[139,148]. A similar diet caused increased secretion of bile, biliary lipids, and increased bile acid pool size. Fecal steroid excretion was also increased.

BILE ACID SYNTHESIS IN THE PIG

(modified from Ref. 106)

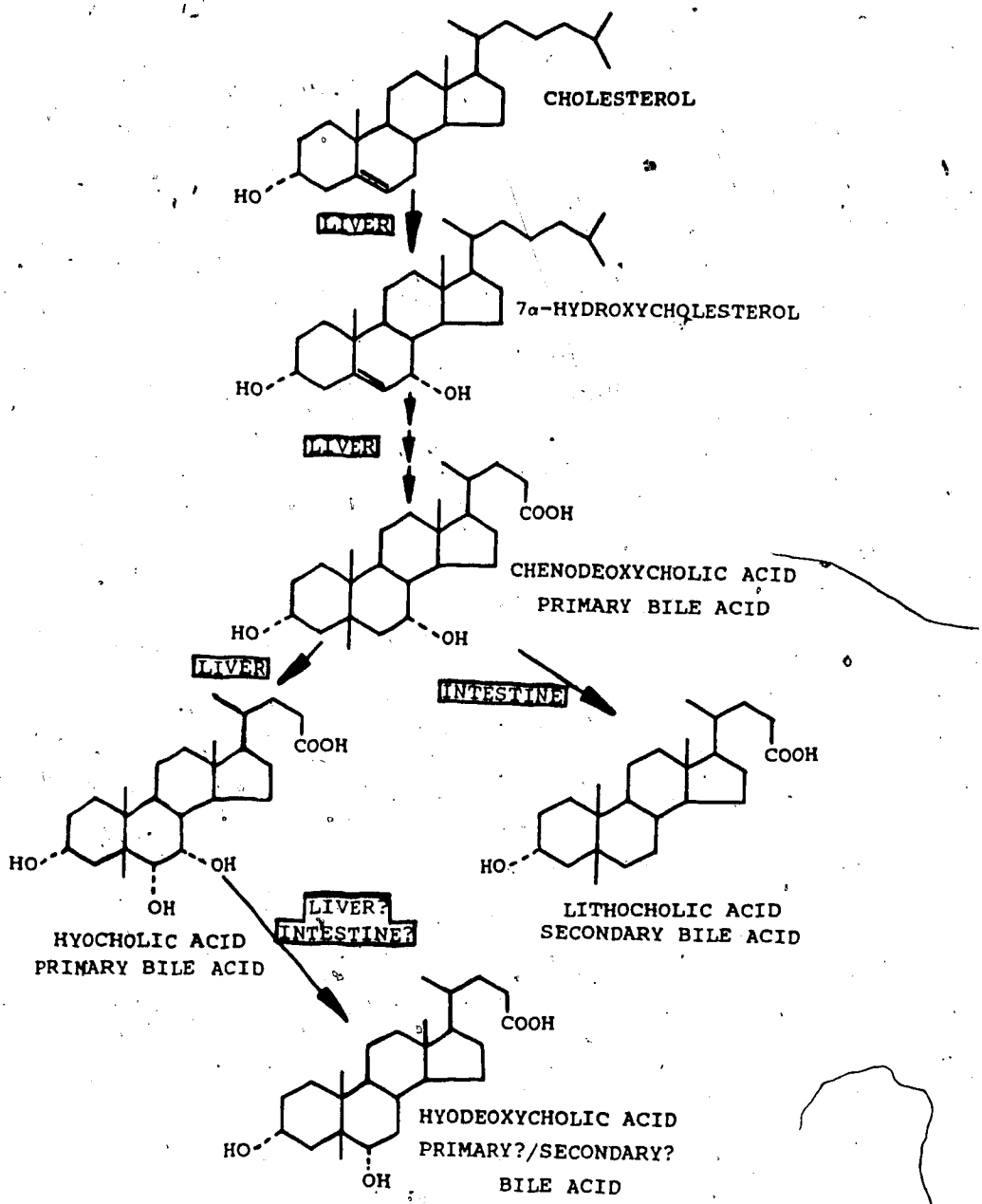


FIGURE 3.

Plasma cholesterol, bile volume, and bile acid pool size all increased in response to the introduction of fat in the diet. Biliary cholesterol secretion increased significantly when the diet was changed to high fat. Phospholipids also increased, as did the bile acid output[140].

RESEARCH PROPOSAL

It has been stated that populations with slow colonic transit time have an increased propensity to form gallstones. In part this may be due to dietary factors, but there is also evidence that the return of secondary bile acids from the colon may also play a role. What has never been studied is the effect of slow colonic transit without altering the diet. It is entirely possible that the fiber that is added to many diets may have chemical as well as its well known laxative effects.

In an effort to resolve this question we propose to mechanically alter the colon of young pigs to impede forward flow of colonic contents. If sufficient slowing occurs this would allow us to study the effects of transit time separate from those of diet. In theory, the slowed transit time should allow for a complete alteration of primary to secondary bile salts, and greatly increase the reabsorption of those bile salts. The increased return of bile salts to the liver will decrease the synthesis of bile salts, and presumably will also change the relative concentrations of the bile salts. This decreased bile salt synthesis will result in an excess of cholesterol in the liver which will have to be excreted in order to maintain the cholesterol homeostasis. Some or all of this excretion will occur via the bile, and the combination of an altered bile spectrum, less bile acid synthesis, and increased cholesterol

excretion should result in an increased cholesterol saturation index of the bile.

In man, increased return of colonic bile salts would increase the return of deoxycholic acid to the liver. This is known to inhibit the synthesis of chenodeoxycholic acid and result in an increase in bile cholesterol saturation. The pig has only small amounts of cholic acid and deoxycholic acid present, and there is no literature commenting on whether any of the pig bile acids (e.g. hyodeoxycholic acid) act in a similar way.

In summary, we propose to mechanically alter the pig colon to achieve prolonged colonic transit time while maintaining the pig on a standard diet. We will then monitor the bile to ascertain whether any change in the biliary lipids occurs.

MATERIALS AND METHODS

i) PRE-OPERATIVE

Young weaned common pigs weighing 10 to 30 kilograms (mean 15 ± 1.2 kg.SEM) were obtained through the University of Alberta Surgical Medical Research Institute. The animals were placed in a holding facility, each pig having a separate pen. The pens measured 1.5 m^2 and all were equipped with a separate feeding dish and water supply. Normal diurnal variation in light was maintained. The animals were placed on a standard trace cholesterol diet (14% hog finisher, University of Alberta Farms) throughout the duration of the experiment.

Once the animals had settled and been on the diet for at least 2 days, the pre-operative transit time was measured. Previous researchers have used dyes[149], and soft radiopaque markers[150]. I found the best results were obtained by directly placing the animal under a light general inhalational anesthesia using halothane 1.2% (vol/vol). The stomach was then intubated using a semi-rigid hollow plastic tube of 10 mm. O.D. Once in the stomach 10 standard markers ("B.B.'s") were instilled. An x-ray was then used to confirm placement of the markers. This procedure had the great advantage that the exact time of marker placement was known, which is not the case when the pig consumes markers placed in its food.

Daily x-rays were then obtained to follow the progression of the markers. The end point was taken as

being the midpoint in hours between the last x-ray to show all markers present, and the first x-ray to show one or more markers absent.

After the transit time had been measured the animals were fasted for two days and then underwent laparotomy for obtaining a bile sample and to undergo the operative procedure. The animals on this protocol were very slow to recover after the operation, and so the protocol was changed slightly in that later animals had a short laparotomy for bile sampling and were then rested for several days on a normal diet before fasting and bowel preparation were undertaken.

Bile was aspirated under direct vision using a 23 gauge needle and syringe. The gallbladder was then observed for several minutes to ensure that no leaking was occurring. The abdomen was then closed using polyglycolic acid sutures. Although percutaneous aspiration of bile has been described in pigs[151,152], I did not have the proper instruments or expertise to perform this.

Our bowel preparation consisted of withholding food but not water for 48 hours. On the morning before surgery the animal was given 300 ml. of magnesium citrate solution ("CITRO-MAG").

ii) OPERATIVE

Formal laparotomy was undertaken using inhalational halothane anaesthesia for both induction and maintenance. The animals were intubated only if airway compromise was

noted once deep anaesthesia was present. The majority did not require intubation. The abdomen was then clipped of all hair, washed with surgical scrub soap and painted with povidone-iodine solution ("BRIDINE"). Normal sterile procedure was maintained throughout.

The abdomen was entered through a midline incision. The pig has a unique colon in that it is held in a conical spiral by peritoneum[153,154]. This entire colonic mass was elevated onto the abdomen. The apex of the spiral is said to be approximately the midpoint of the large intestine[155], and so our next step was to find this point. Once located, we divided the peritoneal membranes on either side of this loop, and were thus able to mobilize it from the rest of the colon. Once sufficient bowel had been mobilized, stay sutures were placed to designate the length of loop to be reversed. The bowel was then divided at these points, and then reanastomosed according to the procedure which we adapted from the literature[156] (Figures 4,5,6.). This resulted in a functionally antiperistaltic loop of colon without twisting the loop on its mesentery. All anastomoses were done using 4-0 silk sutures in standard fashion.

Once the procedure was complete the colon was replaced into the abdomen. The abdominal wall was closed using polyglycolic acid sutures, and the anaesthesia was terminated.

The animals were placed back in their pens and given water only ad libitum for the first day. Once bowel

SURGICAL PROCEDURE

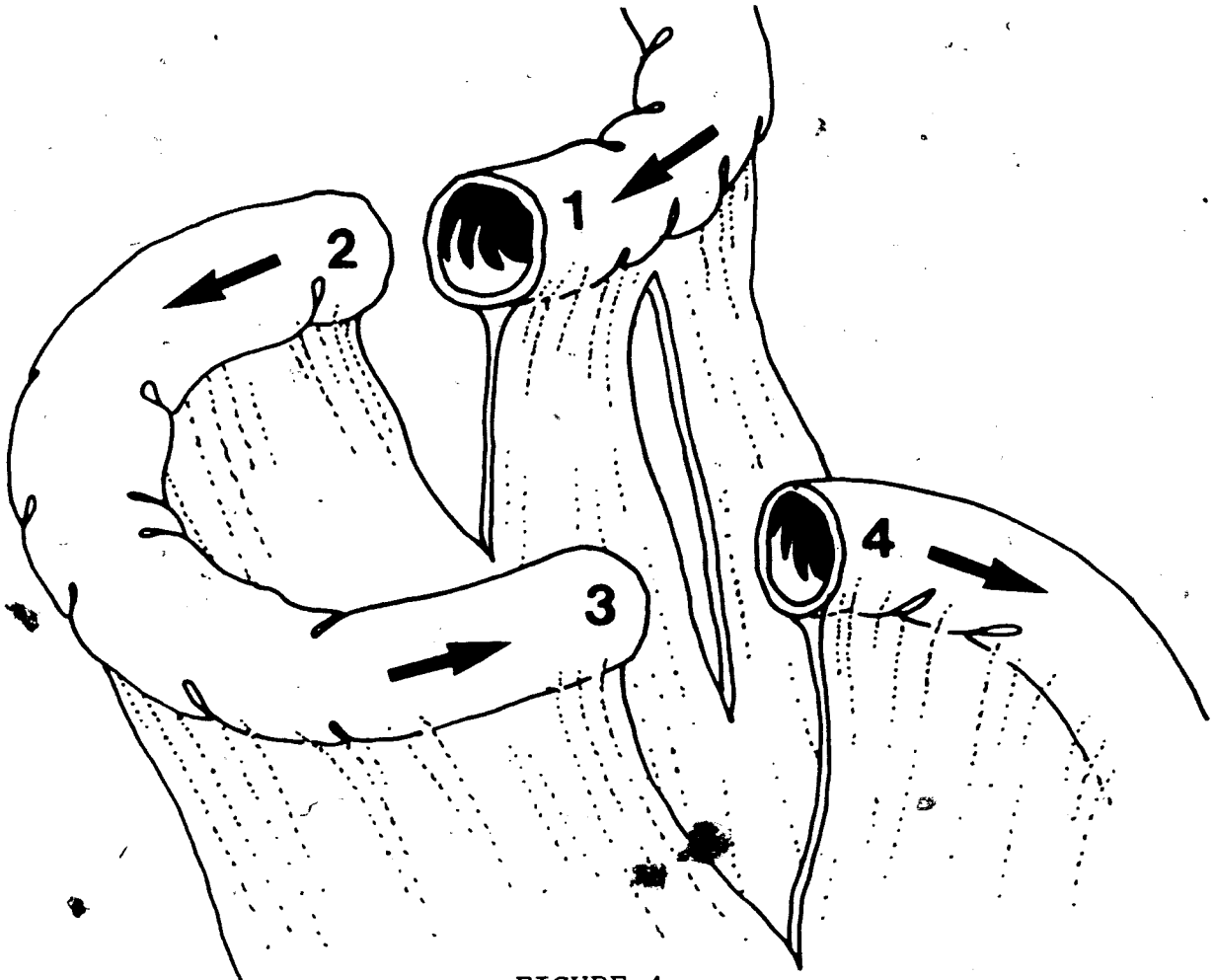


FIGURE 4.

SURGICAL PROCEDURE

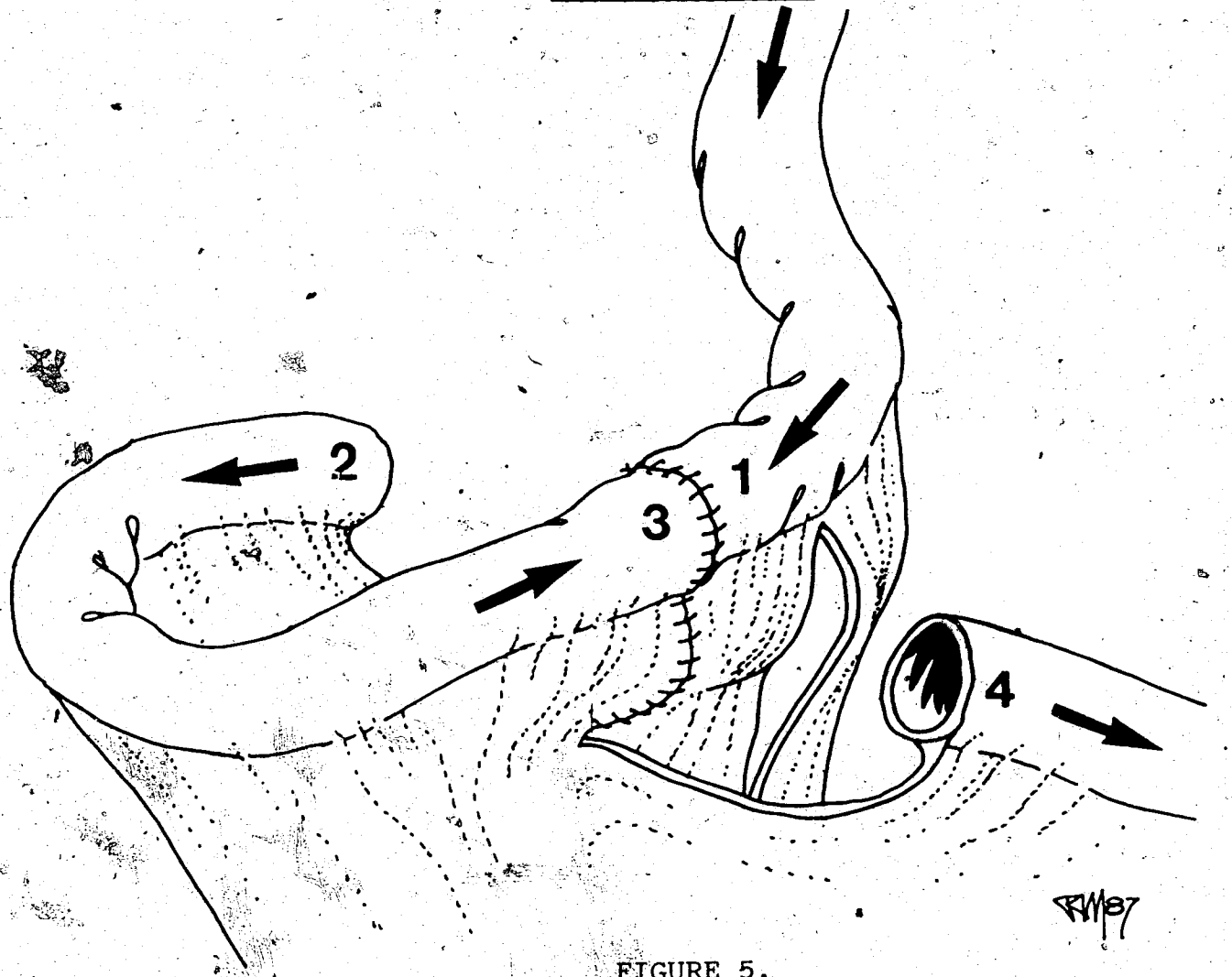


FIGURE 5.

SURGICAL PROCEDURE

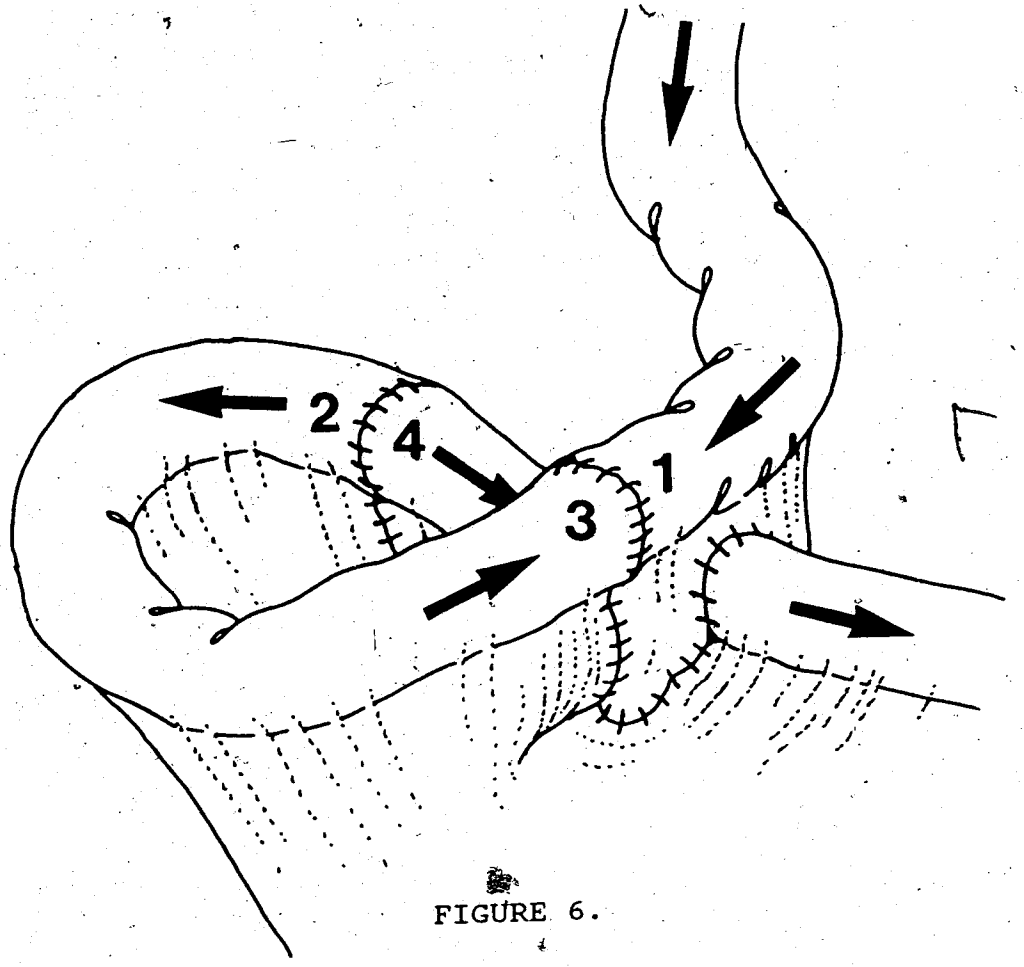


FIGURE 6.

function had returned and they were tolerating fluids, solid food was reintroduced.

iii) POST-OPERATIVE

The animals were maintained on their trace cholesterol diet for 4 weeks. At that time they underwent a laparotomy for bile sampling without prior fasting. If at this point the gallbladder was found to be shrunken and non-functioning the animal was euthanized by lethal injection.

If the gallbladder was normal the bile was obtained and the abdomen closed. The animals were then placed back on normal food rations immediately.

At 6 weeks post-reversal the transit time was again determined using the same technique, except that 10 days was taken as being the absolute end point.

At 8 weeks post-reversal the final bile sample was obtained and the animal was terminated by lethal injection. Measurements of the colon were then obtained to ascertain the position of our antiperistaltic segment.

iv) LABORATORY

Bile samples were stored at -20°C until analysis was performed. All bile samples were analyzed for total cholesterol, phospholipids, and bile salts. Cholesterol levels were measured by an enzymatic colorimetric method utilizing an Abbott Biochromatic Analyzer-ABA-100 (Abbott Laboratories, South Pasadena, California)[157]. Phospholipid concentrations were measured using the method of Sunderman

and Sunderman, utilizing a Unicam spectrophotometer at 675 nm. (Pye Unicam Ltd., Cambridge, U.K.)[158]. The total bile salts were measured using the enzymatic method of Engert and Turner and a Beckman DU-8 spectrophotometer at 340 nm.[159] These methods have been used in the past for the study of biliary lipids[160].

Cholesterol, phospholipids, and total bile salt concentrations were measured as mmol/l. Total biliary lipids were determined from these results using the method of Carey[161], and were expressed as g/dl. The cholesterol saturation index[162,163] was then determined using the method and critical tables of Carey[161].

Comparison of sample data was made using the paired student's t-test where applicable. Unpaired sample means were compared using the standard error of difference between means and tables of standard deviation and probability.

RESULTS

We found that there was considerable intra- and inter-animal variation in all the measured parameters. This has been observed by others[140].

i) ANIMALS

The number of animals studied at 0,4, and 8 weeks was 19, 13, and 9 respectively. The attrition was due to several factors (Table I). In the case of the control animals, they were obtained at an age 4-6 weeks older than our experimental animals and were sacrificed at 4 rather than 8 weeks.

ii) GROWTH

All animals grew well and doubled their weight within 8 weeks as normal pigs have been observed to do[164]. The average weight gain was 259.0 ± 24.1 gm/day in our experimental group. Weight gain in the control animals was 408.8 ± 35.9 gm/day (Table II, Figure 7.). The control animals weight gain differed significantly from that of the experimental animals ($p < 0.01$).

iii) INTESTINAL TRANSIT TIME

Pre-operative transit time in experimental animals was 53.5 ± 7.0 hours. Control animals (which were approximately 4 - 6 weeks older than the experimental animals) had a transit time of 84.0 ± 8.5 hours (Table III). The

TABLE I

DISPOSITION OF ANIMALS

PIG	
1.	Sacrificed at first laparotomy.
2.	Died of mesenteric occlusion due to an adhesive band.
3.	Full protocol.
4.	Full protocol.
5.	Full protocol.
6.	Full protocol.
7.	Full protocol.
8.	Full protocol.
9.	Full protocol.
10.	Died after second laparotomy due to a mesenteric vascular accident - cause unknown.
11.	Full protocol.
12.	Died before entered into the study.
13.	Sacrificed at second laparotomy. Gallbladder small and shrunken, probably nonfunctional.
14.	Died after second laparotomy due to wound infection and peritonitis.
15.	Full protocol.
16.	Died of pneumonia before second laparotomy.
Control-1	Sacrificed at one month.
Control-2	Sacrificed at one month.
Control-3	Sacrificed at one month.
Control-4	Sacrificed at one month.

TABLE II

WEIGHT GAIN OF ANIMALS

PIG	INITIAL WEIGHT (kg)	END WEIGHT (kg)	DIFFERENCE (kg)	DAYS (days)	AVERAGE GAIN (gm/day)
2	10.0	16.8	6.8	28	242.86
3	10.4	27.0	16.6	56	296.43
4	16.0	34.0	18.0	56	321.43
5	12.2	26.1	13.9	56	248.21
6	16.4	38.6	22.2	55	403.64
7	13.2	26.9	13.7	56	244.64
8	16.1	29.1	13.0	56	232.14
9	12.4	29.3	16.9	56	301.79
10	14.0	16.1	2.1	28	75.00
11	16.8	39.5	22.7	63	360.32
13	11.2	16.8	5.6	33	169.70
14	11.7	16.4	4.7	32	146.88
15	11.4	31.8	20.4	63	323.81
TOTALS n=13			176.6	638	3366.84
MEAN					258.99
STANDARD DEVIATION					86.92
STANDARD ERROR OF MEAN					24.11
			CONTROL ANIMALS		
C - 1	29.1	39.5	10.4	26	400.00
C - 2	24.2	37.9	13.7	26	526.92
C - 3	23.2	34.3	11.1	33	336.36
C-4	13.3	27.8	14.5	39	371.80
TOTALS n=4					1635.08
MEAN					408.77
STANDARD DEVIATION					71.85
STANDARD ERROR OF MEAN					35.92

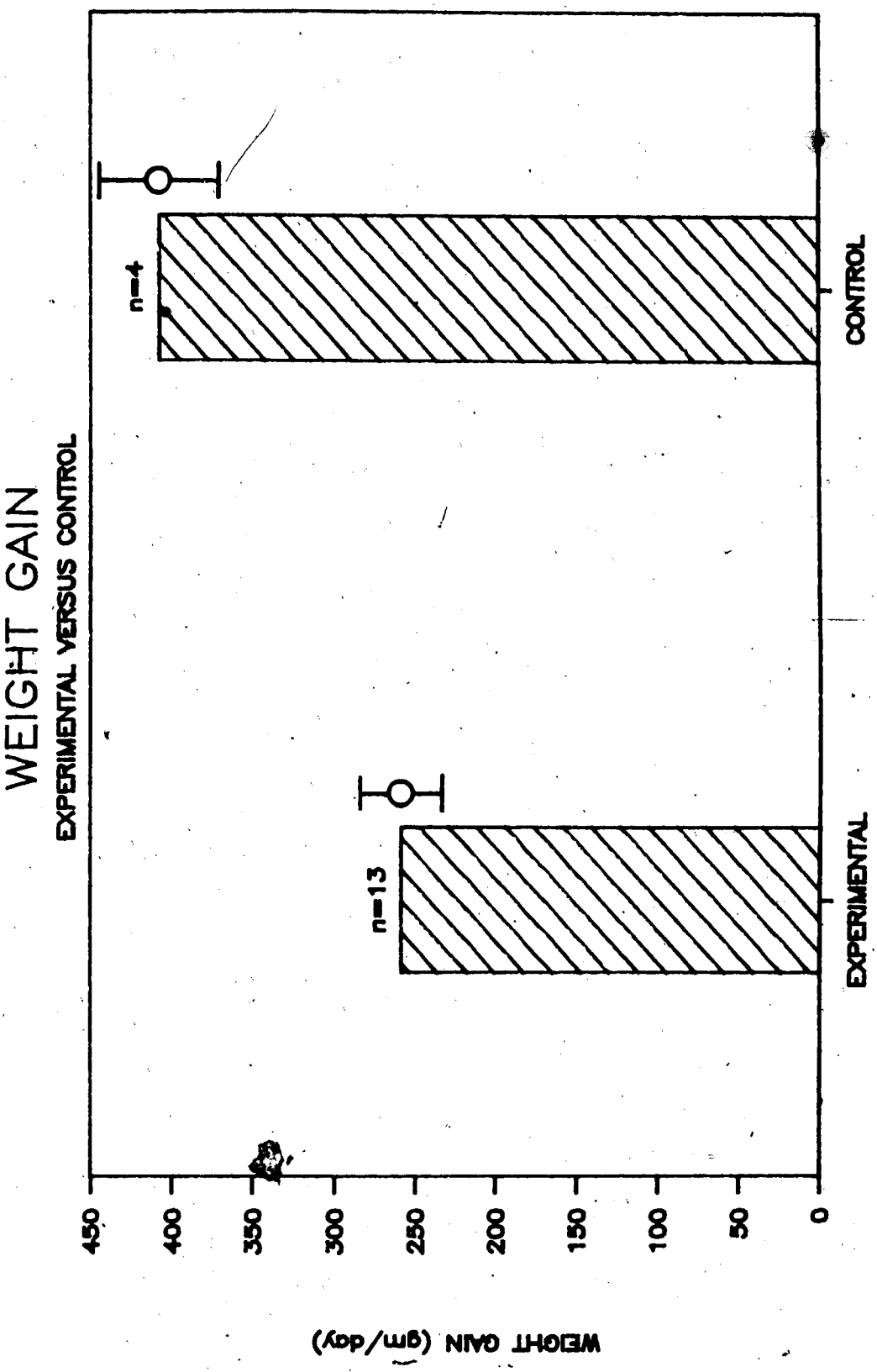

WEIGHT GAIN
EXPERIMENTAL VERSUS CONTROL
WEIGHT GAIN (gm/day)
450
400
350
300
250
200
150
100
50
0
n=13
n=4
EXPERIMENTAL
CONTROL

FIGURE 7.

TABLE III

INTESTINAL TRANSIT TIME (HOURS)

PIG NUMBER	CONTROLS	PRE-OPERATIVE	POST-OPERATIVE
2			96
3			84
4		60	240
6		36	132
7		36	132
8		36	108
9		36	240
10		60	240
11		84	240
13		12	
14		60	
15		84	240
16		84	
CONTROL - 1	108		
CONTROL - 2	84		
CONTROL - 3	84		
CONTROL - 4	60		
TOTAL	336	588	1752
n	4	11	10
MEAN	84	53.46	175.2
STANDARD DEVIATION	16.97	23.09	66.21
STANDARD ERROR OF MEAN	8.49	6.96	20.94

difference is significant ($0.05 > p > 0.02$), suggesting that as the animals grow their intestinal transit time lengthens.

Post-operative transit time was 175.2 ± 20.9 hours. This result is significantly different from the pre-operative ($0.05 > p > 0.02$) and the control animals ($0.05 > p > 0.02$). This result suggests that our operative procedure had the desired effect of significantly prolonging total intestinal transit time (Figure 8.).

iv) LABORATORY ANALYSIS OF BILE SAMPLES

a) Cholesterol

Total biliary cholesterol concentrations are presented in Table IV and Figure 9. The mean values did not differ significantly in comparisons between all groups.

b) Phospholipids

Biliary phospholipid determination results are presented in Table V and Figure 10. The mean values for all groups were compared and they did not differ significantly at the 0.05 level.

c) Total Bile Acids

The total biliary bile acid concentrations are tabulated in Table VI and Figure 11. Comparison between all groups again showed no significant differences.

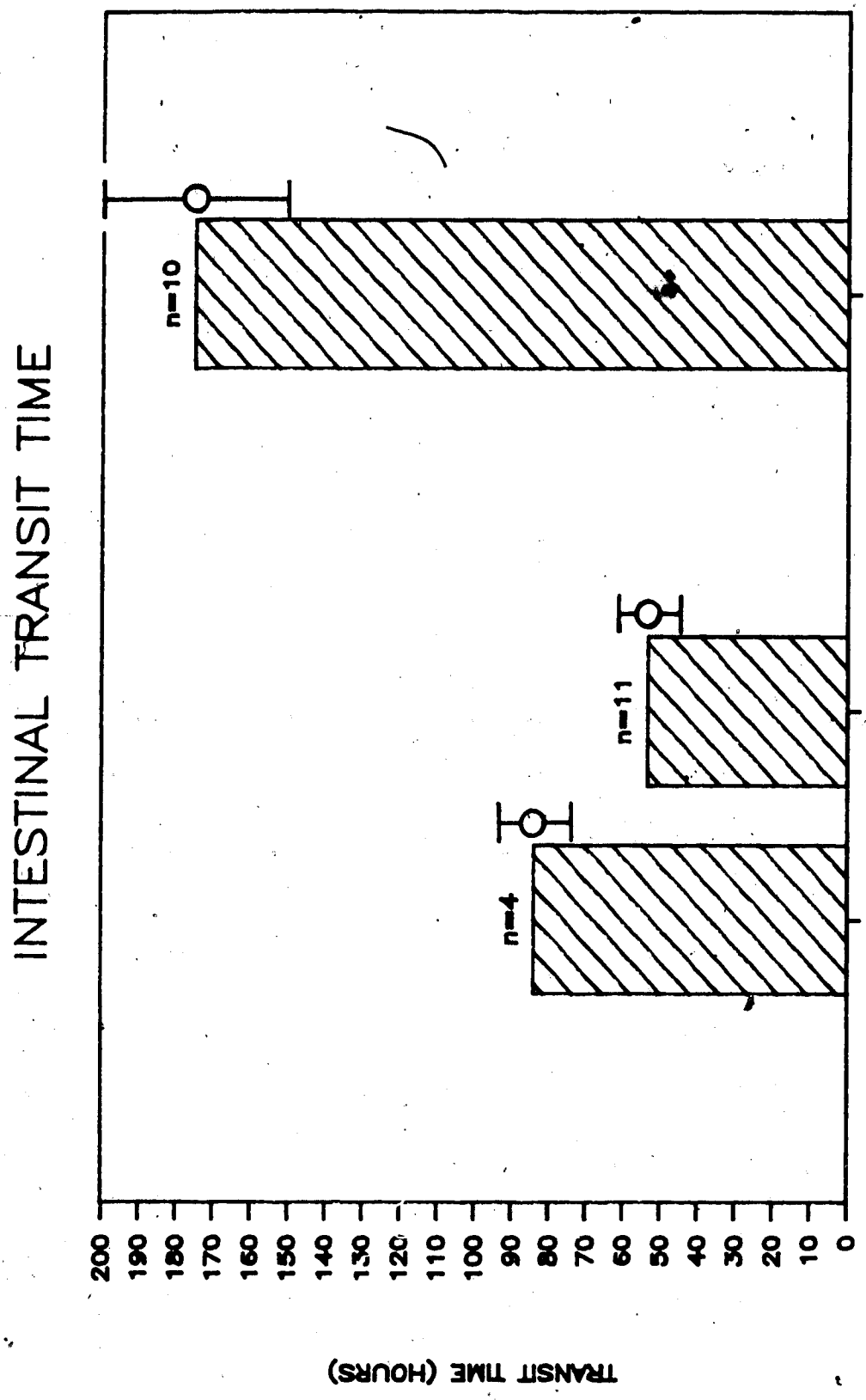

INTESTINAL TRANSIT TIME
TRANSIT TIME (HOURS)
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
n=4
n=11
n=10
CONTROLS
PRE-OP
POST-OP

FIGURE 8.

TABLE IV

BILIARY CHOLESTEROL CONCENTRATION (mmol/l)

PIG NUMBER	0 MONTHS	1 MONTH	1 MONTH (CONTROLS)	2 MONTHS
1	4.90			
2	9.10	2.96		
3	8.40	2.19		10.41
4	9.90	1.00		0.83
5	7.05	8.07		1.40
6	6.47	10.65		3.53
7	5.69	7.88		6.31
8	8.25	5.21		7.32
9	5.73	3.66		2.70
10	9.55	8.20		
11	3.02	1.97		3.04
13	3.96	1.55		
14	1.16	1.63		
15	0.97	1.64		2.03
16	3.21			
CONTROL - 1	2.10		3.31	
CONTROL - 2	1.11		1.77	
CONTROL - 3	1.19		2.69	
CONTROL - 4	2.11		1.80	
TOTALS	93.87	58.93	9.57	37.57
n	19	13	4	9
MEAN	4.941	4.533	2.393	4.174
STANDARD DEVIATION	3.05	3.16	0.65	2.99
STANDARD ERROR OF MEAN	0.70	0.88	0.32	1.00

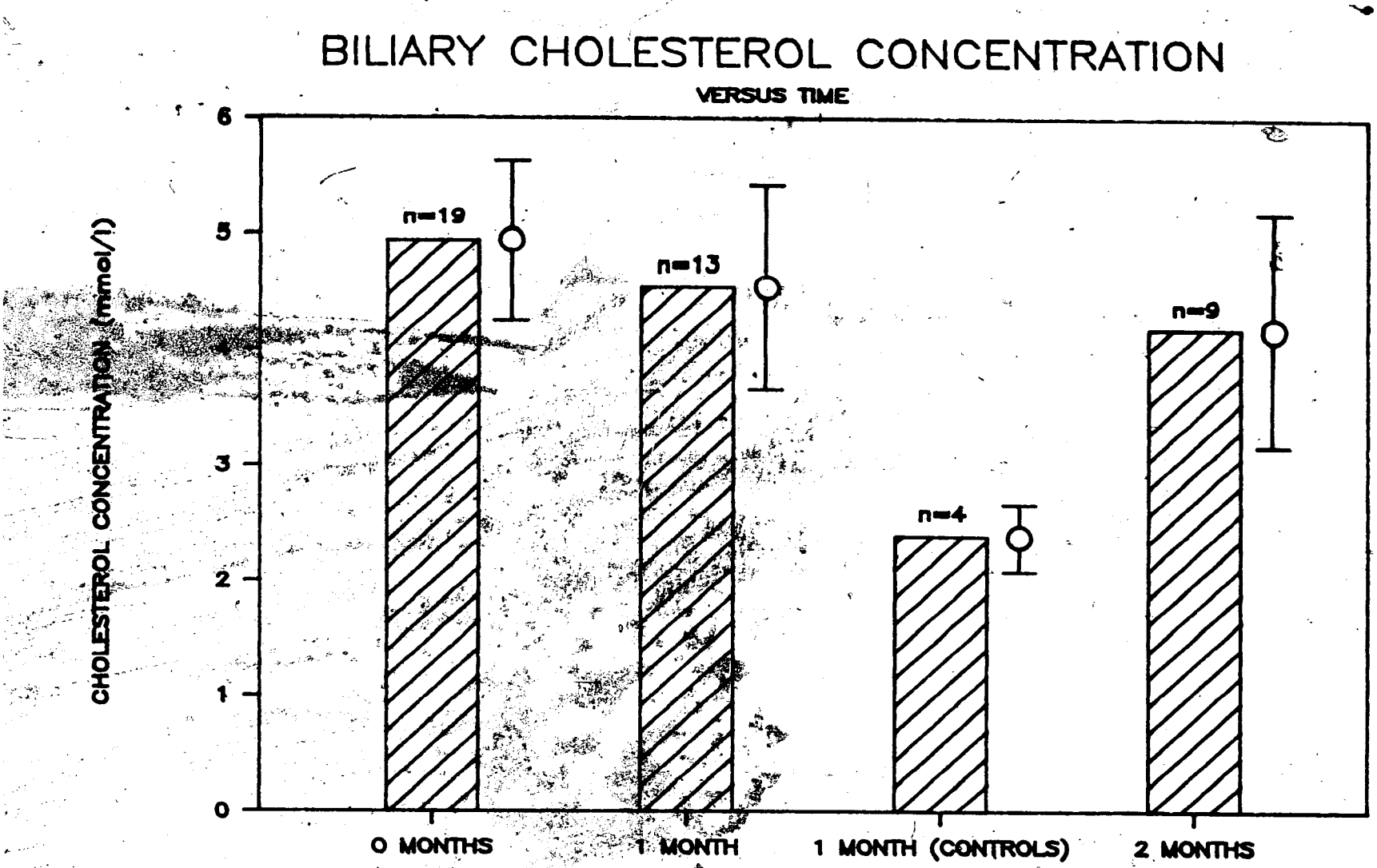

BILIARY CHOLESTEROL CONCENTRATION
VERSUS TIME
CHOLESTEROL CONCENTRATION (mmol/l)
0
1
2
3
4
5
6
n=19
n=13
n=4
n=9
0 MONTHS
1 MONTH
1 MONTH (CONTROLS)
2 MONTHS

FIGURE 9.

TABLE V

BILIARY PHOSPHOLIPID CONCENTRATION (mmol/l)

PIG NUMBER	0 MONTHS	1 MONTH	1 MONTH (CONTROLS)	2 MONTHS
1	44.60			
2	82.60	25.80		
3	49.10	23.00		87.20
4	79.60	12.30		12.50
5	79.20	58.00		10.30
6	41.70	76.60		33.90
7	50.50	49.10		51.80
8	72.20	46.00		53.70
9	59.90	48.10		19.70
10	56.70	42.20		
11	13.90	9.96		8.13
13	14.00	9.29		
14	10.20	6.47		
15	8.40	9.79		12.86
16	17.20			
CONTROL - 1	4.81		14.43	
CONTROL - 2	8.88		5.24	
CONTROL - 3	9.96		10.50	
CONTROL - 4	10.95		12.68	
TOTALS	714.40	416.61	42.85	290.09
n	19	13	4	9
MEAN	37.60	32.05	10.71	32.23
STANDARD DEVIATION	27.58	21.79	3.45	25.50
STANDARD ERROR OF MEAN	6.33	6.04	1.73	8.50

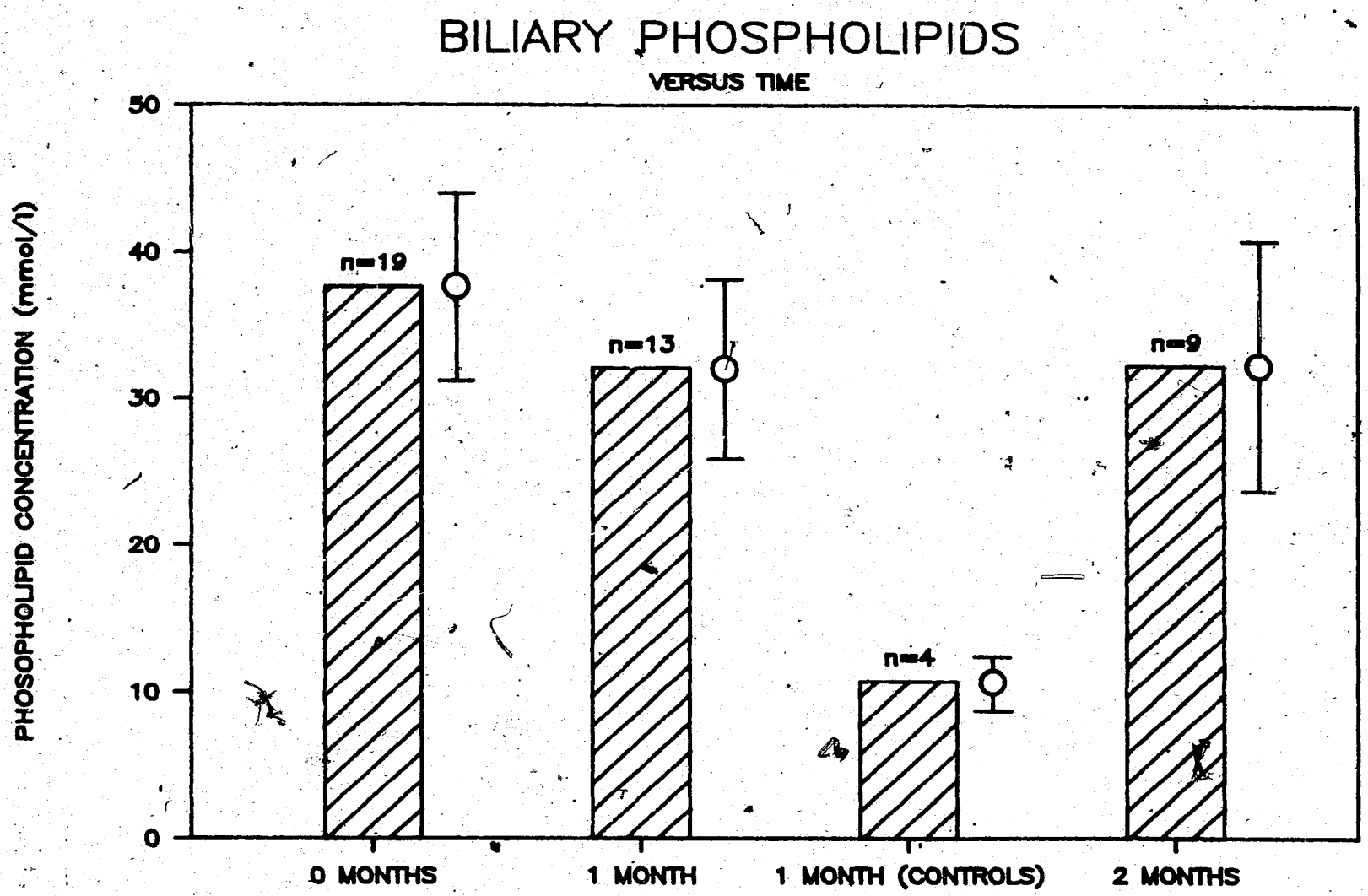

BILIARY PHOSPHOLIPIDS
VERSUS TIME
PHOSOPHOLIPID CONCENTRATION (mmol/l)
50
40
30
20
10
0
n=19
n=13
n=4
n=9
0 MONTHS
1 MONTH
1 MONTH (CONTROLS)
2 MONTHS

FIGURE 10.

TABLE VI

TOTAL BILIARY BILE ACID CONCENTRATION (mmol/l)

PIG NUMBER	0 MONTHS	1 MONTH	1 MONTH (CONTROLS)	2 MONTHS
1	190.0			
2	290.0	137.0		
3	313.0	142.0		332.0
4	282.0	128.0		67.0
5	215.0	304.0		97.0
6	223.0	320.0		190.0
7	275.0	285.0		185.0
8	290.0	196.0		278.7
9	232.0	295.0		158.0
10	320.0	301.0		
11	173.3	108.8		120.0
13	120.0	68.7		
14	78.0	117.0		88.0
15	55.3	73.3		
16	109.3			
CONTROL - 1	129.0		152.0	
CONTROL - 2	72.5		98.0	
CONTROL - 3	91.5		161.0	
CONTROL - 4	86.7		116.0	
TOTALS	3545.60	2475.80	527.0	1515.70
n	19	13	4	9
MEAN	186.61	190.45	131.75	168.41
STANDARD DEVIATION	89.22	92.62	25.75	84.31
STANDARD ERROR OF MEAN	20.47	25.69	12.88	28.10

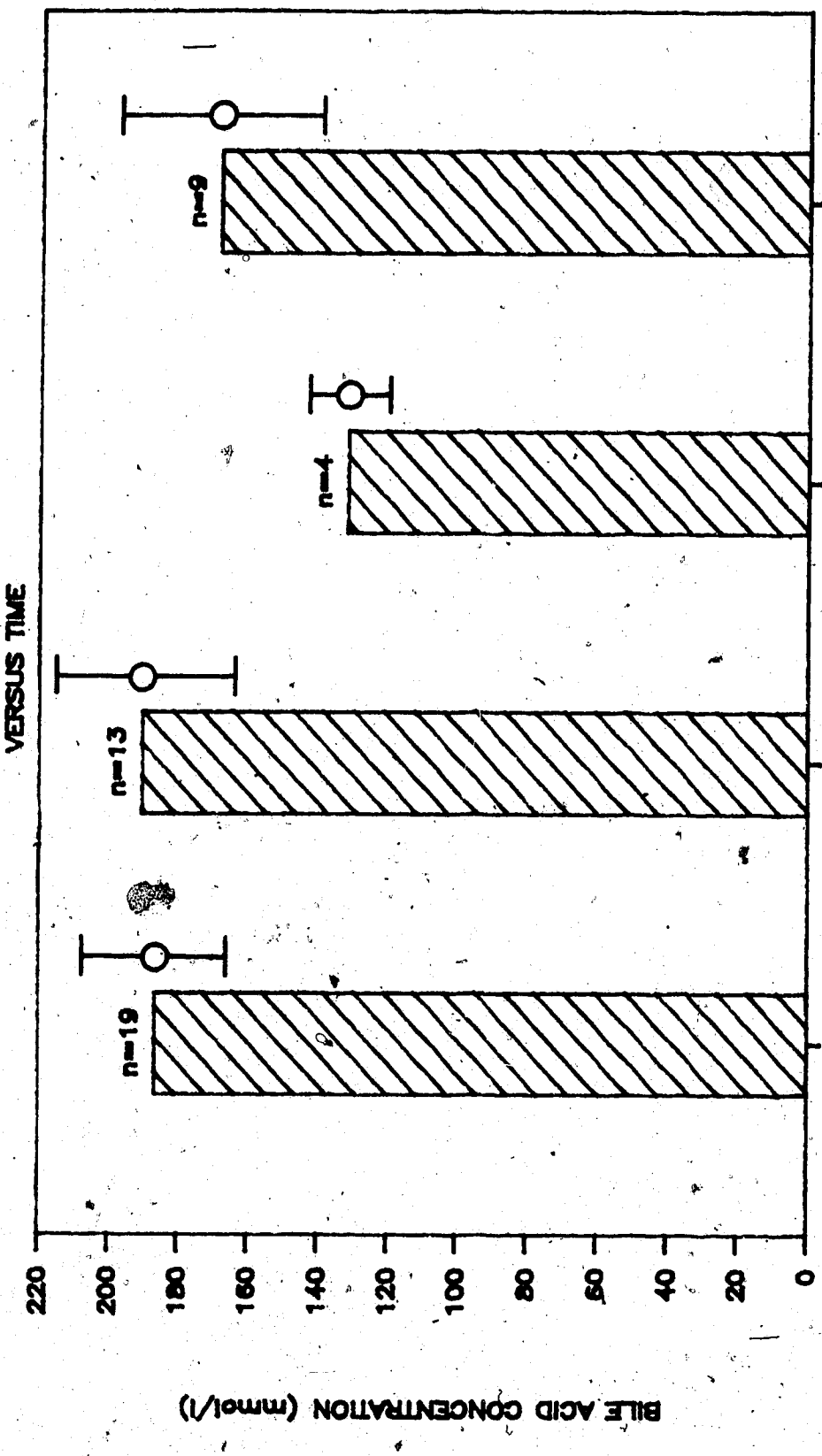

TOTAL BILIARY BILE ACID CONCENTRATIONS
VERSUS TIME
BILE ACID CONCENTRATION (mmol/l)
220
200
180
160
140
120
100
80
60
40
20
0
n=19
n=13
n=4
n=9
0 MONTHS
1 MONTH
1 MONTH (CONTROLS)
2 MONTHS

FIGURE 11.

d) Total Biliary Lipids

The data are presented in Table VII and Figure 12. Comparison between all groups shows no significant differences at the 0.05 level.

v) CHOLESTEROL SATURATION INDEX

The calculated results for the cholesterol saturation index are presented in Table VIII and Figure 13. Mean values at 0, 1, and 2 months were 0.40 ± 0.03, 0.38 ± 0.03, and 0.39 ± 0.05 respectively. The control animals had a value of 0.52 ± 0.07 at 1 month. Comparison of all groups using the student's t-test showed no statistical significance except when comparing the 1 month experimental values with the 1 month control values ($0.05 > p > 0.02$). In light of the non-significance of all other comparisons this is probably an anomaly.

vi) AUTOPSY FINDINGS

At the last laparotomy and after the animal had been sacrificed the colon was removed in total and examined. In all cases the cecum and colon up to the distal anastomosis of the antiperistaltic loop was grossly distended with semi-liquid stool. Just past the distal anastomosis of the reversed segment the diameter of the colon abruptly decreased and the stool became solid.

In all animals where a segment more than 10 cm. was reversed, the segment lengthened. Presumably this is due to

TABLE VII

TOTAL BILIARY LIPIDS (g/dl)

PIG NUMBER	0 MONTHS	1 MONTH	1 MONTH (CONTROLS)	2 MONTHS
1	12.975			
2	20.993	8.841		
3	19.499	8.840		23.462
4	20.398	7.277		4.291
5	16.967	19.734		5.615
6	14.431	22.061		12.093
7	17.637	18.104		13.342
8	20.154	13.395		18.129
9	16.255	18.439		9.389
10	20.476	18.367		
11	9.703	6.190		6.640
13	7.130	4.153		
14	4.665	6.309		
15	3.404	4.421		5.396
16	6.824			
CONTROL - 1	6.788		8.710	
CONTROL - 2	4.291		5.2860	
CONTROL - 3	5.311		8.8230	
CONTROL - 4	5.187		6.7480	
TOTAL	233.088	156.131	29.567	98.357
n	19	13	4	9
MEAN	12.268	12.010	7.392	10.929
STANDARD DEVIATION	6.44	6.26	1.47	6.14
STANDARD ERROR OF MEAN	1.48	1.74	0.74	2.05

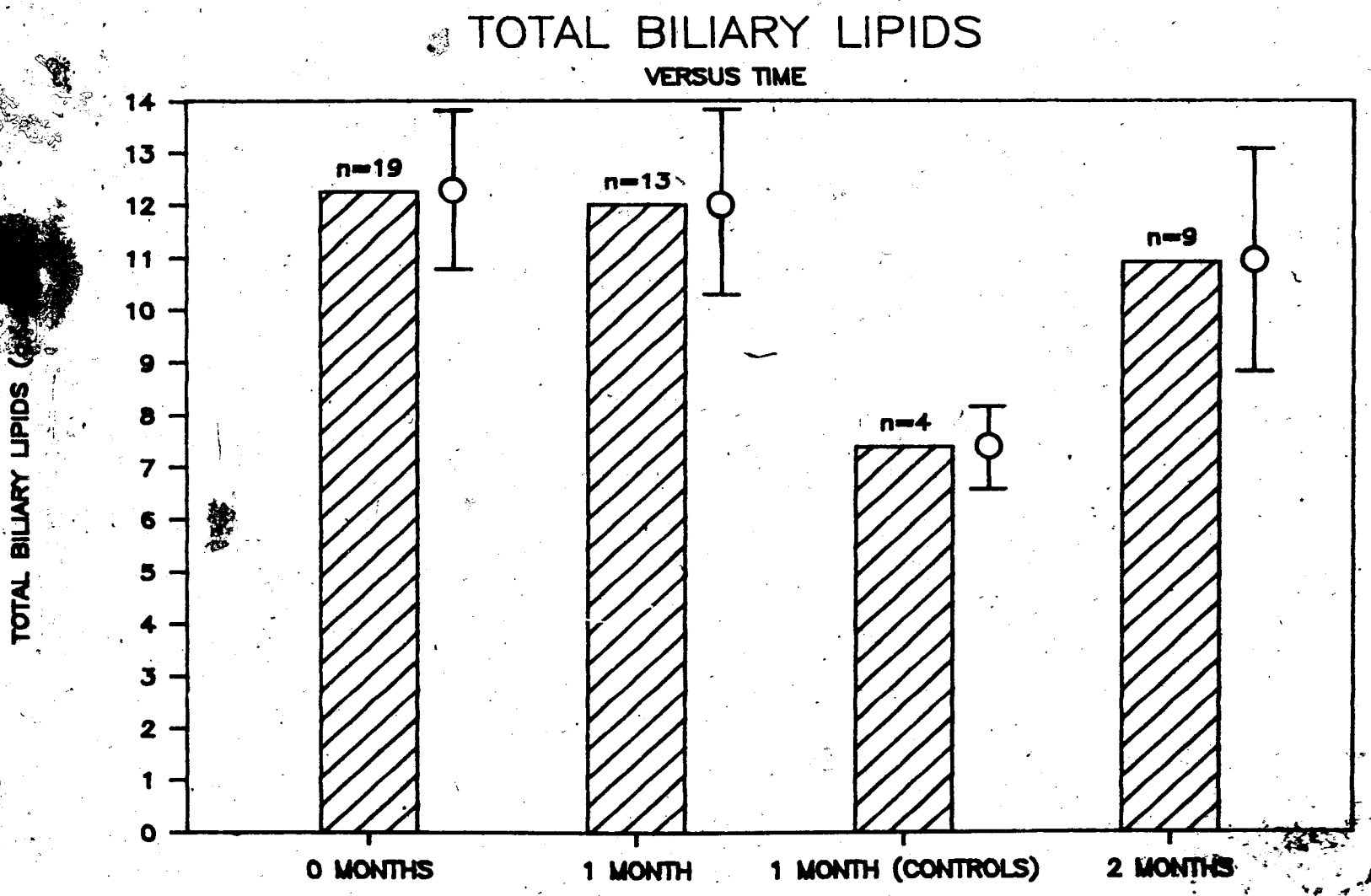

TOTAL BILIARY LIPIDS
VERSUS TIME
TOTAL BILIARY LIPIDS
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
n=19
n=13
n=4
n=9
0 MONTHS
1 MONTH
1 MONTH (CONTROLS)
2 MONTHS

FIGURE 12.

TABLE VIII

CHOLESTEROL SATURATION INDEX

PIG NUMBER	0 MONTHS	1 MONTH	1 MONTH (CONTROLS)	2 MONTHS
1	0.321			
2	0.309	0.334		
3	0.411	0.264		0.313
4	0.349	0.192		0.216
5	0.273	0.351		0.362
6	0.406	0.358		0.272
7	0.284	0.399		0.361
8	0.303	0.335		0.358
9	0.267	0.301		0.351
10	0.414	0.437		
11	0.463	0.466		0.769
13	0.733	0.488		
14	0.335	0.481		
15	0.384	0.482		0.465
16	0.545			
CONTROL - 1	0.664		0.548	
CONTROL - 2	0.349		0.629	
CONTROL - 3	0.328		0.528	
CONTROL - 4	0.545		0.368	
TOTAL	7.683	4.888	2.073	3.467
n	19	13	4	9
MEAN	0.404	0.376	0.518	0.385
STANDARD DEVIATION	0.128	0.[illegible]	0.149	0.150
STANDARD ERROR OF MEAN	0.029	0.[illegible]	0.074	0.050

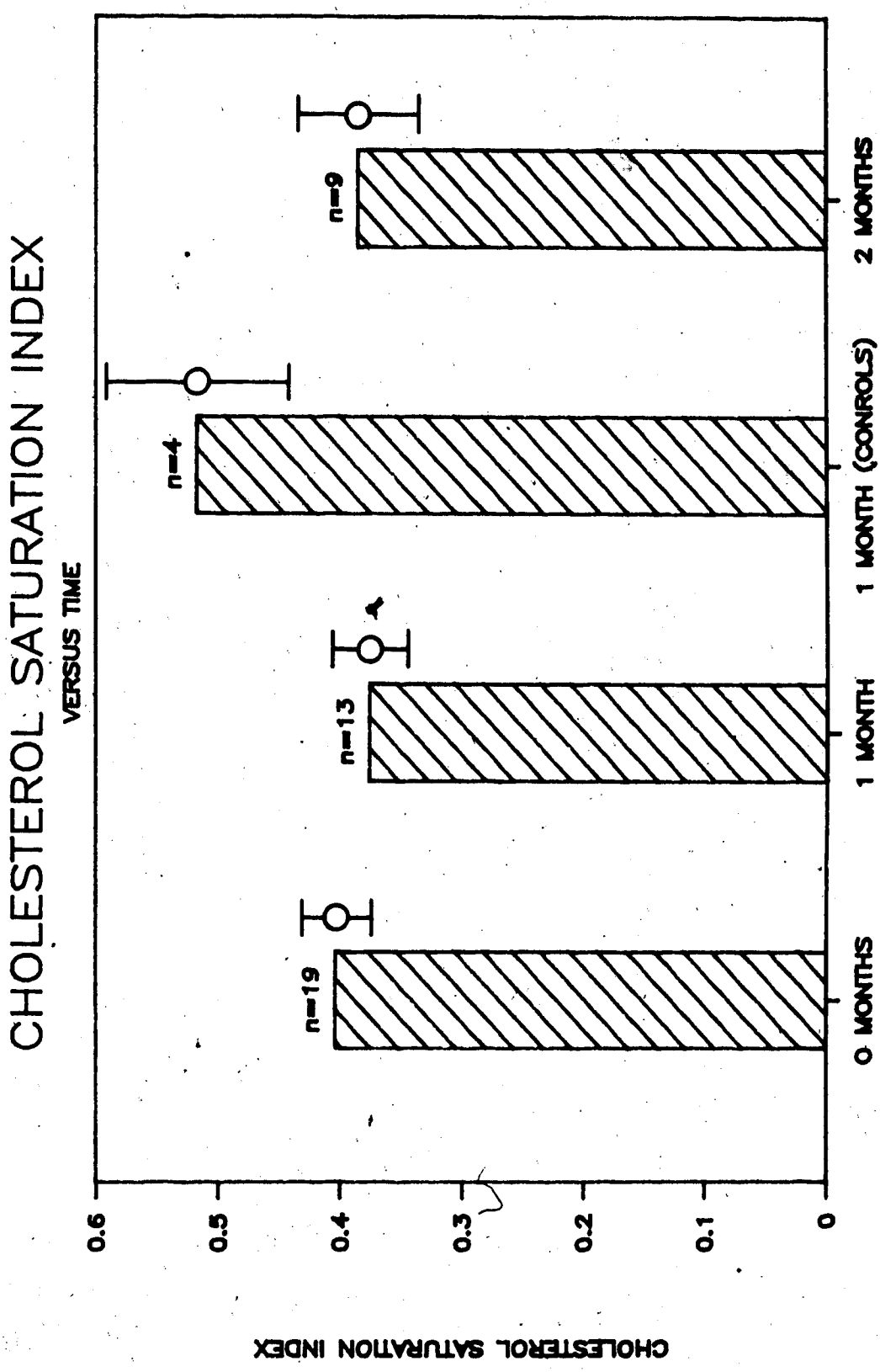

CHOLESTEROL SATURATION INDEX
VERSUS TIME
CHOLESTEROL SATURATION INDEX
0.6
0.5
0.4
0.3
0.2
0.1
0
n=19
n=13
n=4
n=9
0 MONTHS
1 MONTH
1 MONTH (CONTROLS)
2 MONTHS

FIGURE 13.

TABLE IX

AUTOPSY COLONIC MEASUREMENTS (cm.)

PIG NUMBER	LENGTH OF SEGMENT ORIGINALLY REVERSED	AUTOPSY MEASUREMENTS		
		ILEO-CECAL VALVE TO FIRST ANASTOMOSIS	LENGTH OF REVERSED SEGMENT	SECOND ANASTOMOSIS TO RECTUM
2	10	130	10	90
3	15	80	20	120
4	20	125	34	102
5	25	104	63	132
6	20	120	32	150
7	30	110	60	132
8	25	136	44	107
9	30	84	73	141
10	35	99	69	117
11	35	70	71	159
13	35	45	65	97
14	35	112	38	98
15	35	100	87	95

normal growth in the segment. In some cases the segment was more than twice its original length (Table IX).

It was also apparent that the distended segments of colon were grossly different from the normal undistended colon. The distended portion had very thin, almost translucent walls that were easily perforated. As well the mucosa was grossly thinner. We did not study any changes at the microscopic level.

DISCUSSION

i) ANIMALS

In general the animals fared well after the operative procedure. However, some of the deaths might have been due to secondary effects. For instance, pig 10 died shortly after a laparotomy where difficulty was encountered attempting to replace the distended colon into the abdomen. It is conceivable that the blood supply to the bowel may have been impaired by pressure effects. Pig 14 soiled its cage during transport back to its holding pen, and its wound was grossly contaminated with feces. This resulted in an extensive necrotizing infection. Pig 16 died of pneumonia, and at autopsy the massively dilated colon appeared to have displaced the diaphragms upwards thus producing a degree of respiratory impairment.

Judging from the small number of complications, it would appear that the pig can tolerate the presence of an antiperistaltic loop of colon up to 30 - 35 cm. in length with little ill effect.

ii) GROWTH

Young pigs after weaning should double their weight in 8 weeks time[164]. All of our pigs fulfilled this expectation. Our control pigs grew at a rate significantly greater than the experimental animals. The growth rate in our experimental animals was approximately 20 gm/day below the lowest value of 250 gm/day[138] found in the literature, and far below the rate of 590 gm/day[106] expected of swine in

in optimum growth conditions. It should be noted that our control animals did not attain this higher value either. However, since the control and experimental groups differed significantly we must conclude that growth was adversely affected. It is hard to determine why this was so. All animals in both groups ate all food offered to them, so a reduction in appetite does not appear to be a factor. All animals were given the same ration of food.

The large intestine in the pig functions in water and mineral absorption, and the digestion of plant fiber. The pig can satisfy up to one-third of its energy requirements by hind-gut fermentation of fiber[133]. Since our operation slowed intestinal transit time one would expect this energy extraction to be maximal in the experimental pigs. This energy should translate into increased growth. Coupled with the retention of intestinal contents I would have expected the experimental animals to be as large if not larger than the control animals. Since we did not study changes in the colonic environment, we do not know if colonic fermentation and energy extraction from fiber was increased or decreased. We thus do not know what factors were responsible for the lower rate of growth in the experimental animals.

iii) INTESTINAL TRANSIT TIME

The literature gives values of 27 - 52[133], 30[155], and 38 - 45[149] hours for total intestinal transit time in pigs. Our pre-operative value of 53.5 hours is just slightly above the upper range of these values, and our control pigs were

well above this range. Although size might be considered to be a determinant, it is apparently not a significant factor since large pigs (55 kg.) had average transit times of 30 hours[155].

All authors agree that the intestinal contents spend the majority of their time in the large intestine[149]. This fact coupled with the effect of our antiperistaltic loop explains the markedly prolonged transit times obtained in the animals post-operatively.

When comparing the post-operative transit times (Table III) with the length of colon reversed (Table IX) it is apparent that there is not a strong correlation between the two parameters until the maximum tolerable 30 - 35 cm. length is reached. Below this length the effect of a given length of reversed colon is not entirely predictable, but any length of reversed colon will prolong intestinal transit time to some extent.

iv) LABORATORY ANALYSIS OF BILE

The mean values of biliary cholesterol from our animals fall within the range of 0.86 - 1.94 mg/ml (2.22-5.01 mmol/l) found in the literature[138]. The total biliary bile acid levels of 36.52 - 71.67 mg/ml (74.38-145.97 mmol/l) found in the same article are exceeded by the mean values in 3 of our 4 groups. In fact, the only group which falls within this range is the control group at 1 month. However, since analysis of the means showed no statistically significant difference between the groups, it would appear

that our pigs have a range of total biliary bile acids higher than that in the literature. A conclusion that our operative procedure was the cause for this higher range is not supportable. Although in theory the reabsorption of bile acids should go up, the literature review seems to indicate that the resultant decreased synthesis will more than offset this. As well, the higher values were present in our animals at the start of the protocol before the operative procedure had been performed.

I was unable to locate any literature on the normal range of total biliary lipids. The only data on phospholipids was incomplete, giving the levels as a percentage of total biliary lipids, but not giving any values for total biliary lipids[151]. Since no significant change occurred in the parameters we can logically assume that prolonged intestinal transit time does not have a significant effect on them.

v) CHOLESTEROL SATURATION INDEX

Literature values show that pig bile normally has a low cholesterol saturation index. This has been stated to be due to the high fiber content of the pig's diet[152]. In this paper the range of values in young pigs (fasted for 24 hours prior to bile sampling) was 0.36 - 0.48 before addition of more fiber to the diet, and 0.33 - 0.51 after. Our mean values for the cholesterol saturation index agree with these values.

The question of why the cholesterol saturation index did not change remains unanswered. There are, however, several possibilities. We certainly do not yet know about all the metabolic checks and balances that dictate cholesterol and bile salt metabolism. Furthermore, it can be strongly argued that we do not understand fully those factors that we have already identified. This comes about because our knowledge is derived from a mixture of in vitro and in vivo experiments. It has not yet been shown that the in vitro work can be fully applied to the intact animal. Therefore, it is very possible that our experiment was effected by as yet undiscovered overriding control mechanisms.

We clearly showed that intestinal transit time was significantly prolonged. There is good reason to believe that this should have resulted in a decreased loss of bile acids and an increased return of bile acids to the liver. This should have decreased the hepatic bile acid synthesis, and all but closed this outlet for cholesterol excretion. Excess cholesterol would have to be excreted in order to maintain cholesterol homeostasis. Either this excretion did not occur, or it occurred in some other form, as none of our biliary lipid parameters changed significantly. Since we did not study fecal bile acid excretion or bile acid turnover we cannot be absolutely certain that we reduced the losses of bile acids and increased their return to the liver.

Another problem is the pig's bile acid spectrum. Much of the work in bile acids has been done in animals that like humans have a significant amount of cholic and deoxycholic acid present in their bile. Some of the most prominent theories on gallstone formation depend on an increased level of deoxycholic acid effecting hepatic chenodeoxycholic acid synthesis. Since there is no literature studying the effects of hyocholic and hyodeoxycholic acids, we do not know whether they act in the same way as cholic and deoxycholic acids.

Our pigs grew rather rapidly and soon became too large to handle. Thus our period of observation was limited and short. We therefore do not know if the long term effect of our operation might be different from the short term effects.

Another possibility is that the prolonged colonic transit time may have worked against us. Since fermentation was probably increased this may have reduced the pH to levels that inhibited bacterial deconjugation and dehydroxylation. This would have all but stopped the production of secondary bile acids. As well, the large bacterial load from the fermentation may have bound all the bile salts and carried them away rather than allowing their absorption. The distended colon was noted to have thin, rather atrophic appearing walls. It is quite possible that one of the effects of this distension was a loss of the normal functions of the mucosa including reabsorption of bile salts.

What is needed to clarify some of these questions are further studies. To begin with, studying colonic reabsorption in the distended colon and fecal excretion of bile acids would tell us if the distended colon is capable of reabsorbing bile salts, and if changes in the colonic environment are allowing for increased reabsorption of bile acids. Gas chromatographic analysis of the bile samples to identify and quantitate the bile acid spectrum before and after the operation would tell us if colonic metabolism and reabsorption of bile acids was effected. Studies of the effects of hyocholic and hyodeoxycholic acid on hepatic bile salt and cholesterol metabolism would be very important in determining whether we should expect to see any changes in cholesterol and bile salt excretion after the operation. Lastly, the use of mini- or micro-pigs would allow us to study the long-term effects of our operative procedure.

CONCLUSIONS

1. In general our operative procedure was well tolerated.

2. The maximum tolerable length of antiperistaltic colon is 30 - 35 cm.

3. The growth of animals having undergone the procedure is adversely effected, the cause is unknown.

4. The operative procedure was successful in significantly prolonging the intestinal transit time.

5. The length of reversed segment did not correlate well with the degree of prolongation of intestinal transit time.

6. There was no statistically significant change in total biliary cholesterol, phospholipids, or bile acids or in the cholesterol saturation index.

7. Transit time does not appear to be a significant determinant of biliary cholesterol saturation in young pigs.

8. These results do not support the commonly held belief that gallstone disease is linked to prolonged intestinal transit time.

REFERENCES

1. Weisberg HF. Pathogenesis of gallstones. Ann Clin Lab Sci 1984; 14:243-251.

2. Smith BF, Lamont JT. The pathogenesis of gallstones. Hosp Pract 1984; 19:93-99.

3. Heaton KW. The epidemiology of gallstones and suggested etiology. Clin Gastroenterology 1973; 2:67-83.

4. Shaffer EA. Gallstones: Current concepts of pathogenesis and medical dissolution. Can J Surg 1980; 23:517-532, 557.

5. Heaton KW. BILE SALTS IN HEALTH AND DISEASE. Churchill-Livingstone, Edinburgh and London, 1972. Pages 184-195.

6. Admirand WH, Small DM. The physiochemical basis of cholesterol gallstone formation in man. J Clin Invest 1968; 47:1043-1052.

7. Carey MC, Small DM. The physical chemistry of cholesterol solubility in bile. J Clin Invest 1978; 61:998-1026.

8. Holzbach RT, Marsh M, Olszewski M, Holan K. Cholesterol solubility in bile. Evidence that supersaturated bile is frequent in healthy man. J Clin Invest 1973; 52:1467-1479.

9. Burnstein MJ, Ilson RG, Petrunka CN, Taylor RD, Strasburg SM. Evidence for a potent nucleating factor in the gallbladder bile of patients with cholesterol gallstones. Gastroenterology 1983; 85:801-807.

10. Holan KR, Holzbach RT, Hermann RE, Cooperman AM, Claffey NJ. Nucleation time: Key factor in the pathogenesis of cholesterol gallstone disease. Gastroenterology 1979; 77:611-617.

11. Whiting MJ, Watts J. Supersaturated bile from obese patients without gallstones supports cholesterol growth but not nucleation. Gastroenterology 1984; 86:243-248.

12. Gollish SH, Burnstein MJ, Ilson RG, Petrunka CN, Strasberg SM. Nucleation of cholesterol monohydrate crystals from hepatic and gallbladder bile of patients with cholesterol gallstones. Gut 1983; 24:836-844.

13. Sedaghat A, Grundy SM. Cholesterol crystals and the formation of cholesterol gallstones. N Eng J Med 1980; 302:1274-1277.

14. Holzbach RT, Kibe A, Thiel E, Howell JH, Marsh M, Hermann RE. Biliary proteins: Unique inhibitors of cholesterol crystal nucleation in human gallbladder bile. J Clin Invest 1984; 73:35-45.

15. Holzbach RT, Howell JH, Freedman MR, Marsh ME, Thiel ES, Kibe A. Nucleation inhibitors: presence in native human bile and possible role in the pathogenesis of cholesterol gallstone disease. In: BILE ACIDS AND CHOLESTEROL IN HEALTH AND DISEASE, Paumgartner G, Stiehl A, [illegible] eds. MTP Press Limited, Boston, 1983. Page[illegible]

[illegible]be A, Holzbach RT, LaRusso NF, et al. Inhibition of cholesterol crystal formation by apolipoprotiens in supersaturated model bile. Science 1984; 225:514-516.

[illegible]olzbach RT. Recent progress in understanding cholesterol crystal nucleation as a precursor to human gallstone formation. Hepatology 1986; 6:1403-1406.

18. LaMont JT, Smith BR, Moore RL. Role of gallbladder mucin in the pathophysiology of gallstones. Hepatology 1984; 4:51S-56S.

19. Levy [illegible], Smith BF, LaMont JT. Human gallbladder mucin accelerates nucleation of cholesterol in artificial bile. Gastroenterology 1984; 87:270-275.

20. Smith BF, LaMont JT. Identification of gallbladder mucin bilirubin complex in human cholesterol gallstone matrix. J Clin Invest 1985; 76:439-445.

21. Wormack NA. The development of gallstones. Surg Gynecol Obstet 1971; 133:1-9.

22. Wormack NA, Zeppa R, Irvin GL. The anatomy of gallstones. Ann Surg 1963; 157:670-686.

23. Freston JW, Bouchier IA, Newman J. Biliary mucous substances in dihydrocholesterol-induced cholelithiasis. Gastroenterology 1969; 57:670-678.

24. Lee SP, LaMont JT, Carey MC. Role of gallbladder mucus hypersecretion in the evolution of cholesterol gallstones. J Clin Invest 1981; 67:1712-1723.

25. Smith BF, LaMont JT. The central issue of cholesterol gallstones. Hepatology 1986; 6:529-531.

26. Malet PF, Williamson CE, Trotman BW, Soloway RD. Compostion of pigmented centers of cholesterol gallstones. Hepatology 1986; 6:477-481.

27. Holzbach RT, Kibe A. Pathogenesis of gallstones. In: GALLSTONES: CONTEMPORARY ISSUES IN GASTROENTEROLOGY, Volume 4, Cohen S, Soloway RD, eds. New York: Churchill-Livingstone Inc., 1985:73-100.

28. Sodhi HS. Cholesterol metabolism in man. In: HANDBOOK OF EXPERIMENTAL PHARMACOLOGY, Volume 41. Kritchevsky D, ed. Springer-Verlag, Berlin, 1975. Pages 29-107.

29. Borgstrom B. Quantification of cholesterol absorption in man by fecal analysis after feeding of a single isotope-labeled meal. J Lipid Res 1969; 10:331-337.

30. DuPont J. Cholesterol Systems in Insects and Animals. CRC Press Inc., Boca Raton, 1982.

31. Haselwood GAD. The biological importance of bile salts. In: FRONTIERS OF BIOLOGY, Neuberger A, Tatum E, eds. North Holland Publishing Company, Amsterdam, 1978. Volume 47.

32. Kaneko JJ, Ed. Clinical Biochemistry of Domestic Animals, 3rd. Edition. Academic Press, New York, 1980.

33. Wilson JD, Lindsey CA. Studies on the influence of dietary cholesterol on cholesterol metabolism in the isotopic steady state in man. J Clin Invest 1965; 44:1805-1814.

34. Myant NB, Mitropoulos KA. Cholesterol 7-α hydroxylase. J Lipid Res 1977; 18:135-153.

35. Bloch K, Berg BN, Rittenburg D. The biological conversion of cholesterol to cholic acid. J Biol Chem 1943; 149:511-517.

36. Siperstein MD, Jayko ME, Chaikoff JL, Dauben WG. Nature of the metabolic products of C^{14}-cholesterol excreted in bile and feces. Proc Soc Exp Biol Med 1952; 81:720-724.

37. Maton PN, Reuben A, Dowling RH. Relationship between hepatic cholesterol synthesis and biliary cholesterol secretion in man: Hepatic cholesterol synthesis is not a major regulator of biliary lipid secretion. Clin Sci 1982; 62:515-519.

38. Quintao E, Grundy SM, Ahrens EH. Effects of dietary cholesterol on the regulation of total body cholesterol in man. J Lipid Res 1971; 12:233-247.

39. Balasubramanian S, Mitropoulos KA, Myant NB. Evidence for the compartmentation of cholesterol in rat-liver microsomes. Eur J Biochem 1973; 34:77-83.

40. Mitropoulos KA, Myant NB, Gibbons GF, Balasubramaniam S, Reeves EA. Cholesterol precursor pools for the synthesis of cholic and chenodeoxycholic acids in rats. J Biol Chem 1974; 249:6052-6056.

41. Schwartz CC, Vlahcevic ZR, Halloran LG, Gregory DH, Meek JB, Swell L. Evidence for the existence of definitive hepatic cholesterol precursor compartments for bile acids and biliary cholesterol in man. Gastroenterology 1975; 69:1379-1385.

42. Bjorkhem I, Lewenhaupt A. Preferential utilization of newly synthesized cholesterol as substrate for bile acid biosynthesis. J Biol Chem 1979; 254:5252-5256.

43. Bjorkhem I, Danielsson H. 7α-hydroxylation of exogenous and endogenous cholesterol in rat-liver microsomes. Eur J Biochem 1975; 53:63-70.

44. Schwartz CC, Berman M, Vlahcevic ZR, Halloran L, Gregory D, Swell L. Multicompartment analysis of cholesterol metabolism in man. I Characterization of the hepatic bile acid and biliary cholesterol precursor sites. J Clin Invest 1978; 61:408-423.

45. Schwartz CC, Halloran LG, Vlahcevic ZR, Gregory DH, Swell L. High and low density lipoprotein metabolism in man: Preferential utilization of free cholesterol from high density lipoproteins for biliary cholesterol secretion in man. Science 1978; 200:62-64.

46. Halloran LG, Schwartz CC, Vlahcevic ZR, Nisman RM, Swell L. Evidence for high density lipoprotein free cholesterol as the primary precursor for bile acid synthesis in man. Surgery 1978; 84:1-7.

47. Miller LK, Tiell ML, Paul I, Spaet TH, Rosenfeld RS. Side chain oxidation of lipoprotein-bound [25,25-^{3}H]-cholesterol in the rat: Comparison of HDL and LDL and implications for bile acid synthesis. J Lipid Res 1982; 23:335-344.

48. Thornton JR, Heaton KW, MacFarlane DG. A relation between high density lipoprotein cholesterol and bile cholesterol saturation. Br Med J 1981; 283:1353-1355.

49. Turley SD, Spady DK, Dietschy JM. Alteration of the degree of biliary cholesterol saturation in the hamster and rat by manipulation of the pools of preformed and newly synthesized cholesterol. Gastroenterology 1983; 84:253-264.

50. Borgstrom B. Bile salts - their physiological functions in the gastrointestinal tract. Acta Med Scand 1974; 196:1-10.

51. Miettinen TA. Bile acid metabolism. In: HANDBOOK OF EXPERIMENTAL PHARMACOLOGY, Volume 41. Kritchevsky D, ed., Springer-Verlag, Berlin, 1975. Pages 109-150.

52. Long TT, Jakoi L, Stevens R, Quarfordt S. The sources of rat biliary cholesterol and bile acid. J Lipid Res 1978; 19:872-878.

53. Kim DN, Lee KT, Reiner JM, Thomas WA. An evaluation of some of the potential immediate sources of cholesterol for bile acid synthesis in swine. Exp Mol Path 1975; 22:284-293.

54. Hofmann AF. Chemistry and enterohepatic circulation of bile acids. Hepatology 1984; 4:4S-14S.

55. Salen G, Batten AK, Tint GS, Dayal B, Shefer S. The transformation of 3α,7α,12α-trihydroxycoprostanoic acid to cholic acid in humans. In: BILE ACIDS AND CHOLESTEROL IN HEALTH AND DISEASE, Paumgartner G, Stiehl A, Gerik W, eds. MTP Press Limited, Boston, 1983. Pages 91-98.

56. Shefer S, Hauser S, Bekersky I, Mosbach EH. Feedback regulation of bile acid biosynthesis in the rat. J Lipid Res 1969; 10:646-655.

57. Mosbach EH, Salen G. Bile acid biosynthesis, pathways, and regulation. Dig Dis 1974; 19:920.

58. Saghvi A, Grassi E, Warty V, Diven W, Wight C, Lester R. Reversible activation-inactivation of cholesterol 7-α hydroxylase possibly due to phosphorylation-dephosphorylation. Biochem Biophys Res Commun 1981; 103:886-892.

59. Kwok CT, Burnett W, Hardie IR. Regulation of rat liver microsomal cholesterol 7-α hydroxylase: reversible inactivation by ATP + Mg^{2+} and a cytosolic activator. J Lipid Res 1981; 22:580-589.

60. Packard CJ, Shepard J. The hepatobiliary axis and lipoprotein metabolism: Effects of bile acid seqestrants and ileal bypass surgery. J Lipid Res 1982; 23:1081-1098.

61. Scallen TJ, Sanghvi A. Regulation of three key enzymes in cholesterol metabolism by phosphorylation/dephosphorylation. Proc Natl Acad Sci USA 1983; 80:2477-2480.

62. Whiting MJ. Bile Acids. Adv Clin Chem 1986; 25:169-232.

63. Blitzer BL, Boyer JL. Cellular mechanisms of bile formation. Gastroenterology 1982; 82:346-357.

64. Thureborn E. Human hepatic bile. Composition changes due to altered enterohepatic circulation. Acta Chir Scand 1962; 303(Suppl).

65. Duane WC, Pries JM. Regulation of bile salt synthesis. In: BILE ACIDS AND CHOLESTEROL IN HEALTH AND DISEASE, Paumgartner G, Stiehl A, Gerik W, eds. MTP Press, Boston 1983. Pages 73-75.

66. Davis RA, Hyde PM, Kuan JW, McNeal M, Schexnayder JA. Bile acid secretion by cultured rat hepatocytes. Regulation by cholesterol availability. J Biol Chem 1983; 258:3661-3667.

67. Fisher MM, Kakis G, Yousef IM. Bile acid pool in Wistar rats. Lipids 1976; 11:93-96.

68. Lack L, Weiner IM. Intestinal bile salt transport: Structure-activity relationships and other properties. Am J Physiol 1966; 210:1142-1152.

69. Playoust MR, Isselbacher KJ. Studies on the transport and metabolism of conjugated bile salts by intestinal mucosa. J Clin Invest 1964; 43:467-476.

70. Wilson FA. Intestinal transport of bile acids. Am J Physiol 1981; 241:G83-G92.

71. Weiner IM, Lack L. Bile salt absorption, enterohepatic circulation. In: HANDBOOK OF PHYSIOLOGY, Section 6, Volume III. American Physiological Society, Washington D.C., 1968. Page 1439.

72. MacDonald IA, Bokkenheuser VD, Winter J, McLernon AM, Mosbach EH. Degradation of steroids in the human gut. J Lipid Res 1983; 24:675-700.

73. Eastwood MA. Vegetable fiber: its physical properties. Proc of the Nutr Soc 1973; 32:137-145.

74. Norman A, Sjovall J. On the transformation and enterohepatic circulation of cholic acid in the rat. Bile acids and steroids 68. J Biol Chem 1958; 233:872-875.

75. Portman OW. Further studies of the intestinal degradation products of cholic acid-24-C^{14} in rats: Formation of deoxycholic acid. Arch Biochem Biophys 1958; 78:125-137.

76. Beher WT. Bile Acids. Chemistry and physiology of bile acids and their influence of atherosclerosis. In: MONOGRAPHS IN ATHEROSCLEROSIS, Volume 6. Kritchevsky D, Pollak OJ, Simms HS, eds. S. Karger, Basel, 1976. Page 34.

77. Hepner GW, Hofmann AF, Thomas PJ. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. I Cholyglycine. J Clin Invest 1972; 51:1889-1897.

78. Cowen AE, Korman MG, Hofmann AF, Cass OW, Coffin SB. Metabolism of lithocholate in healthy man. II- Enterohepatic circulation. Gastroenterology 1975; 69:67-76.

79. Low-Beer TS. Diet and bile acid metabolism. Clin Gastroenterology 1977; 6:165-178.

80. Hofmann AF, Lachin JM. Biliary bile acid composition and cholesterol saturation. Gastroenterology 1983; 84:1075-1077.

81. Pomare EW, Low-Beer T. The selective inhibition of chenodeoxycholate synthesis by cholate metabolites in man. Clin Sci Mol Med 1975; 48:315-321.

82. Low-Beer TS, Pomare EW. Can colonic bacterial metabolites predispose to cholesterol gallstones? Br Med J 1975; i:438-40.

83. Fromm H. Intestinal bacterial conversion of chenodeoxycholic to ursodeoxycholic acid in man. In: BILE ACIDS AND CHOLESTEROL IN HEALTH AND DISEASE, Paumgartner G, Stiehl A, Gerik W, eds. MTP Press Limited, Boston, 1983. Page 339.

84. Samuel P, Saypol GM, Meilman E, Mosbach EH, Chafizadeh M. Absorption of bile acids from the large bowel in man. J Clin Invest 1968; 47:2070-2078.

85. Eriksson S. Biliary excretion of bile acids and cholesterol in bile fistula rats. Bile acids and steroids. Proc Soc Exp Biol Med 1957; 94:578-582.

86. Shefer S, Hauser S, Lapar V, Mosbach E. Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl-CoA-reductase and cholesterol 7-α hydroxylase in the rat. J Lipid Res 1973; 14:573-580.

87. LaRusso NF, Szczepanik PA, Hofmann AF. Effect of deoxycholic acid ingestion on bile acid metabolism and biliary lipid secretion in normal subjects. Gastroenterology 1977; 72:132-140.

88. Ahlberg J, Angelin B, Einarsson K, Hellstrom K, Leijd B. Influence of deoxycholic acid on biliary lipids in man. Clin Sci Mol Med 1977; 53:249-56.

89. Carulli N, Ponz de Leon M, Zironi F, Iori R, Loria P. Bile acid feeding and hepatic sterol metabolism: Effect of deoxycholic acid. Gastroenterology 1980; 79:637-41.

90. Adler R, Bennion L, Duane W, Grundy S. Effects of low dose chenodeoxycholic acid feeding on biliary lipid metabolism. Gastroenterology 1975; 68:326-334.

91. LaRusso N, Hoffman NE, Hofmann AF, Northfield TC, Thistle JL. Effect of primary bile acid ingestion on bile acid metabolism and biliary lipid secretion in gallstone patients. Gastroenterology 1975; 69:1301-1314.

92. Northfield TC, LaRusso NF, Hofmann AF, et al. Biliary lipid output during three meals and an overnight fast. II: Effect of chenodeoxycholic acid treatment in gallstone subjects. Gut 1975; 16:12-17.

93. Einarrson K, Grundy S. Effects of feeding cholic acid and chenodeoxycholic acid on cholesterol absorption and hepatic secretion of biliary lipids in man. J Lipid Res 1980; 21:23-34.

94. Schoenfield LJ, Bonorris GG, Ganz P. Induced alterations in the rate-limiting enzymes of cholesterol and bile acid synthesis in the hamster. J Lab Clin Med 1973; 82:858-868.

95. Anderson JM. Chenodeoxycholic acid desaturates bile - but how? Gastroenterology 1979; 77:1146-1151.

96. LaRusso N, Hoffman N, Hofmann A, Northfield T, Thistle J. Differing aspects of primary bile acid ingestion on biliary lipid secretion in gallstone patients: Why chenodeoxycholic acid but not cholic acid dissolves gallstones. Gastroenterology 1974; 66:729.

97. Northfield T, LaRusso N, Thistle [illegible] Hofmann A. Effect of chenodeoxycholic acid therapy on biliary lipid secretion in gallstone patients. Gastroenterology 1973; 64:780.

98. Maton PN, Ellis HJ, Higgins MJ, Dowling RH. Hepatic HMG CoA reductase in human cholelithiasis: effects of chenodeoxycholic and ursodeoxycholic acids. Eur J Clin Invest 1980; 10:325-332.

99. Hofmann AF. Medical treat[illegible] of cholesterol gallstones by bile desaturatin[illegible]nts. Hepatology 1984; 4:199S-208S.

100. Dowling RH. Cholelithiasis: m[illegible]l treatment. Clin Gastroenterology 1983; 12:125-1[illegible]

101. Thistle JL, Hofmann AF. Effic[illegible]y and specificity of chenodeoxycholic acid th[illegible]py for dissolving gallstones. N Eng J Med 1973; 289:655-659.

102. Iser JH, Sali A. Chenodeoxycholic acid: A review of its pharmacological properties and therapeutic use. Drugs 1981; 21:90-119.

103. Dowling RH. Chenodeoxycholic acid therapy of gallstones. Clin Gastroenterology 1977; 6:141-163.

104. Vlahcevic ZR, Bell CC, Buhac I, Farrarr JT, Swell L. Diminished bile acid pool size in patients with gallstones. Gastroenterology 1970; 59:165-173.

105. Nicolau G, Shefer S, Salen G, Mosbach EH. Determination of hepatic cholesterol 7-α hydroxylase activity in man. J Lipid Res 1974; 15:146-151.

106. Tracy JD, Allhands RV, Savage DC, Jensen AH. The effects of a dietary antimicrobial on the biological half-life of chenodeoxycholic acid and plasma bile acid concentration patterns in the young pig. J Anim Sci 1986; 62:997-1004.

107. Low-Beer TS, Nutter S. Colonic bacterial activity, biliary cholesterol saturation, and pathogenesis of gallstones. Lancet 1978; ii:1063-65.

108. Thornton JR, Heaton KW. Do colonic bacteria contribute to cholesterol gallstone formation? Effects of lactulose on bile. Br Med J 1981; 282:1018-1020.

109. MacDonald IA, Singh G, Mahony DE, Meier CE. Effect of pH on bile salt degradation by mixed fecal cultures. Steroids 1978; 32:245-56.

110. Pomare EW, Heaton KW. Bile salt metabolism in patients with gallstones in functioning gallbladders. Gut 1973; 14:885-890.

111. Low-Beer TS, Pomare EW, Morris JS. Control of bile synthesis. Nature New Biol 1972; 238:215.

112. Van der Werf SDJ, Van Berge Henegouwen GP, Van Tongeren JHM. Colonic absorption of endogenous deoxychólate and biliary lipid composition in man. In: BILE ACIDS AND CHOLESTEROL IN HEALTH AND DISEASE, Paumgartner G, Stiehl A, Gerik W, eds. MTP Press, Boston, 1983. Pages 275-277.

113. Stanley MM, Paul D, Gacke D, Murphy J. Effects of cholestyramine, metamucil, and cellulose on fecal bile salt excretion in man. Gastroenterology 1973; 65:889-894.

114. Leveille GA, Sauberlich HE. Mechanism of the cholesterol-depressing effect of pectin in the cholesterol-fed rat. J Nutr 1966; 88:209-214.

115. Eastwood MA, Hamilton D. Studies on the absorption of bile salts to nonabsorbed components of the diet. Biochem Biophys Acta 1968; 152:164.

116. Serafin JA, Nesheim MC. Influence of dietary heat-labile factors in soybean meal upon bile acid pools and turnover in the chick. J Nutr 1970; 100:786-796.

117. Miettinen TA. Clinical implications of bile acid metabolism in man. In: THE BILE ACIDS, CHEMISTRY, PHYSIOLOGY, AND METABOLISM, Volume 2. Nain PP, Kritchevsky D, eds., Plenum Press, New York, 1973. Page 191-247.

118. Nestel PJ, Havenstein N, Whyte HM, Scott TJ, Cook LJ. Lowering of plasma cholesterol and enhanced sterol excretion with the consumption of polyunsaturated ruminant fats. N Eng J Med 1973; 288:379-382.

119. Heaton KW. Gallstones and cholecystitis. In: REFINED CARBOHYDRATE FOODS AND DISEASE. SOME IMPLICATIONS OF DIETARY FIBER. Burkitt DP, Trowell HC, eds: London, Academic Press, 1975; pages 173-194.

120. Heaton KW. Diet and gallstones. In: GALLSTONES. Fisher MM, Goresky CA, Shaffer EA, Strasberg SM, eds. Plenum Press, New York, 1979. Pages 371-389.

121. Lafont D, Lairon D, Vigne JL, et al. Effect of wheat bran, pectin, and cellulose on the secretion of bile lipids in rats. J Nutr 1985; 115:849-855.

122. Schaefer O. When the Eskimo comes to town. Nutr Today 1971; 6:8-16.

123. Heaton KW. Food fiber as an obstacle to energy intake. Lancet 1973; 2:1418-1421.

124. Watts JM, Jablonski P, Toouli J. The effect of added bran to the diet on the saturation of bile in people without gallstones. Am J Surg 1978; 135:321-24.

125. Kirwan WO, Smith AN, McConnell AA, Mitchell WD, Eastwood MA. Action of different bran preparations on colonic function. Br Med J 1974; 4:187-189.

126. McDougall RM, Walker K, Thurston OG. Effect of increased dietary fiber on biliary cholesterol in patients with gallstones. Surgical Forum 1977; 28:416-418.

127. McDougall RM, Yakymyshyn L, Walker K, Thurston OG. Effect of wheat bran on serum lipoproteins and biliary lipids. Can J Surg 1978; 5:433-435.

128. Thornton JR, Emmett PM, Heaton KW. Diet and gallstones: Effects of refined and refined carbohydrate diets on bile cholesterol saturation and bile acid metabolism. Gut 1983; 24:2-6.

129. Hillman L, Peters S, Fisher C, Pomare E. Effects of the fiber components pectin, cellulose, and lignin on bile salt metabolism and biliary lipid composition in man. Gut 1986; 27:29-36.

130. Anderson JW, Chen LWY. Plant fiber and carbohydrate and lipid metabolism. Am J Clin Nutr 1979; 32:346-363.

131. Kim DN, Rogers DH, Li JR, Lee KT, Reiner JM, Thomas WA. Some effects of a grain-based mash diet on cholesterol metabolism in swine. Exp Mol Path 1980; 32:143-153.

132. Pomare EW, Heaton KW, Low-Beer T, Espiner H. The effect of wheat bran upon bile salt metabolism and upon the lipid composition of bile in gallstone patients. Am J Dig Dis 1976; 21:521-526.

133. Low AG, Partridge IG, Eds. Current Concepts of Digestion and Absorption in Pigs. National Institute for Research in Dairying, Reading, England. 1980.

134. Harvey RF, Pomare EW, Heaton KW. Effects of increased dietary fiber on intestinal transit. Lancet 1973; 1:1278-1280.

135. Burkitt DP, Walker ARP, Painter NS. Effect of dietary fiber on stools and transit times and its role in the causation of disease. Lancet 1972; 2:1408-1411.

136. Pomare EW, Heaton KW. Alteration of bile salt metabolism by dietary fiber (bran). Br Med J 1973; 4:262-264.

137. Holzbach RT. Animal models of cholesterol gallstone disease. Hepatology 1984; 4:191S-198S.

138. Marsh A, Kim DN, Lee KT, Reiner JM, Thomas WA. Cholesterol turnover, synthesis, and retention in hypercholesterolemic growing swine. J Lipid Res 1972; 13:600-615.

139. Dunne HW, Leman AD, Eds. Diseases of Swine. Iowa State University Press, Ames Iowa. 1975.

140. Cevallos WH, Holmes WL, Myers RN, Smink RD. Swine in atherosclerosis research. Atherosclerosis 1979; 34:303-317.

141. DuPont J, Oh SY, O'Deen L, McClellan MA, Lumb WV, Butterfield AB, Clow DJ. Cholesterol and bile acid turnover in miniature swine. Lipids 1974; 9:717-721.

142. Haselwood GAD. BILE SALTS. Methuen and Company, London 1967.

143. Jones TC, Hunt RD. Veterinary Pathology. Lea & Febiger, Philadelphia, 1983.

144. Splitter GA. Cholelithiasis in a pig. Vet Rec 1972; 90:73.

145. Haselwood GAD. Bile salts of germ free domestic fowl and pigs. Biochem J 1971; 123:15-18.

146. Kidder DE, Manners MJ. Digestion in the Pig. Scientechnica, Bristol, 1978.

147. Tracy JD, Jensen AH, Allhands RV. Bile acid metabolism in the pig. Cornell Vet 1986; 76:128-138.

148. Reitman JS, Mahley RW, Fry DL. Yucatan miniature swine as a model for diet-induced atherosclerosis. Atherosclerosis 1982; 43:119-132.

149. Keys JE, DeBarthe JV. Site and extent of carbohydrate, dry matter, energy and protein digestion and the rate of passage of grain diets in swine. J Anim Sci 1974; 39:57-62.

150. Reele SB, Ruwart MJ, Noah ML. Gastrointestinal propulsion measurement with radiopaque capsules during 16,16-dimethyl PGE$_2$ infusion. Prostaglandins Leukotrienes Med 1982; 9:629-640.

151. Klapdor R, Hein C. Addition of fiber (wheat bran, bassorin) to the standard food does not influence biliary lipid composition in piglets. Res Exp Med (Berlin) 1982; 180:21-24.

152. Klapdor R. The ultrasonically guided puncture of the gallbladder in animals. Endoscopy 1971; 9:166-169.

153. Swindle MM. Basic Surgical Excercises Using Swine. Praeger Publishers, New York, 1983. Page 6.

154. Getty R. Sisson & Grossman's "The Anatomy of the Domestic Animals", Volume 2 - Porcine. W.B. Saunders, Philadelphia, 1975.

155. Hecher JF, Grovum WL. Rates of passage of digesta and water absorption along the large intestine of sheep, cows, and pigs. Aust J Biol Sci 1975; 28:161-167.

156. Rygiak AN, Nasarov LV. Antiperistaltic displacement of an ileal loop without twisting its mesentery. Dis Col & Rect 1969; 12:409-411.

157. Siedel J, Schlumberger H, Klose S, et al. Improved reagent for the enzymatic determination of serum cholesterol. J Clin Chem Clin Biochem 1981; 19:838-839.

158. Sunderman FW, Sunderman FW Jr., eds. Lipids and the Steroid Hormones in Clinical Medicine. Lippincott, Philadelphia, 1960. Pages 23-31.

159. Engert R, Turner MD. Problems in the measurement of bile acids with 3-hydroxysteroid dehydrogenase. Anal Biochem 1973; 51:399-407.

160. Al-Rabeeah A, Thurston OG, Walker K. Effect of total parenteral nutrition on biliary lipids in neonates. Can J Surg 1986; 29:289-291.

161. Carey MC. Critical tables for calculating the cholesterol saturation of native bile. J Lipid Res 1978; 19:945-955.

162. Metzger AL, Heymsfield S, Grundy SM. [redacted] lithogenic index - a numerical expression for the relative lithogenicity of bile. Gastroenterology 1972; 62:499-501.

163. Thomas PJ, Hofmann AF. A simple calculation of the lithogenic index of bile: expressing biliary lipid composition on rectangular coordinates. Gastroenterology 1973; 65:698-700.

164. Tavemor WD, Ed. Nutrition and Disease in Experimental Animals. Bailliere, Tindall, and Cassell. London, 1970.

VITA

Name: Norman D. Causton

Place of Birth: Vancouver, British Columbia, Canada

Year of Birth: 1956.

Post Secondary Education: 1974-1978. University of British Columbia, Faculty of Science. Earned B.Sc. degree in Cell Biology with first class standing, June 1978.

1978-1982. University of British Columbia, Faculty of Medicine. Earned M.D. degree with second class standing, June 1982.

1982-1983. Rotating internship, The General Hospital, St. John's, Newfoundland.

1983-1987. Resident in General Surgery, University of Alberta and University of Alberta Hospitals, Edmonton, Alberta.

Publications: Janusz MT, Jamieson WRE, Causton N, et al. Coronary artery bypass in patients over 65 years of age. Can J Surg 1983; 26:186-188.