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Effect of Dietary Cholesterol and Indomethacin on Cholelithiasis and Gallbladder Motility in Guinea Pig

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This study examines the effects of dietary cholesterol and subcutaneous indomethacin on gallstone formation, gallbladder motility, and bile composition in guinea pigs. Guinea pigs on cholesterol diets developed gallstones which were not primarily composed of cholesterol and were not prevented by indomethacin. Animals receiving cholesterol diets showed significant gallbladder enlargement which was inhibited by indomethacin. Cholesterol did not alter gallbladder pressure–volume relationships or the response to CCK, while indomethacin diminished gallbladder tone. Although cholesterol feeding did not appear to alter smooth muscle contractility in the guinea pig gallbladder, it caused significant gallbladder enlargement by a mechanism which may be dependent on prostaglandins.

Crystal precipitation from saturated bile is a critical step in gallstone formation, but the imperfect correlation between cholesterol saturation in human bile and cholesterol gallstone formation indicates that additional factors are important as well (1). Studies in the cholesterol-fed prairie dog have identified concomitant changes in gallbladder function and motility which may contribute to cholesterol gallstone formation. These include increased mucin secretion by gallbladder epithelium (2), decreased gallbladder emptying (3), and increased cystic duct resistance (4). The contribution of altered gallbladder muscle contractility to stasis has not been clearly defined.

We studied cholesterol-fed guinea pigs to compare gallstone formation in this model with the process described in the prairie dog. It has been reported that gallstones in the cholesterol-fed guinea pig are composed largely of calcium phosphate and are not primarily cholesterol stones (5). We wished to determine whether cholesterol feeding and gallstone formation were accompanied by abnormal gallbladder contractility or other evidence of stasis in this model. Since salicylates appear to prevent gallbladder stasis and gallstone formation in the cholesterol-fed prairie dog (6, 7), we also examined the effect of long-term subcutaneous indomethacin on stone formation and gallbladder motility in control and cholesterol-fed guinea pigs.

We assessed gallbladder motility by measurements of fasting gallbladder volume, pressure–volume relationships, and the pressure response to intravenous CCK in the intact *in vivo* gallbladder. A sensitive hysteresis technique was used to measure gallbladder compliance in the stimulated and unstimulated gallbladder. The effect of dietary cholesterol and indomethacin on gallbladder bile composition and gallbladder histology was also determined.

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MATERIALS AND METHODS

Experimental Design. Fifty-four female Hartley guinea pigs, weighing approximately 300 g, were entered into four experimental groups; groups were then randomly subdivided into animals studied at six weeks, or after 12 weeks of treatment. The first group of control animals received standard Purina guinea pig chow and water *ad libitum;* the second group received a 0.5% cholesterol-supplemented diet (Teklad). The third group of animals received standard guinea pig chow and daily subcutaneous injections of 5 mg/kg indomethacin (Merck, Sharp and Dohme) dissolved 15 mg/ml in 95% ethanol. The effect of cholesterol plus indomethacin was observed in guinea pigs maintained on the 0.5% cholesterol diet plus indomethacin 2.0–5.0 mg/kg/day subcutaneously for a six-week period only.

Experimental Technique. Guinea pigs were studied in acute terminal experiments after either six or 12 weeks of the study. Animals were fasted with water *ad libitum* for 18 hr, weighed, and anesthetized with intraperitoneal urethane, 2 gm/kg. A midline laparotomy was performed, and the contents of the gallbladder were aspirated from the fundus with a needle; the volume was recorded and the bile stored at −20°C for analysis. An intravenous catheter was placed in the inferior vena cava, and normal saline was infused at 30 ml/kg/hr with a Harvard infusion pump. A 1.0-cm anterior duodenotomy was performed and the papilla of Vater was suture ligated to prevent gallbladder emptying without interrupting the nervous and vascular afferents to the gallbladder which accompany the cystic duct. A catheter was then placed in the fundus of the gallbladder and held in position with a pursestring suture; this was connected by a Y-connector to a Harvard infusion–withdrawal pump and to a pressure transducer attached to a Beckman 77540 recorder (Figure 1). The fasting volume of the gallbladder was repetitively infused and withdrawn at a steady rate over 4-min cycles while gallbladder pressure was measured continuously. The pressure curves obtained were highly reproducible and at least three pressure volume cycles were measured for the gallbladder in the unstimulated state for each animal.

CCK Stimulation. CCK-PZ (Karolinska Institute, lot 27822), 0.6 Ivy dog units/kg/min, was given to each animal as a continuous intravenous infusion over 20 min. This dose was chosen since it reliably increased gallbladder pressure in all animals but was a submaximal stimulus as determined by preliminary experiments. Three minutes after beginning the CCK stimulus, the pressure response to infusion of gallbladder volume increased to a new steady state, which persisted as long as CCK was being administered. Three reproducible pressure–volume cycles were recorded during the CCK infusion in all animals, and a pressure–volume cycle was recorded again following a 20-min recovery period to establish return to baseline unstimulated pressure. In all animals examined, the unstimulated tracing following CCK was unchanged from that prior to CCK.

Analysis of Data. Pressure tracings were analyzed using a digitizing program on a Hewlett-Packard 9815A desktop computer equipped with a Hewlett-Packard *x–y* plotter

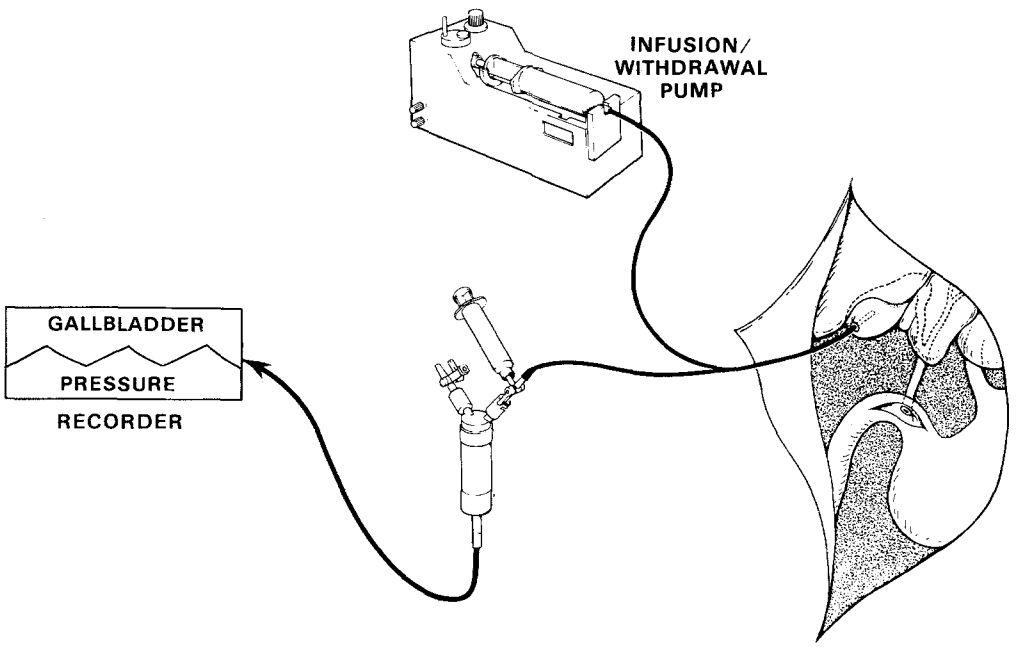

Fig 1. Diagram of guinea pig experimental preparation. The guinea pig is anesthetized with urethane and laparotomy performed. A catheter is sutured into the fundus of the gallbladder after the papilla of Vater is closed through a duodenotomy. The gallbladder catheter is connected by a Y-connector to an infusion–withdrawal pump and a pressure transducer. A separate catheter in the inferior vena cava is used to infuse fluids and drugs.

7225A. Pressures were measured from the infusion and withdrawal curves at increments representing 10% of infused volume. The mean pressure during three infusion–withdrawal cycles was determined at these volume increments for each gallbladder in an unstimulated and stimulated state. The mean and standard error of the pressure measurements in each cohort of animals was then determined.

Pressure–volume curves were developed for individual animals by graphing the mean values for gallbladder pressure at each 10% increment of gallbladder volume during infusion and withdrawal. An example is shown in Figure 2. Pressure–volume curves were generated for cohorts of animals by graphing the mean gallbladder pressure at each 10% volume increment. The area beneath the pressure–volume curves for infusion and withdrawal were calculated using Simpson's equation for integration. The area of the pressure–volume loop was estimated by subtracting the area of the withdrawal curve from that of the infusion curve.

Data are reported as the mean ± SD for each cohort. Statistical significance was determined using analysis of variance with Dunnett's test to compare pressure, compliance, volume, and area of the pressure–volume curve, as well as to compare cholesterol and bile acid concentrations in bile. Where appropriate, the Student's *t* test was used for paired or unpaired comparisons. Difference in stone incidence among the groups of animals was determined using Fisher's exact test.

Gallbladder Histology. At the conclusion of the experiment, the gallbladder was immediately excised and placed in formalin. Specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination.

Analysis of Bile. Uncentrifuged bile specimens from several animals of each group were examined immediately for cholesterol monohydrate crystals under light microscopy. Bile from each animal was separately assayed for total bile salts and cholesterol after storage at −20°C. Total bile salts were measured by the method of Talalay (8) as modified by Admirand and Small (9). For cholesterol assays, the method of Rudel and Morris (10) was employed.

RESULTS

Gallstone Formation and Fasting Gallbladder Volume. No animals in the control group or the group receiving indomethacin developed gallbladder stones, in contrast to a 36% incidence of stones among all of the cholesterol-fed guinea pigs ($P = 0.002$) (Table 1). Four animals of 14 (29%) on high-cholesterol diets developed stones, and among animals receiving indomethacin as well, four of eight (50%) had gallstones. These stones ranged in size between 0.5 and 2 mm, were yellow in color, and soft. Microscopic examination of fragmented stones revealed brown pigmented material. No cholesterol monohydrate crystals were noted in microscopic examination of gallbladder bile in any animal, although bile from cholesterol-fed animals contained small crystals which appeared similar to the calcium bilirubinate crystals photographed by Juniper and Burson (11). X-ray diffraction studies failed to give a diffraction pattern, further indicating the absence of cholesterol monohydrate crystals. Stones were soluble in an ethyl acetate–chloroform mixture or in 0.1 N NaOH but did not dissolve in 0.1 N HCl. These findings are consistent with stones composed largely of bilirubinate.

As shown in Table 2, the fasting gallbladder volume was higher in the cholesterol-fed animals

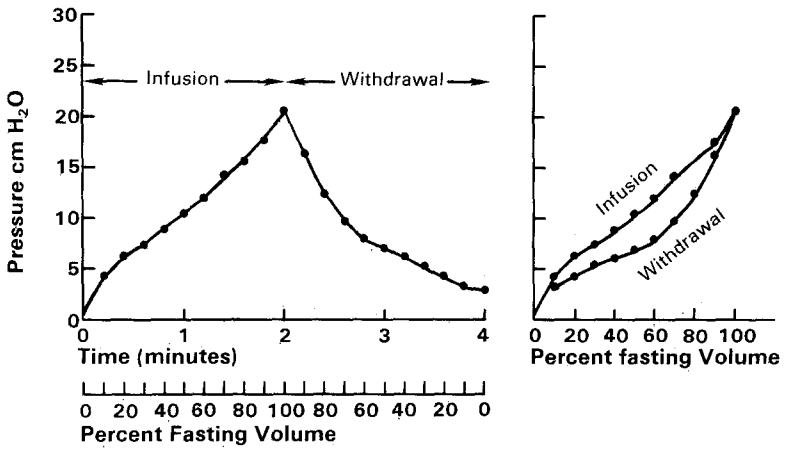

Fig 2. Characteristic pressure tracing from a guinea pig gallbladder during infusion and withdrawal of the gallbladder fasting volume. The infusion and withdrawal pressure curves are superimposed to generate the "pressure–volume loop" which reflects the work done by the gallbladder in accommodating to infused volume.

TABLE 1. ANIMAL WEIGHT, INCIDENCE OF STONES, AND BILE COMPOSITION AFTER SIX TO TWELVE WEEKS IN STUDY

	Controls		*Cholesterol*		*Indomethacin*		*Indo. + chol.*
	*6 wk (7)**	*12 wk (6)*	*6 wk (10)*	*12 wk (7)*	*6 wk (7)*	*12 wk (5)*	*6 wk (8)*
Animal weight (g)	512 ± 18	661 ± 84	499 ± 48	498 ± 51†	483 ± 27	587 ± 100	533 ± 102
Stones	0	0	2	2	0	0	4
Bile cholesterol (mm/100 ml)	1.1 ± 0.7		2.5 ± 1.0†		1.0 ± 0.7		2.4 ± 1.3†
Bile salts (mm/100 ml)	1.51 ± 0.42		1.54 ± 0.53		1.86 ± 0.39		1.76 ± 0.66

*Numbers in parentheses are numbers of animals.
†$P < 0.05$ vs control group.

than in controls ($P = 0.05$), and some animals demonstrated striking gallbladder enlargement. Gallbladder volume in the indomethacin group and cholesterol plus indomethacin group did not differ from controls. Body weights of animals at the time of the experiments are given in Table 1. The increase in gallbladder volume in the cholesterol-fed group could not be attributed to a greater total body weight, since these animals gained weight less rapidly than controls. The cholesterol-fed guinea pigs appeared healthy, with normal coats and activity levels. Laparotomy showed fatty change in their livers, which were grossly enlarged and pale in color, and histologic sections showed multiple vacuoles in hepatocytes.

In contrast, animals receiving both indomethacin and a high-cholesterol diet demonstrated severe toxicity. Twelve 300-g guinea pigs which were placed on a 0.5% cholesterol diet plus indomethacin 5 mg/kg subcutaneously daily developed progressive weight loss with diarrhea, and most died in the third and fifth weeks of the study. A subsequent group of 400-g guinea pigs were successfully maintained on a high-cholesterol diet plus indomethacin 2.0 mg/kg/day subcutaneously for six weeks and then studied.

Gallbladder Pressure–Volume Relationships. From each guinea pig, data obtained in both the unstimulated state and during CCK stimulation included (1) gallbladder pressure at fasting volume, (2) "stiffness" defined as the slope of the pressure–volume curve *(P/V)*, and (3) area between the infusion and withdrawal curves.

Gallbladder pressure at fasting volume for six-week and 12-week animals is shown in Table 3. There were no significant differences seen between pressures in control and cholesterol-fed animals. In contrast, both groups of animals that received indomethacin showed decreased gallbladder pressure compared to controls which was statistically significant. This occurred despite virtually identical fasting gallbladder volumes in the control and indomethacin groups. Stimulation with CCK produced a prompt rise in gallbladder pressure in response to infused volume which was significant in all groups ($P < 0.0005$). Mean gallbladder pressure at fasting volume during CCK infusion is shown in Table 4. Again, no significant difference was noted between control and cholesterol-fed animals. Animals which received indomethacin with or without cholesterol diet showed significantly lower peak pressure with CCK, consistent with the lower pressures in the baseline state. The mean increase in pressure produced by CCK stimulation is given in Table 4 and was not significantly different among the four groups.

Figure 2 shows a characteristic pressure tracing from a control gallbladder, along with resulting

TABLE 2. FASTING GALLBLADDER VOLUME AFTER SIX OR TWELVE WEEKS IN STUDY (NUMBER OF ANIMALS IS IN PARENTHESIS)

Time in study	*Control*	*Cholesterol*	*Indomethacin*	*Cholesterol + indomethacin*
6 wk	1.71 ± 0.59 (7)	2.21 ± 0.94 (7)	1.60 ± 0.51 (7)	1.80 ± 0.70 (7)
12 wk	2.03 ± 0.37 (6)	2.68 ± 0.81 (4)	1.82 ± 1.00 (5)	
All animals	1.86 ± 0.51 (13)	2.39 ± 0.89* (11)	1.69 ± 0.72 (12)	1.80 ± 0.70 (7)

*$P = 0.05$ vs control group.

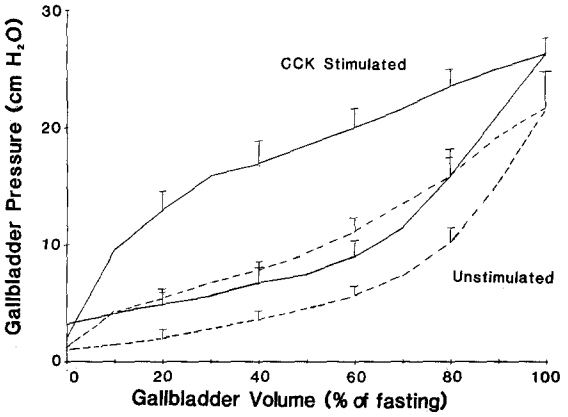

Fig 3. Effect of CCK stimulation (0.6 units/kg/min intravenous infusion) on gallbladder pressure during infusion and withdrawal of the fasting gallbladder volume in control guinea pigs. CCK increased pressure during infusion and increased the size of the pressure–volume loop.

pressure–volume curve. The contour of the pressure volume curves generated by infusing and withdrawing gallbladder volume was similar for all groups of animals. The pressure response to volume infusion in the unstimulated gallbladder was approximately linear, but rose sharply if the fasting volume was exceeded. During CCK stimulation, infusion of fluid into the gallbladder produced an initial rapid rise in pressure, followed by a more gradual linear rise which paralleled the response in the unstimulated state (Figure 3). Pressure–volume curves for cholesterol-fed animals were identical to those in controls, in both the unstimulated and stimulated state. In animals receiving indomethacin, peak pressures were lower but the tracings were otherwise similar (Figure 4).

Chemical Analysis of Bile. Gallbladder bile specimens from each animal were separately analyzed for bile salt and cholesterol as shown in Table 1. Levels of bile salt and cholesterol were markedly lower in guinea pig bile than levels described in primates (9). Thirteen of 38 bile samples (34%) demonstrated cholesterol concentrations at the lower accuracy limit of our assay (< 1 μmol/100 ml). All but two of these low values were obtained in animals which were not receiving cholesterol supplements. Animals receiving high cholesterol diets had significantly increased biliary cholesterol relative to controls. In addition, animals on cholesterol diets which developed stones had higher biliary cholesterol than animals receiving cholesterol diets that did not develop stones (biliary cholesterol 3.21 ± 1.24 vs 2.11 ± 1.01 μmol/dl, $P < 0.05$). Indomethacin did not appear to affect biliary cholesterol concentrations. There was no statistically significant difference in bile salt concentration among the four groups.

Gallbladder Histology. Histologic examination of selected gallbladders demonstrated no differences among the four experimental groups. At the conclusion of the experiment, all the gallbladders exhibited edema of the muscular wall. Mild acute inflammatory changes were present, and the mucosa was intact in all specimens. Although the gallbladders were examined after several hours of manipulation, the cellular architecture was preserved well enough to rule out major differences in mucosal integrity or degree of inflammation.

DISCUSSION

In this study, dietary cholesterol supplementation induced gallstone formation in guinea pigs over a six- to 12-week period. Although cholesterol feeding elevated biliary cholesterol, the bile did not contain cholesterol crystals, and the stones were probably composed largely of bilirubinate. Cholesterol feeding also induced significant gallbladder enlargement without altering gallbladder smooth muscle tone or contractile response to CCK. Daily injections of indomethacin diminished resting tone in the gallbladder and prevented gallbladder enlargement during cholesterol feeding. Indomethacin did not prevent gallstone formation in animals receiving a high-cholesterol diet.

To assess gallbladder motility, we used a sensitive technique adapted from Schoetz et al (12) which describes pressure–volume relationships in the gallbladder with a hysteresis loop. Schoetz

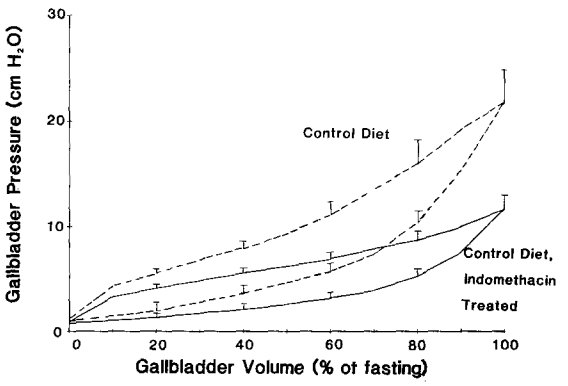

Fig 4. Comparison of gallbladder pressure during infusion and withdrawal of the fasting gallbladder volume in control and indomethacin-treated guinea pig.

TABLE 3. GALLBLADDER PRESSURE AT FASTING VOLUME (UNSTIMULATED) (NUMBER OF ANIMALS IS IN PARENTHESES)

Time in study	*Control*	*Cholesterol*	*Indomethacin*	*Cholesterol + indomethacin*
6 wk	21.51 ± 6.78 (7)	21.79 ± 6.21 (7)	11.71 ± 3.47* (7)	13.67 ± 3.71* (7)
12 wk	14.98 ± 5.28 (4)	21.59 ± 4.99 (4)	13.19 ± 6.51 (5)	
All animals	19.13 ± 6.84	22.07 ± 5.71	12.33 ± 4.76*	13.67 ± 3.72

*$P < 0.05$ vs control

demonstrated the sensitivity of this method to small variations in smooth muscle tone of the primate gallbladder. Davison and al-Hassani (13) and Ryan and Cohen (14) used a similar technique and were also able to distinguish minor changes in gallbladder tone. Using this method, we demonstrated a normal pressure–volume relationship and an apparently normal response to CCK in cholesterol-fed animals, although the use of only one dose of CCK may obscure small variations in response. There was no evidence that alterations in dietary cholesterol altered smooth muscle function in the gallbladder, even in animals which formed stones. This agrees with the findings of Doty et al (4) who observed an apparently normal or increased pressure response to CCK in the gallbladders of cholesterol-fed prairie dogs. In contrast, Fridhandler et al noted a diminished response to CCK-OP *in vitro* in gallbladders from cholesterol-fed ground squirrels (15).

Gallbladder enlargement was noted in guinea pigs receiving dietary cholesterol, even in the absence of stones or cholesterol crystals in the bile. Although the gallbladder enlargement noted in this study was of borderline statistical significance, our subsequent observations have confirmed its reproducibility. This is consistent with some other small animal models for cholelithiasis where gallbladder size has been measured (16, 17). Obstruction is unlikely to cause gallbladder distension in our model since guinea pigs which received indomethacin and cholesterol developed stones but had normal gallbladder volumes. The ability of indomethacin to prevent gallbladder enlargement suggests a prostaglandin-mediated mechanism for cholesterol-induced gallbladder enlargement.

Indomethacin also markedly decreased unstimulated gallbladder tone, but this apparent increase in compliance did not cause gallbladder enlargement. Indomethacin may cause a parallel decrease in tone in the cystic duct or may alter bile secretion or gallbladder absorption (18) which experimentally are influenced by prostaglandins. The gallbladder is known to synthesize prostaglandins E_2, F_{2a}, and I_2, and thromboxane A_2 (19); endogenous prostaglandin production may affect tone in the cystic duct and in the gallbladder itself. We failed to observe any effect of indomethacin on the *in vivo* contractile response to CCK, despite reports that indomethacin decreased smooth muscle contractility *in vitro* (20). The indomethacin dose used for reported *in vitro* studies was several times higher than estimated peak concentration in our model, and tissue levels of indomethacin were probably much lower when our experiments were performed 18 hr after the last dose.

We measured low concentrations of cholesterol and bile salt in guinea pig bile which agree with the previous studies of Schoenfield and Sjovall (21). Other studies in which the lithogenic index was calculated in cholesterol-fed guinea pigs have dem-

TABLE 4. GALLBLADDER PRESSURE AT FASTING VOLUME WITH CCK STIMULATION (cm H_2O) (NUMBER OF ANIMALS IS IN PARENTHESES)

Time in study	*Control*	*Cholesterol*	*Indomethacin*	*Cholesterol + indomethacin*
6 wk	25.84 ± 3.14 (7)	26.62 ± 3.34 (7)	21.67 ± 2.78* (7)	20.93 ± 2.35* (7)
12 wk	23.35 ± 4.08 (4)	29.45 ± 4.82 (4)	20.2 ± 6.66 (5)	
All animals	24.85 ± 3.57	27.76 ± 4.01	21.06 ± 4.38	20.93 ± 2.35
Increase in pressure produced by CCK stimulation (cm H_2O)				
All animals	7.16 ± 5.02	5.79 ± 4.33	8.73 ± 3.28	7.25 ± 3.96

*$P < 0.05$ vs controls.

onstrated that gallbladder bile is far from saturation in this model (22). Dietary cholesterol induces a mild hemolytic anemia in the guinea pig (23), and the resultant pigment load may contribute to the formation of bilirubinate stones.

In conclusion, we found that contractility and response to CCK in the guinea pig gallbladder were not affected by cholesterol feeding. Despite this, significant gallbladder enlargement occurred which was prevented by indomethacin and may reflect stasis due to altered cystic duct motility or alterations in bile flow or gallbladder absorption in cholesterol-fed animals.

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