

— Original Article —

Gallbladder contractility and gallstone formation in the Richardson Ground Squirrel

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Summary: The pathogenesis of cholesterol cholelithiasis in man is probably multifactorial and the mechanism by which gallbladder stasis occurs in gallstone patients has not been studied in detail. In the present study, time-dependent changes in gallbladder motility of the Richardson Ground Squirrels were investigated. 100 of animals were examined for their contractile responses of gallbladders to CCK-OP and Ach in vitro. Also, biochemical changes of serum and bile were investigated. There were no significant differences in motility of gallbladders to CCK-OP or Ach between control and cholesterol-fed animals. The results of this study indicate that gallbladder muscle contractility remains unchanged during the dietary induction of cholesterol gallstones in the Richardson Ground Squirrel. *Gastroenterol Jpn* 1990;25:93-103

Key words: CCK-OP; gallbladder contractility; gallstone formation

Introduction

There is evidence of increased cholesterol cholelithiasis in accordance with western food life in Japan. However, the pathogenesis of cholelithiasis in man is probably multifactorial and the mechanism by which gallbladder stasis occurs in gallstone patients has not been studied in detail. Increased cystic duct resistance^{1,2}, decreased gallbladder muscle contractility³, and gallbladder mucus hypersecretion⁴ have each been suggested as the most probable cause of gallbladder stasis. The contribution of altered gallbladder muscle contractility to stasis has not been clearly defined. The purpose of the present study was to quantify any time-dependent changes occurring in gallbladder motility during the dietary induction of cholesterol gallstone in the Richardson Ground Squirrel.

Materials and Methods

One hundred Richardson Ground Squirrels (300~600 g) of both sexes were captured in the wild near Edmonton, Canada during spring-time and quarantined at the university animal holding facilities for 3 weeks. The animals were caged individually and allowed continuous access to food and water. The study lasted from April until February. Normally, the Richardson Ground Squirrel hibernates during the winter but this was prevented by housing them at a constant temperature of 23°C in rooms that were kept light for 12 hours per day.

After a baseline period (2 weeks) on a standard rat chow diet (Lab Blox Chow, Allied Mills Inc., Chicago, Ill.), the animals were divided into 2 dietary groups.

The cholesterol diet group received a high cholesterol diet that contained 2% cholesterol (w/w) (U.S. Biochemicals Corp.). Half of the an-

imals were maintained on a standard rat chow diet and served as a control group. Animals were maintained on their respective diets for 1 week, 2 weeks, 3 weeks, 10 weeks, and 20 weeks and sacrificed by cervical dislocation after a 17 hour fast. Blood was rapidly aspirated from the heart and serum cholesterol was analysed. The gallbladder was exposed through a midline laparotomy. After aspiration of gallbladder bile, cholesterol, bile acids, and phospholipids were measured and analysed.

Serum and bile cholesterol were determined using the Beckman Spinchem method using Dri-STAT Cholesterol-ES Reagent⁵. Bile acids was determined by the methods of Engert and Turner⁶, and Sheltawy and Losowsky⁷. The determination of phospholipids was performed by the method of Bartlett⁸. The concentrations were expressed as the millimolar concentration for each lipid component. The lithogenic index was calculated from the method of Thomas and Hofmann⁹.

Gallbladders were immediately removed and rinsed with Krebs solution (20°C) (NaCl 116mM, KCl 5.4mM, CaCl₂ 2.5mM, MgCl₂ 1.2mM, NaH₂PO₄ 1.2 mM, NaHCO₃ 22.0mM, D-glucose 10.1mM). This solution was examined for the presence of cholesterol crystals and stones using a polarizing microscope.

Then, whole gallbladders were suspended in 5ml organ baths containing Krebs solution and equilibrated with 95%O₂-5%CO₂ at 37°C. The pH of the solution was adjusted to 7.4. The cystic duct part of the gallbladder was connected to the bottom of the aerator tube and the fundic part was attached to the force-displacement transducer (Grass FTO3C, Grass Instrument Co., Quincy, Mass) which was connected to a multichannel recorder (BECKMAN R611, Beckman Electronic Instruments Division., Schiller Park, IL.) (Fig. 1).

Baseline tension was adjusted to 0.7g. This was chosen from the length-tension study in which the gallbladder showed maximal contractile responses to 10⁻³M Acetylcholine (Ach) at 0.7g baseline tension. After a 30 min. equilibration time, gallbladders were examined for

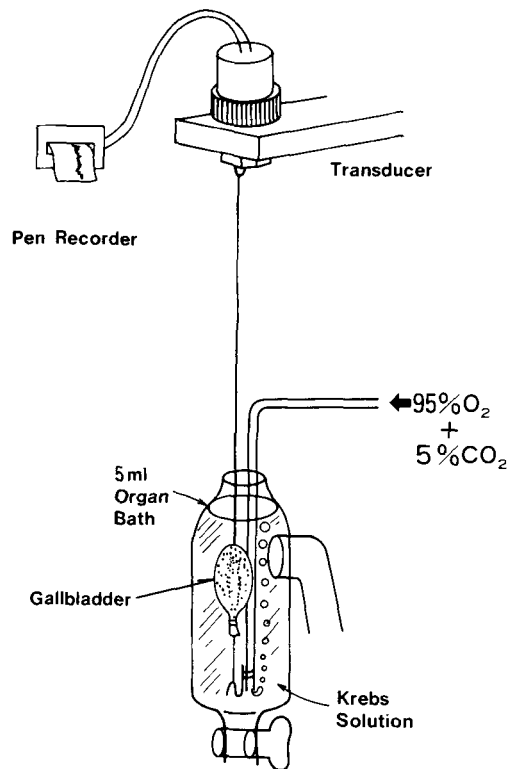


Fig. 1 The method of examination for the gallbladder contractility. The cystic duct part of gallbladder was connected to the bottom of the aerator tube and the fundic part was attached to the transducer. Baseline tension was adjusted to 0.7g.

their contractile responsiveness to Ach (10⁻¹⁰~3 × 10⁻³M) and Cholecystokinin-octapeptide (CCK-OP) (SIGMA) (10⁻¹²~3 × 10⁻⁵M). Ach was added to the organ baths at first and CCK-OP was added after washing.

All data were analyzed statistically using two-tailed, unpaired Students t-test. Differences were considered significant at P<0.05.

Results

1. Biochemical studies

Animals appeared healthy during the study periods. No macroscopic diseases were found at sacrifice. There were no significant differences in body weights or weights of gallbladder between control and cholesterol diet groups except at 10 weeks. Volume of aspirated bile

Table 1 Details of the control and cholesterol diet groups in which gallbladder contractility was examined.

		n	Gender (M:F)	Body weight at sacrifice (g)	Weight of gallbladder (mg)	Volume of bile (ml)
1 week	Control group	10	3: 7	355±17	29±3	0.27±0.04
	Cholesterol Diet group	10	3: 7	374±29	36±4	0.32±0.07
2 week	Control group	10	1: 9	365±27	25±3	0.24±0.04
	Cholesterol Diet group	10	2: 8	419±18	33±4	0.21±0.06
3 week	Control group	15	4:11	349±18	25±1	0.23±0.03
	Cholesterol Diet group	15	4:11	404±21	29±2	0.24±0.02
10 week	Control group	10	5: 5	357±18	30±2	0.29±0.04
	Cholesterol Diet group	10	5: 5	469±41*	45±6*	0.38±0.08
20 week	Control group	5	4: 1	409±25	36±3	0.33±0.05
	Cholesterol Diet group	5	4: 1	422±37	34±3	0.60±0.11

* Significantly different from control value ($P<0.05$)**Table 2** Effect of cholesterol diet on biochemical changes

		Serum-cholesterol (mM/L)	Bile-cholesterol (mM/L)	Bile acids (μ M/mL)	Phospholipids (mM/L)	Lithogenic index
1 week	Control group	9.51±0.43 (n=10)	8.01±0.70 (n=10)	246.2±11.9 (n=10)	33.23±4.18 (n=10)	0.340±0.027 (n=10)
	Cholesterol Diet group	15.30±1.55* (n=10)	19.57±1.46* (n=10)	257.2±20.8 (n=10)	48.88±2.59* (n=10)	0.673±0.054* (n=10)
2 week	Control group	11.01±0.64 (n=10)	8.31±0.41 (n=10)	241.2± 4.9 (n=10)	30.03±2.95 (n=10)	0.364±0.012 (n=10)
	Cholesterol Diet group	15.37±1.42* (n=10)	20.77±2.44* (n= 7)	269.2±37.3 (n= 8)	46.01±6.11* (n= 7)	0.757±0.062* (n= 7)
3 week	Control group	10.53±0.43 (n=15)	10.57±0.64 (n=15)	250.9±10.2 (n=15)	36.81±2.12 (n=15)	0.421±0.029 (n=15)
	Cholesterol Diet group	17.23±3.02* (n=15)	20.67±1.20* (n=15)	246.2±12.9 (n=15)	46.86±1.93* (n=15)	0.723±0.031* (n=15)
10 week	Control group	9.83±0.44 (n=10)	8.77±1.03 (n=10)	219.9±10.9 (n=10)	26.21±1.58 (n=10)	0.415±0.032 (n=10)
	Cholesterol Diet group	16.95±2.29* (n=10)	13.97±1.70* (n=10)	179.9±24.0 (n=10)	31.04±4.72* (n=10)	0.722±0.030* (n=10)
20 week	Control group	9.38±0.78 (n= 5)	6.63±0.41 (n= 5)	249.7±32.2 (n= 5)	29.96±2.56 (n= 5)	0.305±0.051 (n= 5)
	Cholesterol Diet group	15.64±3.43 (n= 5)	11.01±1.24* (n= 5)	201.4±25.8 (n= 5)	24.41±2.51 (n= 5)	0.569±0.022* (n= 5)

* Significantly different from control value ($P<0.05$)

(mean±SEM)

from the cholesterol diet groups were not significantly different from controls (**Table 1**).

The effects of cholesterol-enriched diet on biochemical measurements are shown in **Table 2**. Serum cholesterol increased significantly ($P<0.05$) in the cholesterol diet groups (1~10 weeks) compared to the corresponding control

groups. However, in the 20 weeks group, serum cholesterol was not significantly different from control. Bile cholesterol also increased significantly ($P<0.05$) in all the cholesterol diet groups. Phospholipids increased significantly ($P<0.05$) in the cholesterol diet groups up to and including 3 weeks, but did not show any differences

Table 3 Effects of cholesterol diet on responses of gallbladder to CCK-OP

	Control Group		Cholesterol Diet Group	
	ED50 (nM)	Max. tension (G)	ED50 (nM)	Max. tension (G)
1 week	79.8 (38.6–165.0)	2.00±0.18	114.6 (63.7–206.0)	1.58±0.36
2 week	80.0 (29.3–218.0)	2.41±0.19	77.1 (51.5–115.3)	2.27±0.29
3 week	56.6 (19.1–161.4)	1.79±0.40	66.4 (47.0– 93.8)	2.25±0.21
10 week	73.8 (28.4–191.4)	2.17±0.33	100.5 (44.6–226.5)	1.69±0.17
20 week	63.1 (42.7– 93.3)	3.09±0.43	64.6 (26.3–158.5)	2.63±0.15

ED50 values (and 95% confidence limits) and maximal tensions

All groups: n=5

between controls and cholesterol diet groups in 10 and 20 weeks. There were no differences in bile acids among all control and cholesterol diet groups. Lithogenic index of gallbladder bile was significantly increased in all the cholesterol diet groups ($P<0.05$). Even after a 1 week, the lithogenic index of the cholesterol diet group increased significantly ($P<0.05$) from a control value of 0.340 ± 0.027 to 0.673 ± 0.054 . The lithogenic index in the 20 week period cholesterol diet group was lower (0.569 ± 0.022) than other cholesterol diet groups, but it was still significantly increased ($P<0.05$) from the control group (0.305 ± 0.051).

In animals from the cholesterol diet group, cholesterol crystals were detected in the bile from gallbladders after 2 weeks. Stones were found in every gallbladder after 20 weeks and these primarily consisted of cholesterol in the range of 58–74%. No crystals or stones were found in any of the control animals.

2. Motility studies

During the initial equilibration period (30 min.), the tone of the gallbladder preparations was maintained at 0.7g baseline tension. No marked rhythmical contractions were observed.

CCK-OP induced concentration-dependent contractions of gallbladders from both control and cholesterol diet groups. ED50 values (95% confidence limits) and maximal contractile responses of gallbladders to CCK-OP are shown in **Table 3**. There were no significant differ-

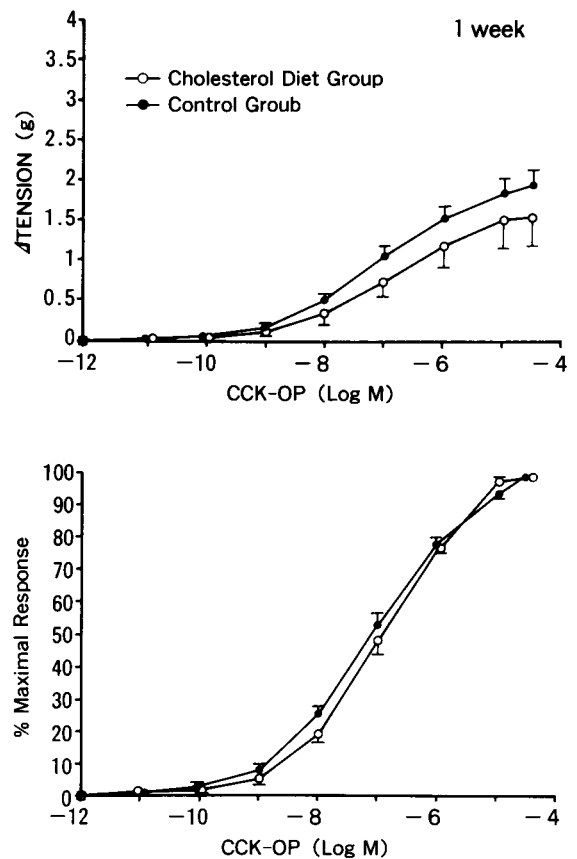


Fig. 2 Effect of cumulative concentrations of CCK-OP on gallbladders from animals maintained on their respective diets for 1 week. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal response.

ences in ED50 values or maximal tensions between control and cholesterol diet groups.

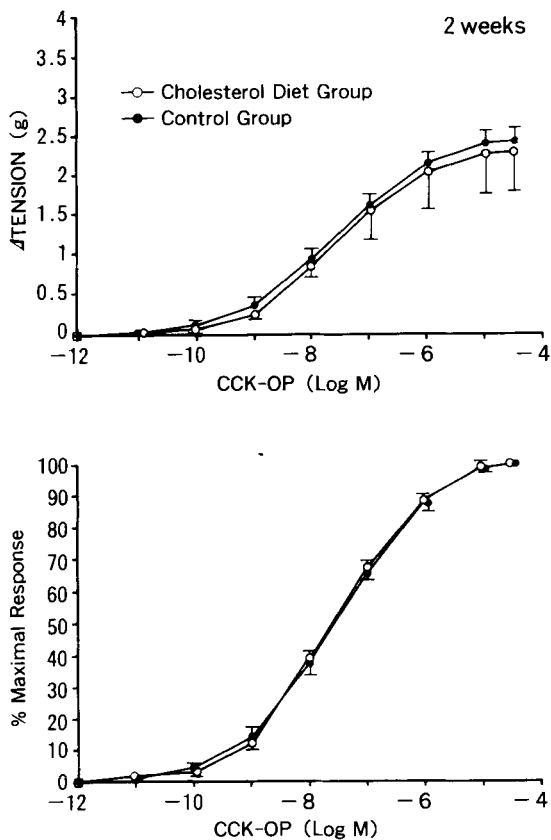


Fig. 3 Effect of cumulative concentrations of CCK-OP on gallbladders from animals maintained on their respective diets for 2 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal response.

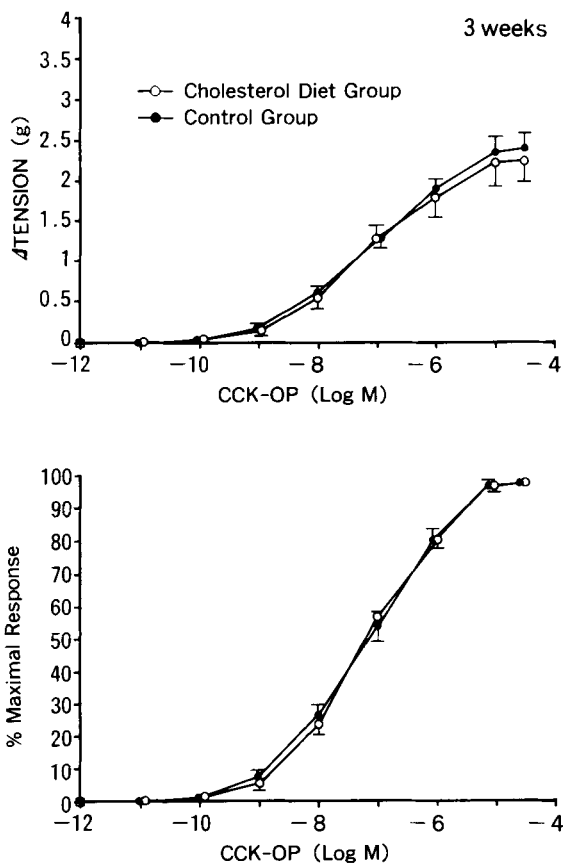


Fig. 4 Effect of cumulative concentrations of CCK-OP on gallbladders from animals maintained on their respective diets for 3 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal response.

The concentration-effect curves of gallbladders to CCK-OP for animals maintained on their respective diets for 1 week are shown in **Figure 2**. Upper panel shows responses expressed as increases in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal contraction (sensitivity). There were no significant differences in the contractility or sensitivity of gallbladders between control and cholesterol diet groups.

Figure 3 shows the concentration-response curves to CCK-OP for animals maintained on their respective diets for 2 weeks. The maximal contraction and sensitivity of gallbladders from

the cholesterol diet group were similar to those from the control group.

There were also no significant differences in contractility or sensitivity changes of gallbladders to CCK-OP between the two groups fed for 3 weeks. (**Fig. 4**).

In the 10-week groups, the maximal contractile response of gallbladders to CCK-OP from the cholesterol diet group was lower than that of the control group, but it was not significantly different (**Fig. 5**).

Although stones were found in all gallbladders from the cholesterol diet group at 20 weeks, there were no significant differences in contrac-

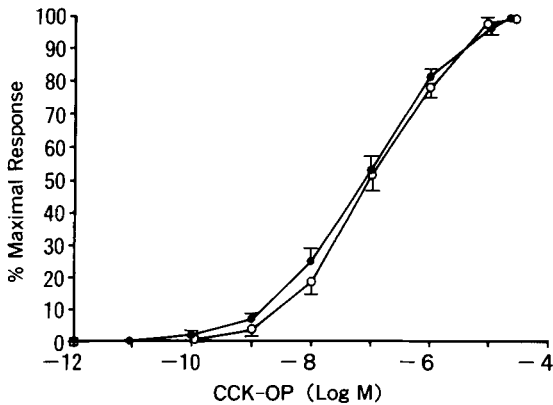
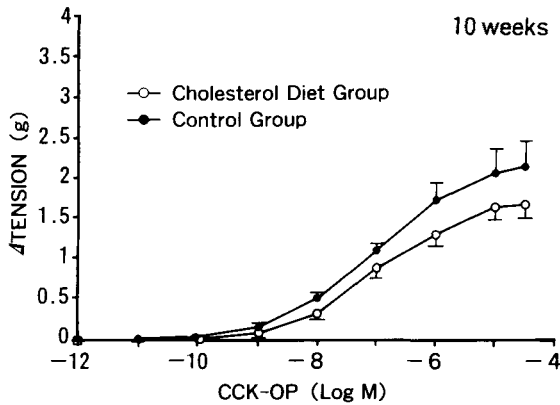


Fig. 5 Effect of cumulative concentrations of CCK-OP on gallbladders from animals maintained on their respective diets for 10 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal response.

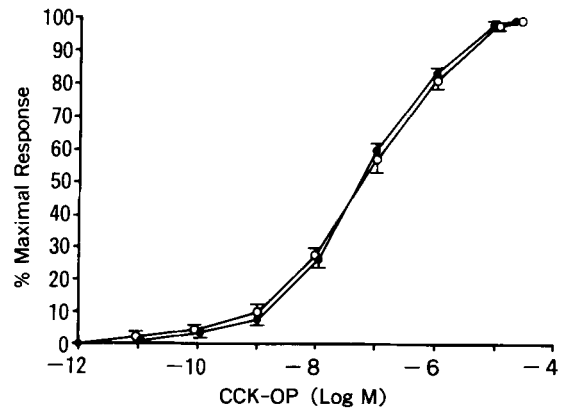
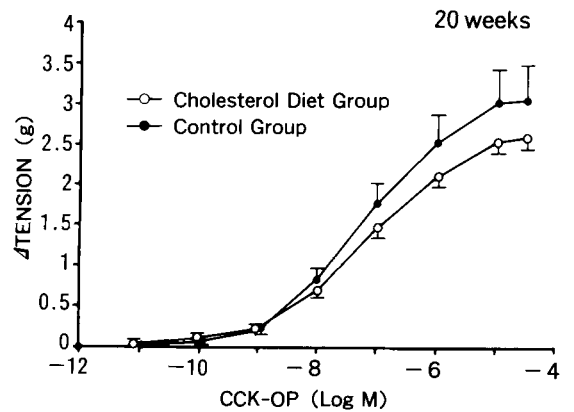


Fig. 6 Effect of cumulative concentrations of CCK-OP on gallbladders from animals maintained on their respective diets for 20 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal response.

Table 4 Effects of cholesterol diet on responses of gallbladder to Acetylcholine

	Control Group		Cholesterol Diet Group	
	ED50 (μ M)	Max. tension (G)	ED50 (μ M)	Max. tension (G)
1 week	7.9 (5.5– 11.2)	0.46 \pm 0.06	5.5 (2.2–13.9)	0.44 \pm 0.09
2 week	19.8 (6.2– 62.8)	0.63 \pm 0.14	18.0 (5.6–57.7)	0.77 \pm 0.14
3 week	17.8 (2.9–109.9)	0.66 \pm 0.06	17.0 (6.1–47.6)	0.59 \pm 0.08
10 week	12.7 (4.5– 35.5)	0.60 \pm 0.09	15.0 (3.9–57.4)	0.54 \pm 0.05
20 week	47.9 (30.9– 74.1)	1.15 \pm 0.08	28.2 (17.0–46.8)*	1.02 \pm 0.17

ED50 values (and 95% confidence limits) and maximal tensions

* Significantly different from control value

All groups: n=5

tility or sensitivity changes of gallbladders to CCK-OP between control and cholesterol diet

groups (**Fig. 6**).

Ach also induced concentration-dependent

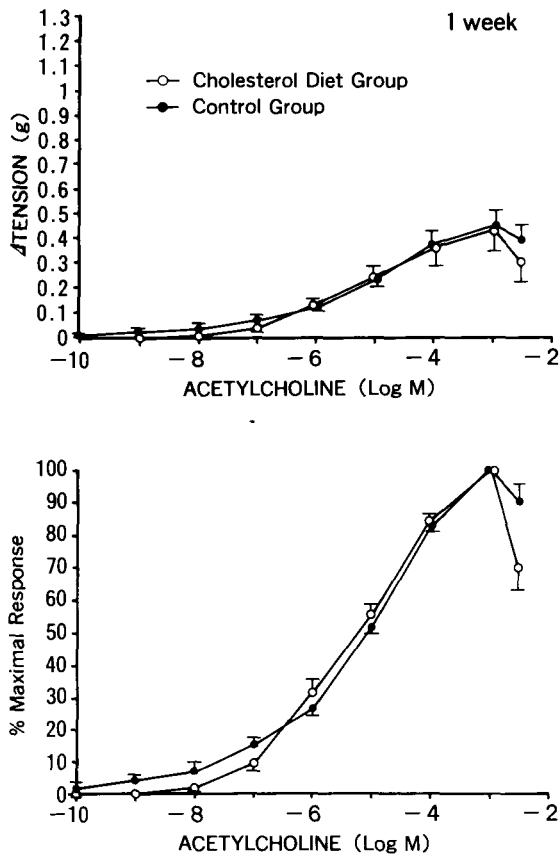


Fig. 7 Effect of cumulative concentrations of Ach on gallbladders from animals maintained on their respective diets for 1 week. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas, in the lower panel, responses are expressed as a percentage of the maximal response.

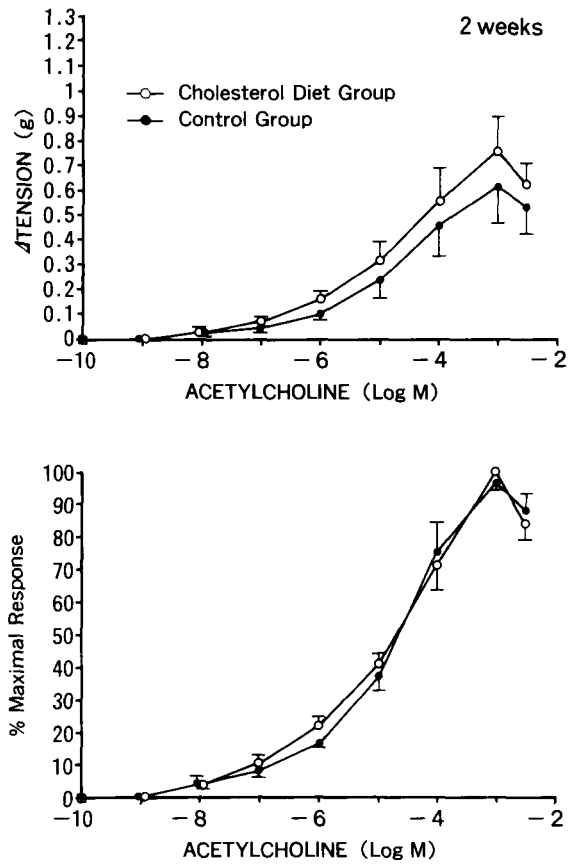


Fig. 8 Effect of cumulative concentrations of Ach on gallbladders from animals maintained on their respective diets for 2 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas in the lower panel, responses are expressed as a percentage of the maximal response.

contractions of gallbladders from both control and cholesterol diet groups. ED50 values (95% confidence limits) and maximal contractile responses of gallbladders to Ach are shown in **Table 4**. There were no significant differences in maximal tensions among all the control and cholesterol diet groups. There were also no significant differences in the ED50 of gallbladders to Ach except 20 weeks.

Figure 7 shows the concentration-response curves of gallbladders to Ach for animals maintained on their respective diets for 1 week. There were no significant differences in the contractility or sensitivity changes between control and cholesterol diet groups.

In the 2-week group, the maximal contractile response of gallbladders to Ach from cholesterol diet group was higher than that from the control group, but it was not significantly different (**Fig. 8**).

Figures 9 and 10 show the concentration-response curves of gallbladders to Ach for animals maintained on their respective diets for 3 and 10 weeks. Again, there were no significant differences in contractility or sensitivity between the two groups.

There were also no significant differences in contractility or sensitivity changes of gallbladders to Ach between the two groups of the 20

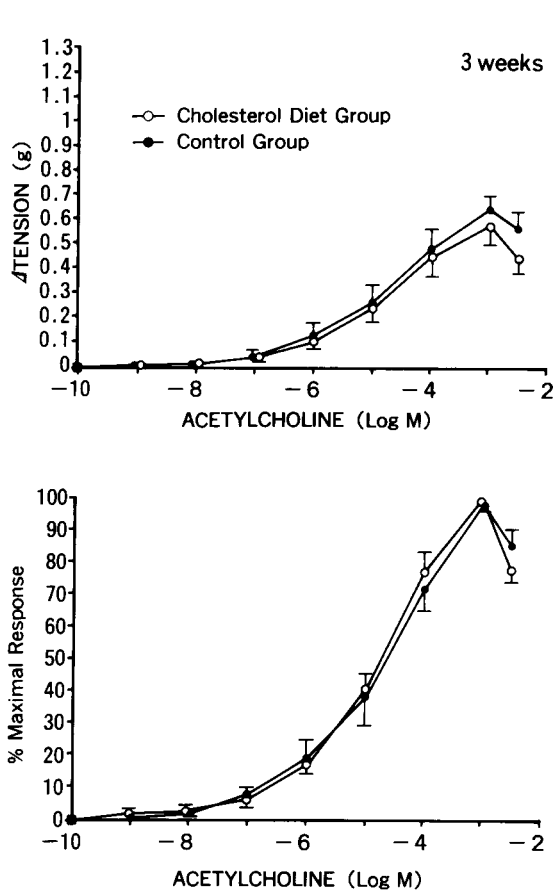


Fig. 9 Effect of cumulative concentrations of Ach on gallbladders from animals maintained on their respective diets for 3 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas in the lower panel, responses are expressed as a percentage of the maximal response.

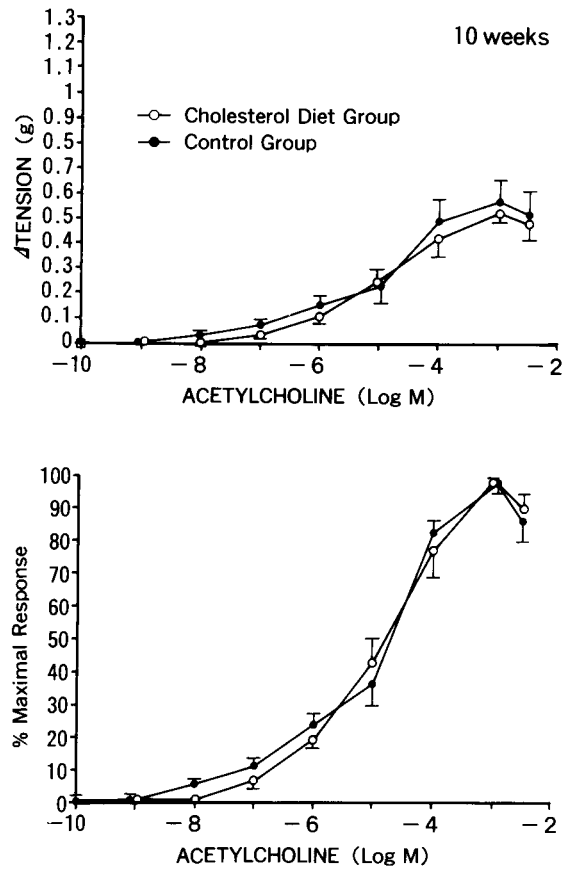


Fig. 10 Effect of cumulative concentrations of Ach on gallbladders from animals maintained on their respective diets for 10 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas in the lower panel, responses are expressed as a percentage of the maximal response.

week period when stones were found in all gallbladders from the cholesterol diet group (**Fig. 11**).

Then, by the polarizing microscopic analysis of krebs solution in which gallbladders were rinsed, 43 gallbladders from cholesterol-fed animals were classified into three groups.

- 1) No cholesterol crystals (n=19)
- 2) Cholesterol crystals but no gallstones (n=9)
- 3) Cholesterol gallstones (n=15)

Maximal contractile responses of gallbladders to CCK-OP and Ach were reinvestigated based on these three groups against 50 control animals since there were not any time-dependent

changes in the motility of the cholesterol-fed animals. There were no significant differences in the maximal contractile responses of gallbladders to CCK-OP or Ach among these three cholesterol diet groups and the control group (**Fig. 12**).

Discussion

Since Meckel von Helmsbach¹⁰ speculated that stasis of bile was involved in gallstone formation, many attempts to account for the pathogenesis of cholesterol cholelithiasis focused on the gallbladder.

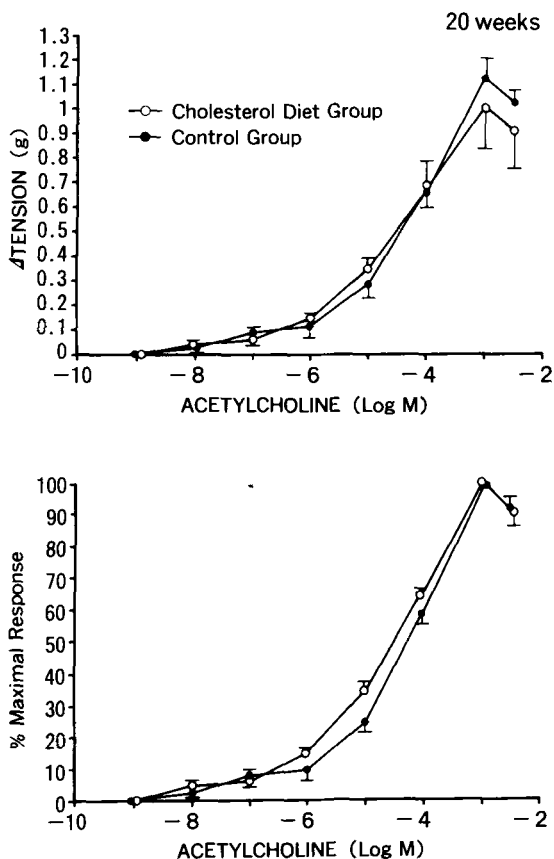


Fig. 11 Effect of cumulative concentrations of Ach on gallbladders from animals maintained on their respective diets for 20 weeks. Each point represents the mean \pm SEM. Upper panel shows responses expressed as an increase in tension in grams, whereas in the lower panel, responses are expressed as a percentage of the maximal response.

In 1968, the physical state of bile (the presence or absence of insoluble cholesterol) was determined by the relative concentrations of cholesterol, bile salt, and lecithin¹¹. Abnormal bile supersaturated with cholesterol was shown to be associated with cholesterol gallstone formation. This directed more attention to the liver as the site of the abnormality in the pathogenesis of gallstones.

This is necessary but not sufficient by itself to produce gallstones since normal humans without gallstones were demonstrated to have supersaturated bile for prolonged periods of the day¹². The additional requirement for stone formation appear to be nucleating factors for crys-

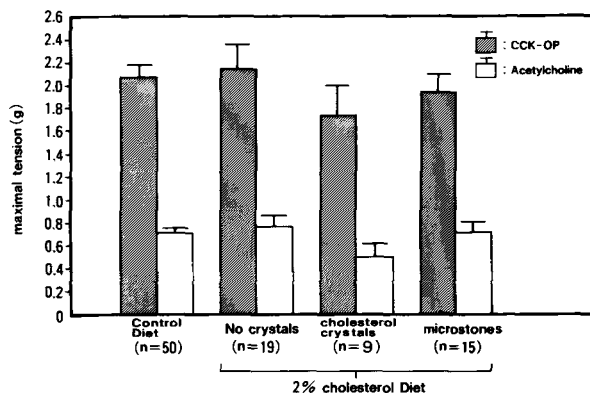


Fig. 12 Effect of cholesterol diet on responses of gallbladder to CCK-OP and Acetylcholine. Maximal tensions \pm SEM are reported.

talization, entrapment of crystals and microstones.

In humans conditions in which it is presumed that there are changes in motility (i.e., "stasis"), there is an increased incidence of gallstones. For example, gallbladder volume during fasting and residual volume after contraction were increased during the latter trimesters of pregnancy¹³. Gallbladder sludge was detected in 100% of patients during parenteral nutrition for 6 weeks¹⁴.

The mechanism by which gallbladder stasis occurs in gallstone patients has not been studied in detail. Previous investigations on gallbladder emptying in humans with gallstones have presented conflicting results; some demonstrating impaired gallbladder emptying¹⁵, others normal emptying¹⁶.

Gallstones have been produced in many species by dietary manipulation. In a study using guinea pigs, Brotschi¹⁷ demonstrated that 0.5% cholesterol diet for 12 weeks induced gallbladder enlargement without altering gallbladder smooth muscle tone or the contractile response to CCK. However, guinea pigs on cholesterol diets developed gallstones which are not primarily composed of cholesterol but largely of bilirubinate. Gallstones were induced in mice by feeding a cholesterol-cholic acid containing diet^{18,19}.

Richardson Ground Squirrel and Prairie dog are more suitable models for the study of cholesterol gallstone formation because these animals make gallstones rapidly by cholesterol diet. Moreover, stones in these animals primarily consisted of cholesterol^{3,20}.

In the present study, time-dependent changes in gallbladder motility of the Richardson Ground Squirrels were investigated. There were no significant differences in the contractility and sensitivity of gallbladders to CCK-OP or Ach between control and cholesterol-fed animals. The results of this study indicate that gallbladder muscle contractility remains unchanged during the dietary induction of cholesterol gallstones in this species.

However, Fridhandler et al.³ demonstrated a defect in gallbladder contractility associated with increase in bile lithogenicity in the same species. In that study, gallbladders from animals on 1% cholesterol diet showed reductions in tension generated in response to CCK-OP and Ach.

That result is contrary to the result of the current study. We could not show any impairment in the gallbladder muscle contractility before, during, and after gallstone formation by the cholesterol-enriched diet in this species.

Biochemical results showed that the cholesterol diet was effective in these animals and bile cholesterol increased significantly in the cholesterol diet group. Also, the lithogenic index increased within the first week commencement of the 2% cholesterol diet. The changes in lithogenic index in each cholesterol diet group were similar to those reported by Fridhandler. Cholesterol crystals were detected in the gallbladders even after a 2-week cholesterol diet period.

There might be methodological differences between the current study and that of Fridhandler. For example, the concentration of cholesterol in the diet (2% cholesterol (w/w) in our study: 1% in their study) and the stimulant used in each experiment (CCK-OP, Sigma Chemical Co. in our study: CCK-OP, KINEVAC, Squibb Co. in their study) were dif-

ferent but not significantly.

A possible explanation for the lower lithogenic index and smaller increase in serum cholesterol for the 20-week cholesterol diet group may be that animals became tolerant to the diet. Alternatively, animals in the 20-week group may have consumed less food as there were larger losses in body weight in both control and cholesterol diet animals (**Table 1**).

A previous study in cholesterol-fed Prairie dogs demonstrated a correlation between lithogenic index and cystic duct resistance. However, the mechanism of increased cystic duct resistance remained obscure².

Decreased gallbladder emptying and increased cystic duct resistance were shown concurrently by the perfusion method in the same species with cholesterol crystals or gallstones induced by 0.4% cholesterol diet²¹. These changes were not present in the cholesterol-fed animals without crystals. The presence of cholesterol crystals, but not lithogenic bile, was associated with impaired gallbladder emptying. It was suggested by Doty et al.²¹, therefore, that the cause of increased cystic duct resistance may be due to the crystals themselves which could have obstructed the duct.

However, our results demonstrated that the maximal contractile responses of gallbladders with cholesterol crystals or gallstones to Ach or CCK-OP were similar to those of gallbladders in the control animals or in the cholesterol-fed animals without crystals (**Fig. 12**).

We conclude that the cause of impaired gallbladder emptying may be due to disorder of bile flowing out of the gallbladder by the physicochemical changes of bile itself, rather than the impairment of gallbladder smooth muscle contractility. Gallbladder muscle contractility changes do not play an important role in the pathogenesis of cholesterol gallstones in the Richardson Ground Squirrel.

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