The Formation of Abnormal Bile and Cholesterol Gallstones from Dietary Cholesterol in the Prairie Dog

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ABSTRACT To study the pathogenesis of cholesterol gallstones, we fed 24 adult male prairie dogs a high cholesterol, egg yolk diet. 13 control animals received a cholesterol-free diet. All animals fed the egg yolk diet formed multiple gallstones in 2–6 months' time. These stones contained cholesterol, 77±14% by dry weight. No stones ocurred in the control group.

The egg yolk-fed animals developed bile of altered chemical composition. The cholesterol concentration of hepatic and gallbladder bile increased significantly. The molar ratios of bile acid/cholesterol and phospholipid/cholesterol decreased in hepatic and gallbladder bile. The predominant bile acid shifted from cholic acid, 78% of the total bile acids, to chenodeoxycholic acid, 60% of the total. In common bile duct cannulated animals the high cholesterol diet produced increased secretion of cholesterol by the liver and increased bile flow.

In animals fed the egg yolk diet for 2 months, cholesterol-4-^{14}C was included in the daily diet for the next 4 months to establish an isotopic steady state. At autopsy the mean specific activity of cholesterol was similar in serum, liver, hepatic bile, gallbladder bile, and gallstones. Thus the cholesterol of gallstones apparently equilibrated constantly throughout the study and was not sequestrated as a static pool.

The high cholesterol, egg yolk diet caused the secretion of an "abnormal bile" which led to precipitation of cholesterol from micellar solution. The increased bile cholesterol relative to bile acid and phospholipid favored stone formation. This dietary induction of cholesterol gallstones provided a unique animal model, in part but not completely analogous to human cholelithiasis.

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INTRODUCTION

Despite growing knowledge of gallbladder physiology and of the physiochemical nature of bile, the pathogenesis of cholelithiasis in man remains obscure. A contributing factor to this lack of progress is the paucity of animal models which form gallstones analogous to the human disease. This point has been stressed by Dam in a recent review (1). Of special interest to us was the possibility of inducing cholesterol gallstones in an animal by a high cholesterol, high fat diet which would be similar to the diet of population groups with a high incidence of gallstones.

Gallstones have been produced in many species by dietary manipulation. However, the experimental production of cholesterol gallstones has usually required either unusual additives such as cholestyramine (2) and cholic acid (3) or a diet fat-free and high in sucrose (4). These additives and such a diet are unphysiological circumstances and do not create an experimental situation particularly analogous to human cholelithiasis. A preliminary report suggested the possibility of a more suitable animal model, the prairie dog, which developed gallstones from the feeding of a diet high in egg yolk (5).

In the experiments to be described, a high cholesterol diet containing egg yolk invariably produced cholesterol gallstones in prairie dogs. Such a high cholesterol diet taxed the capacity of the animal to excrete and metabolize cholesterol and led to "cholesterol overloading." Gallstones developed because the bile became chemically abnormal and could not retain cholesterol in solution. The abnormal bile was initially elaborated in the liver. By virtue of cholesterol-4-^{14}C experiments, the cholesterol of the diet was shown to be incorporated into the cholesterol of the gallstones.

TABLE I

*Composition of Semisynthetic Diets**

	Diet 1—control diet	Diet 2—lithogenic diet
	g/100 g	*g/100 g*
Ingredients		
Soya assay protein	20.23	7.48
Egg yolk powder	—	36.60
Crystalline cholesterol	—	0.40
Corn starch	—	14.00
Cane sugar	70.57	33.94
Corn oil	1.62	—
Salt mix (Phillips Hart)	4.00	4.00
Vitamin supplement GBI	1.00	1.00
Nonnutritive fiber	2.58	2.58
Cholesterol source		
Egg yolk	0	0.8
Crystalline	0	0.4
Total	0	1.2
Calories	Per cent total	Per cent total
Fat	4	41
Carbohydrate	76	44
Protein	20	15

* Especially prepared by General Biochemicals Div., Chagrin Falls, Ohio.

METHODS

37 adult male prairie dogs[1] were caged individually in thermoregulated (23°C) rooms. After a baseline period of a low cholesterol diet,[2] the animals were divided into two dietary groups. Each group was fed a different semisynthetic diet (Table I). The control diet (diet 1) was free of cholesterol, and fat provided 4% of the total calories. The lithogenic diet (diet 2) contained a large quantity of egg yolk. Its cholesterol content was 1.2% by weight, and fat provided 41% of the total calories. Both diets contained ample essential fatty acids. These constituted 2.2% of the total calories in the control diet and 3.28% of the total calories for the lithogenic diet. 13 animals received the control diet and 24 animals received the lithogenic (egg yolk) diet. The duration of these experiments was either short term (2–3 months) or long term (6 months).

Cholesterol-4-^{14}C was added to the egg yolk diet for the last 4 months of the experiment in six animals to establish an isotopic steady state. Portions of the radioactive diet were prepared by mixing 1500 g of chow with 10 μCi of purified cholesterol-4-^{14}C (footnote 3) dissolved in ether. The ether was evaporated and the chow fed ad lib. as previously indicated. The animals consumed an average of 0.1 μCi of cholesterol-4-^{14}C per day. Venous blood samples were obtained from a superficial leg vein at intervals of 2 wk to 2 months. For this procedure the animals were fasted for 16 hr and placed in a squeeze cage.

Each dietary experiment was concluded with an acute study to obtain gallbladder and hepatic bile. After a 16 hr fast the animals were anesthetized with 0.6 ml/kg dosage of Dial with urethane[4] i.p. The cystic duct was ligated and the gallbladder bile was aspirated. The common duct was cannulated with No. 50 polyethylene tubing and 1 hr specimens of hepatic bile were collected in chilled tubes. The gallbladder was then excised and examined for the presence of stones. The contents of the gallbladder were blotted on filter paper to make the collection as complete as possible. Samples of gallstones varied in quantity from 5 to 70 mg of dry weight. The bile, gallstones, serum, and liver were frozen immediately after collection.

For analysis, the stones and tissues were dried under vacuum at 100°C, weighed, ground to a powder, and extracted 3 times with 2:1 chloroform:methanol (6). The cholesterol content of serum, bile, liver, and gallstones was measured by the method of Abel, Levy, Brodie, and Kendall (7). Each bile sample was centrifuged at 2000 rpm for 5 min and the sediment examined for aggregates of crystals under low power (100×). Cholesterol crystals were identified by criteria stated by Juniper and Burson (8).

Bile was analyzed for the three major components (cholesterol, phospholipid, and bile acids) in five control and seven experimental animals. The cholesterol content was determined before and after centrifugation when the quantity of bile was sufficient. Total bile acids were measured by the steroid dehydrogenase method of Iwata and Yamasaki (9) as modified by Rhodes, Barnado, Philips, Rovelstad, and Hofmann (10). Individual bile acids were determined by gas-liquid chromatography using a method previously described (11). Lipid phosphorus was measured from a 2:1 chloroform:methanol extract of bile by the method of Dryer, Tammes, and Routh (12). Phospholipid values were calculated by multiplying the phosphorus value by 25.

For the determination of cholesterol radioactivity, the sample was saponified with alcoholic KOH. The nonsaponifiable residue was extracted with hexane, dried, and then dissolved in 10 ml of scintillation mixture (4 g of 2,5-diphenyl-oxazole and 100 mg of 1,4-bis (2-(5-phenyloxazole)) benzene in 1 liter of toulene). Samples were counted in a Packard Tri-Carb scintillation spectrometer[5] at an efficiency of 89%. Cholesterol specific activity was expressed in disintegrations per minute per milligram of cholesterol.

Animals fed the egg yolk-rich diet for 2–3 months increased in body weight from 992±152 (SD) to 1100±165 g and appeared healthy. Control animals gained steadily in weight from 1022±220 (SD) to 1218±235 g. However, the long term animals fed the egg yolk rich diet decreased in body weight from 1159±260 (SD) to 1067±204 g. This weight loss occurred predominately during the 5th and 6th months and occurred in 4 of 10 animals. Several long term animals fed the lithogenic diet also developed diarrhea and loss of fur during the latter months.

All data were expressed as the mean ±SD. Statistical comparisons were made by means of Student's *t* test (13).

RESULTS

Gallstones invariably developed in all prairie dogs fed the high cholesterol egg yolk diet. No gallstones formed

[1] *Cynomys ludovicianus,* trapped in the wild state and obtained from Otto Marten Locke of New Branfels, Tex.

[2] Lab Blox Chow, Allied Mills, Inc., Chicago, Ill.; cholesterol content, 28.3 mg/g and plant sterol content, 85.6 mg/g.

[3] New England Nuclear Corp., Boston, Mass.

[4] CIBA Pharmaceutical Co., Summit, N. J.; each ml contained 100 mg allobarbital, 400 mg monoethylurea, 400 mg urethane.

[5] Packard Instrument Co., Inc., Downers Grove, Ill.

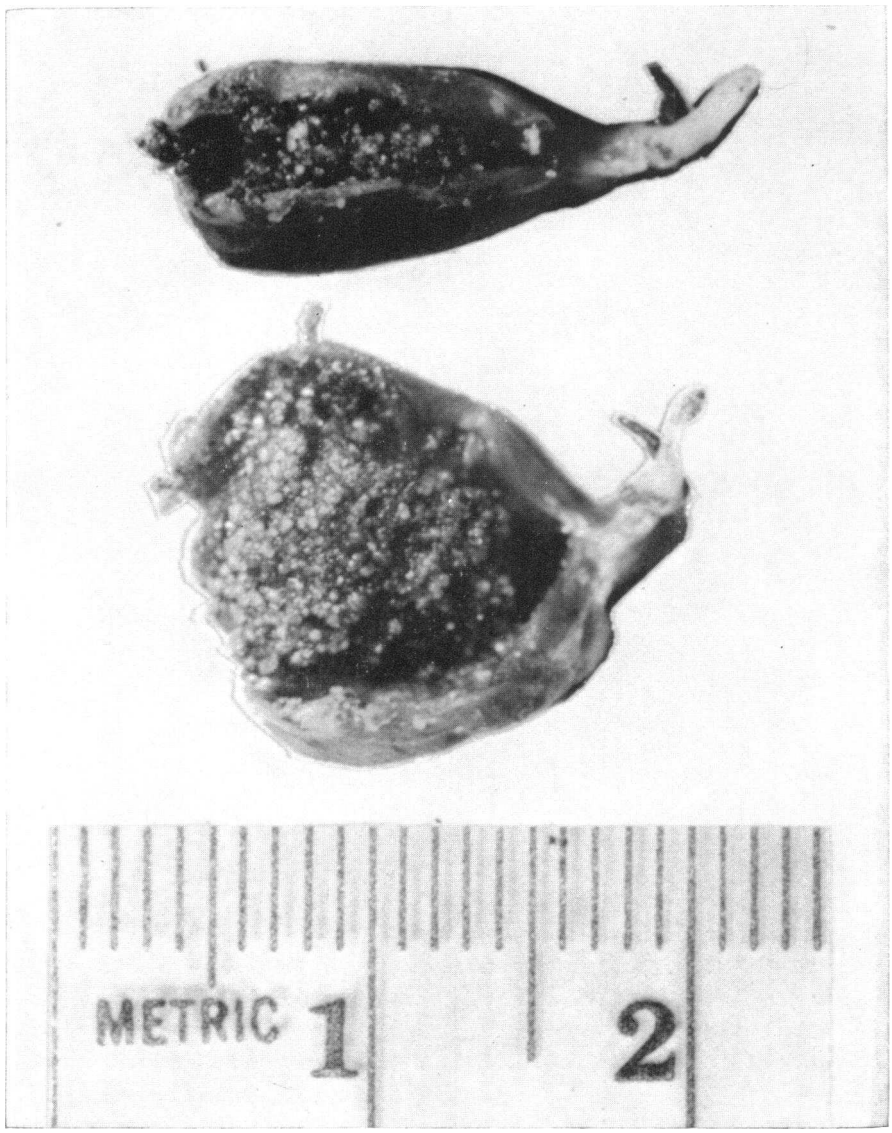

FIGURE 1 The gallbladder and gallstones of a prairie dog fed the high cholesterol, egg yolk diet for 6 months.

in the animals fed the cholesterol-free low fat diet. Gallstones were found only in the gallbladder. They were multiple, pigmented, round or amorphous concretions, about 1 mm in diameter. Typical gallbladders and their contents are shown in Fig. 1. Microscopically, the stones appeared either as laminated crystals with irregular shapes which were characteristic for cholesterol (Fig. 2) or as amorphous pigmented particles. When the latter were crushed, a crystalline nidus was found. This nidus was indistinguishable from the crystalline stones. These gallstones were primarily cholesterol by chemical analysis. The cholesterol content of the gallstones from animals fed 6 months was 74±17% of dry weight and that of stones from animals fed 2–3 months, 80±12%. There were no apparent differences in composition or weight of the gallstones in the animals fed the egg yolk-rich diet for these different time periods.

The cholesterol concentration of gallbladder bile from animals fed the lithogenic diet significantly increased after the egg yolk diet: from 165 to 408 mg/100 ml in short term animals and from 180 to 664 mg/100 ml in long term animals (Table II). Animals fed the egg yolk-rich diet for 2–3 months secreted hepatic bile with higher cholesterol concentrations than did control animals: 173 vs. 65 mg/100 ml. The same comparison in animals fed 6 months also revealed an apparent increase

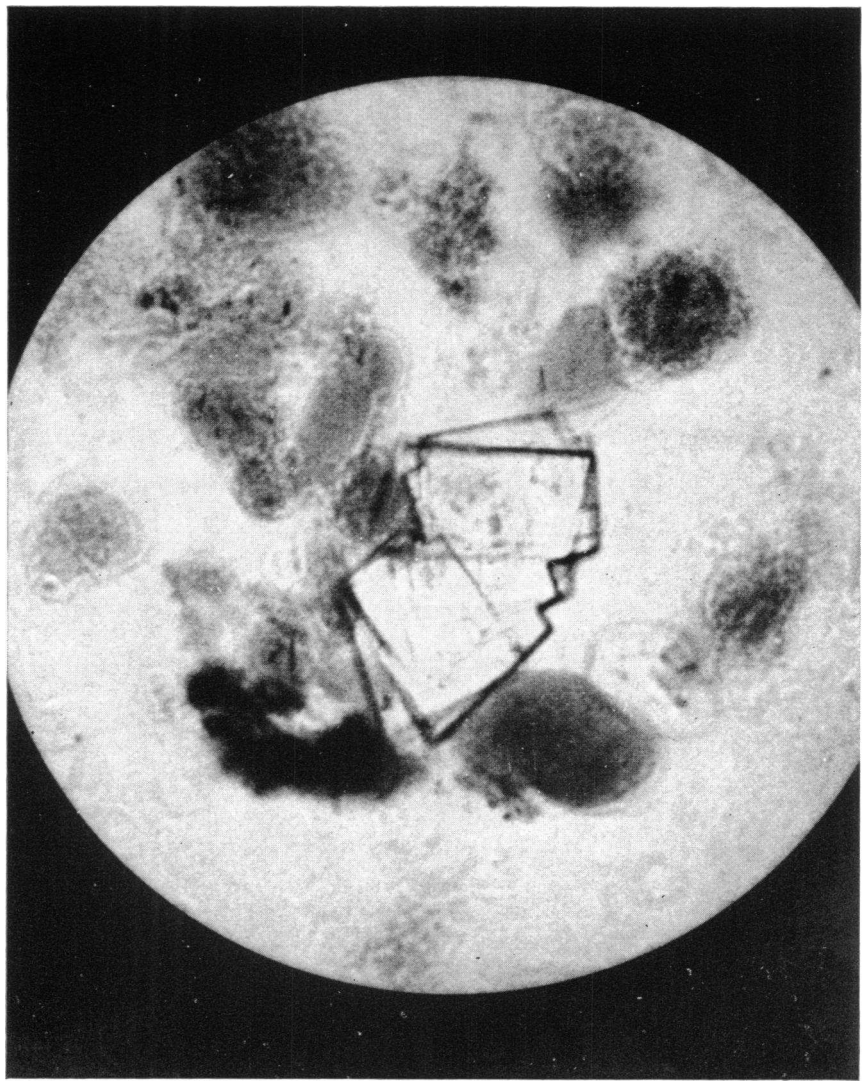

FIGURE 2 Microscopic view of typical crystalline and amorphous pigmented gallstones from prairie dogs fed the egg yolk diet.

for the animals fed egg yolk, but the difference was not statistically significant. However, if the data from long and short term animals fed the same diet were combined, the hepatic bile cholesterol concentration in the egg yolk—fed group was significantly increased from the control group: 175±90 (SD) vs. 61±28 mg/100 ml ($P < 0.001$). The values for Table II related to noncentrifuged bile.

Complete analyses of the three major bile components (cholesterol, phospholipid, and bile acids) were determined in five control animals and in seven prairie dogs fed the egg yolk diet (Table III). This bile was centrifuged before analysis. Although the mean cholesterol, bile acid, and phospholipid concentrations in both hepatic and gallbladder bile appeared to be elevated in the egg yolk-fed group, no statistically significant differences from the control group were found. The apparent difference in cholesterol concentration between centrifuged and noncentrifuged bile results from the fact that the abnormal bile contained large quantities of crystalline cholesterol. This is consistent with microscopic observations of crystalline cholesterol aggregates in gallbladder bile with gallstones. In egg yolk-fed animals, the cholesterol concentration of noncentrifuged hepatic bile was 175 mg/100 ml and that of centrifuged hepatic bile 156 mg/100 ml. Cholesterol crystals were identified in the noncentrifuged bile from the experimental group and this finding probably accounted for the lesser cholesterol content of the centrifuged bile. The cholesterol content of control bile was not significantly changed by

TABLE II

Cholesterol Content of Gallbladder Bile, Hepatic Bile, Serum, and Liver in Prairie Dogs Fed the Cholesterol-Free Control Diet and those Fed the High Cholesterol, Egg Yolk Diet

Diet and duration	Number of animals	Gallstone incidence	Gallbladder bile*	Hepatic bile*	Serum	Liver
				mg/100 ml		*mg/g*
Short term						
Control	8	0	165±74 (SD)	65±31	179±84	11.60±1.77
Egg yolk	14	14	408±264	173±62	1165±430	30.43±14.00
P values			<0.05‡	<0.01	<0.001	<0.01
Long term						
Control	5	0	180±82	54±23	156±53	12.88±0.84
Egg yolk	10	10	644±338	177±111	1572±684	37.51±11.16
P values			<0.02	NS	<0.001	<0.001

* Analyses were carried out on bile which had not been centrifuged.

‡ The *P* value is derived from the statistical comparison between the control and egg yolk-fed animals.

centrifugation since it did not contain cholesterol crystals.

More importantly, from the point of view of cholesterol solubility in bile, when each of the three major constituents of bile was expressed as a molar percentage of the total, a significant increase in the bile cholesterol content was found (Table IV). This significant increase in cholesterol relative to the solubilizing components (phospholipid and bile acids) held true for both the hepatic and gallbladder bile. Furthermore, the bile acid/cholesterol molar ratios decreased significantly in both gallbladder and hepatic bile. The phospholipid/cholesterol ratio was statistically less only in hepatic bile. These findings all indicated that the solubility of bile cholesterol was critically jeopardized and its precipitation into gallstones predictable from its lessened solubility in relationship to the solubilizing components of bile.

The relative concentration of the four individual bile acids were changed greatly by the egg yolk diet (Table III). Cholic acid was the predominant bile acid in control animals and comprised 78% of the total bile acids. Only 18–19% of the total was derived from chenodeoxycholic acid. Small amounts of the secondary bile acids, deoxycholic, and lithocholic acid, were found (3% or less). In prairie dogs fed the egg yolk-rich diet, chenodeoxycholic acid became the major bile acid: in hepatic bile, 60% of the total and in gallbladder bile, 68%. Cholic acid decreased to 30% of the total. The mean concentration of lithocholic acid (the secondary bile acid of chenodeoxycholic acid) in the egg yolk-fed group increased almost 10 times that of the control group, but wide variations precluded statistical significance.

Other consequences of the high cholesterol diet were increased bile flow and cholesterol excretion as measured for 1 hr after common bile duct cannulation in 8 control animals and 10 fed the egg yolk-rich diet. Information from both long term and short term animals is included in these data. Bile flow increased from 0.65±0.12 (SD) ml/hr in control animals to 0.90±0.25 ml/hr in animals fed egg yolk ($P < 0.02$). The mean hepatic bile cholesterol output was 1444±470 μg/hr

TABLE III

Cholesterol, Bile Acid, and Phospholipid Composition of Centrigufed Gallbladder and Hepatic Bile of Prairie Dogs Fed Either the Control Diet or the Egg Yolk-Rich Diet for 3–6 Months

Source of bile	Number of animals	Diet	Cholesterol	Phospholipid	Bile acids	Percentage of total bile acid			
						Cholic	Chenodeoxycholic	Deoxycholic	Lithocholic
				μmoles/100 ml					
Gallbladder	5	Control	325±97 (SD)*	1803±671	8157±2605	77.7±5.3	18.0±3.5	1.21±1.4	1.2±1.6
	7	Egg yolk	707±403	2277±1012	8448±2935	30.3±11.6	67.7±11.3	1.1±0.9	2.6±1.1
P values			NS*	NS	NS	<0.001	<0.001	NS	NS
Hepatic	4	Control	123±58	715±377	1919±1207	78.0±5.1	19.9±3.3	1.6±1.9	0.8±0.5
	5	Egg yolk	408±291	1423±815	3641±2435	31.1±17.0	60.2±14.4	0.9±0.6	7.8±6.5
P values			NS	NS	NS	<0.001	<0.001	NS	NS

* All statistics compare the control and egg yolk-fed animals.

TABLE IV

Relative Molar Percentages and Molar Ratios of Cholesterol, Bile Acid, and Phospholipid of Prairie Dogs Fed Either the Egg Yolk-Rich or Control Diets for 3–6 Months

Source of bile	Number of animals	Diet	Per cent of total moles			Molar ratios	
			Cholesterol	Phospholipid	Bile acid	Bile acid/ cholesterol	Phospholipid/ cholesterol
Gallbladder	5	Control	3.3±1.1 (SD)*	17.5±2.3	79.2±2.0	26.35±10.13	5.97±3.00
	7	Egg yolk	5.7±2.1	18.1±3.6	76.0±5.5	14.76±7.35	3.44±0.97
P values			<0.05*	NS	NS	<0.05	NS
Hepatic	4	Control	4.7±0.7	26.8±3.2	68.5±3.9	14.76±2.83	5.68±0.32
	5	Egg yolk	7.3±1.5	26.7±4.0	65.9±2.6	9.22±1.75	3.88±1.58
P values			<0.02	NS	NS	<0.02	<0.01

* All statistics compare the control and egg yolk-fed animals.

in animals with gallstones compared with 396±215 μg/hr in control animals ($P < 0.001$).

The prairie dogs fed cholesterol-4-^{14}C reached an isotopic steady state in 4–7 wk as indicated by plateauing serum cholesterol specific activities (Fig. 3). Thus, the animals were in the isotopic steady state for 2–3 months before the termination of the experiment. The cholesterol-specific activities of serum, bile, gallstones, and liver were remarkably similar (Table V). However, the specific activity of cholesterol in gallbladder bile was less than that of the serum in several animals. For example, one prairie dog had a gallbladder bile cholesterol specific activity of 694 as compared with a specific activity of 1000 for serum. Nevertheless, the mean gallbladder bile cholesterol specific activity for all the animals was not significantly different from that of serum.

The percentage of cholesterol in serum, bile, gallstones, and liver that originated from the diet was calculated by dividing the cholesterol specific activity of the substance in question by the dietary cholesterol specific activity. The dietary cholesterol specific activity was 1350 dpm/mg of cholesterol. As indicated in Table VI, the diet provided the major portion of the cholesterol of serum, liver, bile, and the gallstones. The percentages of dietary cholesterol incorporated in the cholesterol of liver, gallstones, hepatic bile, and the majority of gallbladder bile samples were similar to those of the corresponding serum. Two exceptions had 51 and 41% of the gallbladder bile cholesterol from dietary origin as compared with 74 and 71% for the corresponding serum.

The egg yolk diet produced an immediate and sustained rise in serum cholesterol concentrations (Fig. 4). After 2 wk of feeding, the mean serum cholesterol level increased from 143 to 747 mg/100 ml. The animals receiving the low fat, cholesterol-free control diet continued to have low serum cholesterol levels similar to the baseline period. The terminal serum cholesterol

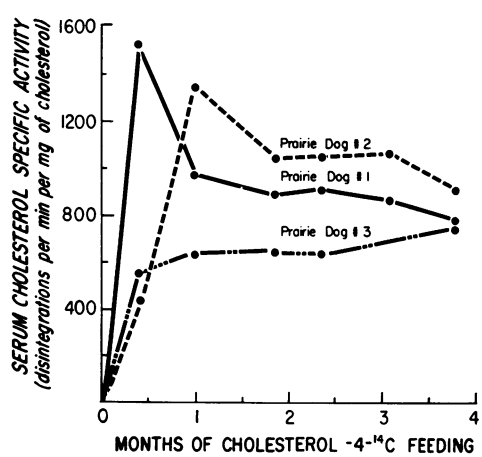

FIGURE 3 The specific radioactivity curves of the serum cholesterol in three representative prairie dogs fed a constant amount of cholesterol-4-^{14}C in conjunction with the high cholesterol, egg yolk diet.

TABLE V

Specific Activities of Cholesterol in Serum, Hepatic Bile, Gallbladder Bile, Gallstones, and Liver in Prairie Dogs Fed Cholesterol-4-^{14}C

	Specific activity*
Serum	923±199
Hepatic Bile‡	
1st hr	908±149
2nd hr	933±224
5th hr	905±204
Gallbladder bile	838±270
Gallstones	893±200
Liver	948±236

* Expressed as dpm (±SD) per mg of cholesterol.

‡ Collection period after common duct cannulation.

TABLE VI

Percentage of Cholesterol in Serum, Bile, Gallstones, and Liver that Originated from the Diet

	Mean per cent	Range
Serum	69	50–92
Hepatic bile*		
1st hr	67	57–80
2nd hr	69	50–92
5th hr	67	47–84
Gallbladder bile	62	47–97
Gallstones	66	48–83
Liver	70	47–96

* Collection period after common bile duct cannulation.

levels of both the short and long term animals with gallstones were significantly increased over control levels but were not significantly different from each other (Table II). The liver cholesterol content was likewise increased by the egg yolk diet.

DISCUSSION

Cholesterol is the major constituent of most human gallstones. An error in the metabolism of this sterol has long been hypothesized as one of the prime factors in the etiology of cholelithiasis. Many investigators have attempted to produce cholesterol gallstones in experimental animals by feeding cholesterol but, to date, this technique has not been uniformly successful. Recently cholesterol stones have been produced in squirrel monkeys by feeding a diet high in butter and cholesterol, but long term feeding (> 9 months) was necessary for a high incidence of gallstones (14). Even then gallstones did not invariably result. When a 1% cholesterol diet was fed to guinea pigs, stones high in calcium phosphate were produced (15). The high cholesterol diets used in other studies to produce cholesterol stones have required the addition of cholic acid or cholestyramine before stones occurred (3, 2). The prairie dog is unique in that gallstones were invariably produced when the animals were fed a high cholesterol, egg yolk diet for only a 2–3 month period of time.

The lithogenic diet used in this study had several distinctive features. The caloric distribution of the diet was equivalent to most American diets; i.e., 41% fat, 44% carbohydrate, and 15% protein. No drugs or other supplements were used. In addition, most of the cholesterol in the diet was from a natural food source, egg yolk. The lithogenic agent of the diet responsible for stone development appeared to be in the high cholesterol content of egg yolk which led to the formation of an abnormal bile.

A basic hypothesis for the formation of cholesterol gallstones in the prairie dog could be derived from the results of this study. The egg yolk diet produced "cholesterol overloading" of the hepatic system for the disposition of cholesterol. Cholesterol in the liver cell may be disposed of in three ways: (*a*) the secretion of cholesterol-containing lipoproteins into the blood and hepatic lymph, (*b*) the conversion of cholesterol into bile acids, then to be excreted in the bile, and (*c*) the secretion of cholesterol itself into the bile. Clearly, gallstones would not form if the first two pathways were successfully followed and excessive cholesterol not excreted into the bile. Should the secretion of bile acid and phospholipid become also abnormal, then any excreted cholesterol would be rendered less soluble. Paramount to the development of gallstones in these studies was the great increase in the cholesterol concentration of hepatic bile (possibility 3 for cholesterol disposition by the liver) plus a reduction in the over-all capacity of the bile to solubilize cholesterol. The accumulation of cholesterol in serum and liver always accompanied stone formation. Isotopic data indicated that 70% of this cholesterol was from the diet. Evidently, the large quantity of exogenous cholesterol being transported to the liver overwhelmed this organ's capacity for cholesterol disposition. The end result was that hepatic cells secreted increased quantities of cholesterol into the bile, and presumably this bile cholesterol when concentrated in the gallbladder provided the cholesterol of the gallstones. Some of the gallstone cholesterol, however, might certainly have been derived from cholesterol transferred from the plasma through the gallbladder mucosa. Damage to the gallbladder mucosa, perhaps from the abnormal hepatic bile, may have also been a factor in stone formation.

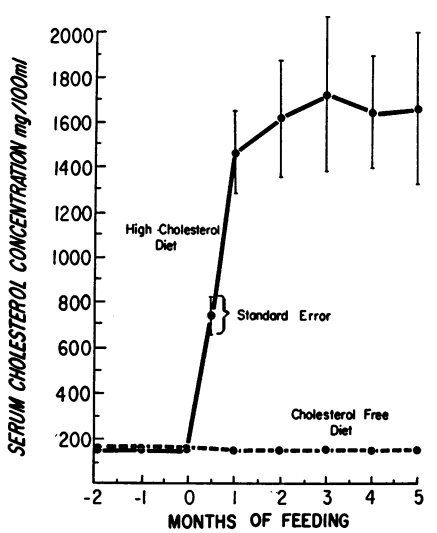

FIGURE 4 Comparative effects of diet 1 (broken line) and diet 2 (solid line) on serum cholesterol levels. Data are from five animals on each diet.

Inherent to the formation of cholesterol gallstones were other pathogenic changes in the composition of bile. It has been established that cholesterol is held in solution in bile in combination with bile salts and phospholipids (16, 17). A decrease in the concentration of these substances relative to cholesterol results in cholesterol precipitation and stone formation (18). In this study, as the result of the egg yolk diet, both hepatic and gallbladder bile changed to a composition which favored cholesterol precipitation. The molar ratios of phospholipid and bile acid to cholesterol became less and the cholesterol molar percentages of the total increased.

The changes in the individual bile acids may have implications with regard to the etiology of cholelithiasis. The major bile acid of the control animals was cholic acid whereas chenodeoxycholic predominated in animals with gallstones. A similar shift has been reported in humans with gallstones (19, 20). It has been speculated that the cholesterol-containing micelles with dihydroxycholanic bile acids are larger and less stable than the micelles with trihydroxy bile acids (21). In contrast, some other in vitro investigations suggest that the type of bile acid makes little difference in the solubilization of cholesterol (22, 23). Complete information regarding the size, shape, and stability of the various mixed micelles existing in bile is still lacking (24). The combination of increased cholesterol concentration relative to the solubilizing components and the predominance of the possibly less stable dihydroxy micelles might have provided the milieu which caused gallstone formation in the prairie dog. The high concentrations of dihydroxycholanic acids in both human and animal gallstones support this hypothesis (25, 26). On the other hand, the formation of micelles containing predominately chenodeoxycholic in animals with gallstones may be the product of a compensatory mechanism for "cholesterol overloading" in the liver. When presented with an increased cholesterol load, cholesterol catabolism may favor the pathway to chenodeoxycholic acid, perhaps because it can solubilize more cholesterol. Further work is needed for ultimate clarification of this problem.

The incorporation of dietary cholesterol-4-^{14}C into gallstones supported to some extent the basic hypothesis of the primary causative role of dietary cholesterol. Since all prairie dogs fed the egg yolk diet and examined 2–3 months later had already formed gallstones, it is probable that labeling of stones in long term animals continued to occur after the stones had initially formed. The cholesterol specific activity of most gallstones was similar to that of bile, serum, and liver despite the fact that the isotope had been added to the diet 2 months after the beginning of the egg yolk diet. The cholesterol of gallstones presumably present at 2 months equilibrated with bile cholesterol over the next 4 months. This evidence indicated that the cholesterol of gallstones might not be sequestrated as a static pool and further suggested the possibility of gallstone regression if the bile composition were altered in the direction of greater cholesterol solubility. Gallstones placed in "solubilizing" bile have been known to dissolve (27).

An alternative explanation might be that the stones are continually being formed and either lost or dissolved. The similarity of specific activities might merely reflect the incorporation of newly precipitated isotopic cholesterol. In either event, the origin of the cholesterol in the stones (even if recent) was largely from cholesterol derived from the diet.

For comparative purposes, it may be useful to list many of the characteristics of an "ideal" animal model in the experimental cholesterol cholelithiasis. Such criteria include: (*a*) the chemical composition of the bile should approximate that of human bile; (*b*) the composition of the experimental gallstones should be similar to that of gallstones in humans; (*c*) the method of induction should be reliable and rapid; (*d*) there should be no toxicity other than that of the biliary tract; (*e*) sufficient quantities of bile, stones, and tissue should be present for analysis. Previous animal models have been compared as to these criteria by Freston and Bouchier (28).

No animal completely satisfies all these criteria, including the prairie dog. However, the prairie dog may more completely fulfill these criteria than other models previously reported. With regard to the first criterion, that bile composition be similar to human bile, most animal models fail. For example, the bile of many animals contains bile acids not present in human bile (the guinea pig, the pig, and the rabbit) (2, 26, 29). The prairie dog has the same four major bile acids as does human bile. In addition, the relative concentration of cholesterol, bile acids, and phospholipids in the prairie dog is similar to those reported in normal and abnormal human bile (19). For example, the phospholipid/cholesterol ratios in normal humans and in patients with cholesterol gallstones were 7.3 and 5.0, respectively, in gallbladder bile. In control and egg yolk-fed prairie dogs these ratios were 6.0 and 3.4, respectively. From the same human study, the bile acid/cholesterol ratio was 19.6 in normals and 11.8 in patients with cholesterol gallstones. This corresponds to 26.4 in control prairie dogs and 14.7 in animals with stones. The prairie dog (as well as the hamster, mouse, squirrel monkey, and guinea pig) meets the second criterion of forming cholesterol gallstones. Concerning the third point, the prairie dog invariably formed gall-

stones when fed the egg yolk-rich diet. Stones were induced as early as 2 months of feeding in this study. Toxic effects did occur in the prairie dog fed egg yolk. Fatty liver and hypercholesterolemia always accompanied the induction of gallstones. In addition, there was a high incidence of atherosclerosis in the animals fed egg yolk. The final criterion for the ideal animal model of having sufficient material for analysis was met in this small animal by the use of chromatographic methods.

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REFERENCES

1. Dam, H. 1969. Nutritional aspects of gallstone formation with particular reference to alimentary production of gallstones in laboratory animals. *World Rev. Nutr. Diet.* **11**: 199.
2. Schoenfield, L. J., and J. Sjövall. 1966. Bile acids and cholesterol in guinea pigs with induced gallstones. *Amer. J. Physiol.* **211**: 1069.
3. Tepperman, J., F. T. Caldwell, and H. M. Tepperman. 1964. Induction of gallstones in mice by feeding a cholesterol-cholic acid containing diet. *Amer. J. Physiol.* **206**: 628.
4. Dam, H., and F. Christensen. 1952. Alimentary production of gallstones in hamsters. *Acta Pathol. Microbiol. Scand.* **30**: 236.
5. Patton, O. H., K. Plotmen, G. E. Cox, and C. B. Taylor. 1961. Biliary cholesterol deposits in ground squirrels and prairie dogs. *Fed. Proc.* **22**: 248.
6. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226**: 497.
7. Abel, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195**: 357.
8. Juniper, K., and E. N. Burson. 1957. Biliary tract studies. II. The significance of biliary crystals. *Gastroenterology.* **32**: 175.
9. Iwata, T., and K. Yamasaki. 1964. Enzymatic determination and TLC of bile acids in blood. *J. Biochem. (Tokyo).* **56**: 424.
10. Rhodes, J., D. E. Barnado, S. F. Phillips, R. A. Rovelstad, and A. F. Hofmann. 1969. Increased reflux of bile into the stomach in patient with gastric ulcer. *Gastroenterology.* **57**: 241.
11. Ivey, K. J., L. DenBesten, and J. A. Clifton. 1970. Effects of bile salts on ionic movement across the human gastric mucosa. *Gastroenterology.* **59**: 683.
12. Dryer, R. L., A. R. Tammes, and J. I. Routh. 1957. The determination of phosphorous and phosphatase with N-phenyl-*p*-phenylenediamine. *J. Biol. Chem.* **225**: 177.
13. Hill, B. H. 1967. Principles of medical statistics. Oxford University Press, Inc., New York. 143.
14. Osuga, T., and O. W. Portman. 1971. Experimental formation of gallstones in the squirrel monkey. *Proc. Soc. Exp. Biol. Med.* **135**: 722.
15. Okey, R. 1952. Gallstone formation and intake of B vitamins in cholesterol fed guinea pigs. *Proc. Soc. Exp. Biol. Med.* **51**: 349.
16. Isaksson, B. 1954. On the lipids and bile acids in normal and pathological bile. Doctoral thesis. Lund, Sweden.
17. Juniper, K. 1965. Physicochemical characteristics of bile and their relation to gallstone formation. *Amer. J. Med.* **39**: 98.
18. Admirand, W. H., and D. M. Small. 1968. The physicochemical basis of cholesterol gallstone formation in men. *J. Clin. Invest.* **47**: 1043.
19. Burnett, W. 1965. Pathogenesis of gallstones. *Biliary Syst. Symp. NATO Advan. Study Inst. 1963.* 601.
20. Tera, H. 1960. Stratification of human gallbladder bile in vivo. *Acta. Chir. Scand. Suppl.* **256**: 1.
21. Tamesue, N., and K. Juniper. 1967. Concentrations of bile salts at the critical micellar concentrations of human gallbladder bile. *Gastroenterology.* **52**: 473.
22. Neiderhiser, D., and H. P. Roth. 1968. Cholesterol solubilization by solutions of bile salts and bile salts plus lecithin. *Proc. Soc. Exp. Biol. Med.* **128**: 221.
23. Bourges, M., D. M. Small, and D. G. Dervichian. 1967. Biophysics of lipid associations. III. The quaternary systems lecithin-bile salt-cholesterol-water. *Biochim. Biophys. Acta.* **144**: 189.
24. Carey, M., and D. Small. 1970. The characteristics of mixed micellar solutions with particular reference to bile. *Amer. J. Med.* **49**: 590.
25. Nakayama, F. 1968. Quantitative microanalysis of gallstones. *J. Lab. Clin. Med.* **72**: 602.
26. Nakayama, F. 1969. Composition of gallstone and bile: species difference. *J. Lab. Clin. Med.* **73**: 623.
27. Naunyn, B. 1896. A treatise on cholelithiasis. New Sydenham Society, London.
28. Freston, J. W., and I. A. D. Bouchier. 1968. Experimental cholelithiasis. *Gut.* **9**: 2.
29. Hofmann, A. F., and E. H. Mosbach. 1964. Identification of allodeoxycholic acid as the major component of gallstones induced in rabbits by 5α-cholestan-3β-ol. *J. Biol. Chem.* **239**: 2813.