Arch. Jap. Chir. 38 (4), 565~580 Juli 1969

Cholesterol Gallstone Formation in Hamsters Correlated with Histological Findings in Livers and Gallbladders

by

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Received for Publication Apr. 30, 1969

INTRODUCTION

A vast amount of observation and experiment on gallistone formation have been reported over the past one hundred year. It is generally stated that three factors are involved : infection, stasis, and metabolic disturbances. Since NAUNYN's classical theory[1], many investigators[2)3)] have suggested that inflammation is the main initiating factor. There is evidence that infection plays a part in the formation of some stones. MAKI[4)] showed experimentally that calcium bilirubinate stones are induced by bile stasis and infection of the bile duct. Pure cholesterol gallstones, however, are most likely to be due to metabolic disturbances. DAM and CHRISTENSEN[5)] succeeded in producing almost pure cholesterol gallstones regularly in hamsters by feeding them a generally deficient diet. In spite of these and many other studies, the mechanism of occurrence of these stones is still not clear. HIKASA et al.[6)7)] demonstrated that cholesterol gallstone formation might be due to a deficiency or metabolic disturbances of essential fatty acids (EFA) and showed that in animals or patients with cholesterol gallstones, cholesterol synthesis in the liver was markedly increased, and some aspects of their metabolism resembled that seen in diabetes mellitus.

In the present study, cholesterol gallstones were produced in golden hamsters by feeding them various diets and the histological appearence of their livers and galibladders was studied. The factors favoring the formation of cholesterol gallstones are discussed.

MATERIALS AND METHODS

Golden hamsters of both sexes, weighing 30 to 60 g, were divided into groups and maintained in individual cages. All animals were given food and water ad libitum, and were weighed weekly. The composition of the diets used in this experiment is shown in Table 1. Control animals were fed commercial rat chow diet, CE-2 (Central Laboratories of Experimental Animals, Tokyo). The same commercial diet was also fed prior to the start of the trial. After different periods on these diets, the animals were sacrificed by decapitation, and the livers and gallbladders were removed. Gallstones, if pesent, were classified and specimens of liver and gallbladder were fixed in 10% formalin solution except for those stained for glycogen. All the specimens were embedded in paraffin, cut into 5 μ sections and stained with hematoxylin-eosin or Perl's Prussian blue for iron. In all

Table 1 Composition of diets

Groups / Composition	A-1 A-2	B-1 B-2	C	D	E	F	G	H	I (CE-2)
Glucose	73.5	63.5	63.5						
α-Starch						73.5	58.5	53.5	
β-Starch				73.5	53.5				
Butter*		10.0			20.0		15.0	20.0	
Sesame oil**			10.0						
Crude casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	24.0
Salt mixture***	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	6.0
Vitamins****	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
Choline chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
Fats									3.5
Wheat and Corn									ca. 60.0
Cellulose									4.5

* Butter : canned by Snow Brand Milk Products Co.
** Sesame oil : by Takemoto Purified Oil Co.
*** Salt Mixture

NaCl	4.6
NaH_2PO_4	9.2
K_2HPO_4	25.3
$CaH_4(PO_4)_2H_2O$	14.3
Ca lactate	36.9
$MgSO_4$	7.0
KI	2.6
	100.0 g

**** Vitamine Mixture (in 100g diet)

Vitamin	Our Laboratory	CE-2
B_1	1mg	0.7mg
B_2	1.5mg	1.0mg
B_6	1.0mg	0.4mg
B_{12}	1γ	2γ
Folic acid	0.15mg	0.02mg
Niacin	10.0mg	8.0mg
C	37.5mg	—
Ca pantothenate	2.5mg	3.0mg
E	1.0mg	1.5mg
A	2500I.U.	1000I.U.
D	200I.U.	200I.U.
Choline	(500mg)	140mg

Table 2 Histological Findings in the Livers and Gallbladders. Each findings was graded from ± to ⅲ.

Histological changes / Feeding period		
Distortion of liver cords		
Damage of liver cells	Degeneration	
	Necrosis	
	Vacuolization of cell nuclei	
Sinusoid	Hypertrophy of kupffer cells	
	Dilatation	
Inflammatory response	in parenchyma	
	in portal area	
Biliary thrombosis		
Proliferation of biliary duct		
Fibrosis		
Hemosiderin deposits		
Fatty metamorphosis	Diffuse	
	Central	
	Peripheral	
Gallbladder	Thickening of wall	
	Inflammatory response	
	Gallstone	

instances, frozen sections were made for Sudan III stains, and some sections were stained with Van Gieson's stain. Best's carmine stains for glycogen were applied to selected sections. The histological changes in the livers and gallbladders are specified in Table 2. Each finding was graded from ± to ###. A diagnosis of fatty liver was made when more than 10% of the cells were fat-laden as described by LEEVY[30] and by KOSAKA[20].

RESULTS

I RELATION OF DIET TO INCIDENCE OF GALLSTONES (Table 3)

In the animals fed a fat free diet with glucose as the carbohydrate, a very high incidence of cholesterol gallstones (85%) was observed. In the animals fed a glucose diet with 10 % butter, the incidence of cholesterol gallstones was even higher (100 %). In these two groups, the animals gained weight for the first two weeks, then gradually lost weight and some of them developed symptoms of acrodynia in the later stages. Their feces were frequently acholic. When straw was spread on the floor of the cages of some of the animals in these two groups to keep them warm when the room heater failed, it was unexpectedly noted that there was a marked decrease in the incidence of cholesterol gallstones. Furthermore, their weight gain remained relatively normal and their general condition was good. The substitution of sesame oil for butter as the source of fat in the glucose diet significantly lowered the incidence of cholesterol gallstones. The general condition of this group was fair, and weight loss was prevented. In the animals fed a fat free diet with β-starch as the carbohydrate, cholesterol gallstones did not form. In the

Table 3 Incidence of gallstones in hamsters fed various diets

Groups	Type of diet	No. of animals	Average initial body weight (g)	Average weight gain (g)	Feeding period	No. of survivors	Incidence of cholesterol gallstones among survivors
A-1	Glucose fat free	20	53	−7.8	2～7 weeks	12	84 %
A-2	Glucose fat free*	10	48.7	12.5	2～6 weeks	10	50
B-1	Glucose 10% butter	20	47.5	4.8	2～5 weeks	14	100
B-2	Glucose 10% butter*	18	45.9	20	5～45 days	12	25
C	Glucose 10% sesame oil	15	52.7	12.7	5～45 days	15	20
D	β-starch fat free	10	46.3	7.5	2～6 weeks	10	0
E	β-starch 20% butter	25	39.8	48.5	10～55 days	18	6
F	α-starch fat free	10	51.2	39.3	2～5 weeks	10	0
G	α-starch 15% butter	15	48.6	45.8	3～6 weeks	10	30
H	α-starch 20% butter	35	45.8	35	5 weeks	26	54
I	Rat chow diet	20	52.9	33	1～7 weeks	20	0

* In groups A-2 and B-2, straw was spread on the floor of each cage.

group fed a β-starch diet with 20% butter, a very low incidence of cholesterol gallstones was noted. In the β-starch diet groups all animals gained weight almost normally. In the animals fed an α-starch diet without fat, no gallstone was found. However, in the animals fed an α-strach diet with butter, there was a relatively high incidence of cholesterol gallstones. In the former the general condition remained very good, while in the latter the animals lost weight after 4 to 5 weeks.

II HISTOLOGICAL FINDINGS

1) Incidence of Vacuolated Nuclei in Liver Sections

In ordinary paraffin sections of the liver, several peculiar vacuolated and empty-appearing nuclei were distributed throughout the liver lobule, never as many as ++ to +++, but enough to be graded + or ++ in most sections (Fig. 7). The correlation between the diet and the incidence of vacuolated nuclei in liver sections is summarized in Table 4. Animals fed the α-starch diet without fat had no vacuolated nuclei in their liver cells. However, in fhe animals fed the α-starch diet with much butter fat, there was a relatively high incidence of vacuolated nuclei.

On the whole, the liver cells of animals on the lithogenic diets tended to contain a few vacuolated nuclei, as shown in Table 4.

Table 4 Relationship between diet and incidence of vacuolated nuclei in liver sections

Type of diet	Incidence of vacuolated nuclei (%)	Coincidence of vacuolated nuclei and cholesterol gallstones (%)
α-starch 20% butter diet	43	19
α-starch 15% butter diet	20	10
α-starch fat free diet	0	0
β-starch 20% butter diet	17	0
β-starch fat free diet	0	0
Glucose 10% butter diet (B-2)	17	17
Glucose 10% butter diet (B-1)	14	14
Glucose fat free diet (A-1)	17	8
Glucose fat free diet (A-2)	0	0
Glucose 10% sesame oil diet	0	0
Rat chow diet (CE-2)	10	0

2) Relationship between Cholesterol Gallstone Formation and Inflammatory Response in Liver Sections (Figure 1 and 6)

Sections were divided into 3 groups as specified in Figure 1 : no change, slight, and moderate to marked.

Of the 15 animals with cholesterol gallstones in the glucoses fat free diet group, none showed any inflammatory change in the liver parenchyma, and only two showed a slight inflammatory response in the portal area. Of the 17 animals with cholesterol gallstones in the glucose butter diet group, three showed a slight inflammatory response in the parenchyma and in the others there was no change in any of the fields. Of the three animals with cholesterol gallstones in the glucose sesame oil diet group, none showed any inflammatory response in the parenchyma, and in one there was a slight change in the portal areas.

Of the 17 animals with cholesterol gallstones in the α-starch butter diet groups, 14 showed almost no inflammatory change, and three showed a slight inflammatory response in the parenchyma. In the portal areas, 13 showed no change, three a slight, and one a moderate inflammatory response. Of the 20 animals without gallstones in the control rat chow diet group, 17 showed no change and three a slight change in the parenchyma. In the portal areas, 13 showed no change, six showed slight, and one moderate changes. Of the 84 remaining hamsters without gallstones, some showed a slight to marked degree of inflammatory response in the liver sections, which may have been related to factors other than diet.

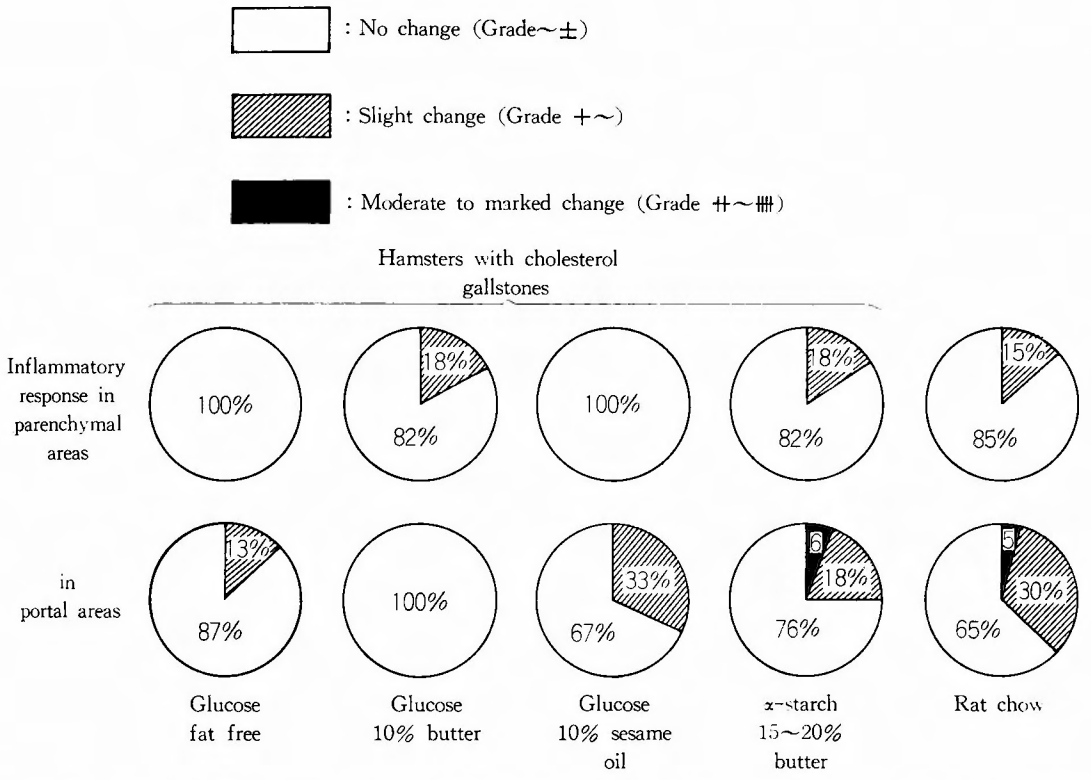

Fig. 1 Relationship between cholesterol gallstone formation and inflammatory response in liver sections

3) Relationship between Cholesterol Gallstone Formation and Hepatic Fibrosis

None of the 15 animals with cholesterol gallstones in the glucose fat free diet group had any hepatic fibrosis. Of the 17 animals with cholesterol gallstones in the glucose butter diet group, only one had any hepatic fibrosis and it was very slight. Of the three animals with cholesterol gallstones in the glucose sesame oil diet group, two had no fibrosis and one had slight fibrosis. Of the 17 animals with cholesterol gallstones in the α-starch butter diet groups, 14 had no hepatic fibrosis and three had slight fibrosis. Of the 20 animals without cholesterol gallstones in the control rat chow diet group, 14 had no hepatic fibrosis and six had slight fibrosis. Some of the 84 others without cholesterol gallstones showed moderate to marked hepatic fibrosis, which closely paralleled the degree of inflammatory response and was presumably due to factors other than diet.

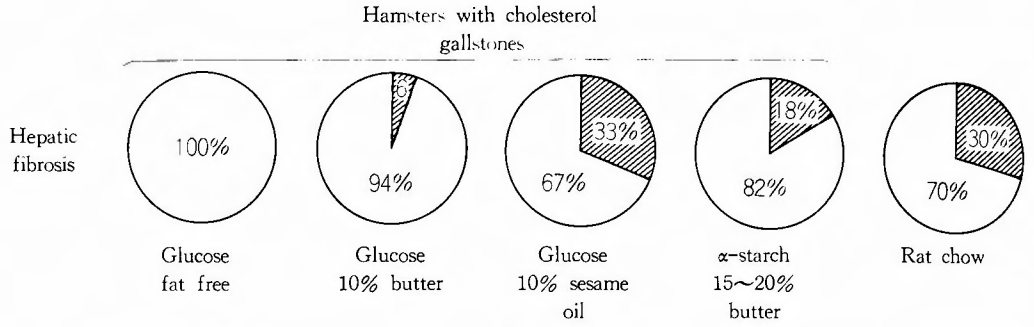

Fig. 2 Relationship between cholesterol gallstone formation and hepatic fibrosis

4) Relationship between Cholesterol Gallstone Formation and Fatty Metamorphosis in Liver Sections

Of the 15 animals with cholesterol gallstones in the glucose fat free diet group, five showed no change, six showed slight, three moderate, and one marked fatty metamorphosis (fatty liver). Of the 17 animals with cholesterol gallstones in the glucose butter diet group, there was no change in six, slight fatty metamorphosis in five, moderate fatty metamorphosis in four and marked fatty metamorphosis (fatty liver) in two. Of the three animals with cholesterol gallstones in the glucose sesame oil diet group, two showed no change and one had slight fatty metamorphosis. Of the 17 animals with cholesterol gallstones in the α-starch butter diet group, nine had moderate, four marked (fatty liver), and two slight fatty metamorphosis, while two showed no change. Of the 20 animals without gallstones in the control rat chow diet group, eight showed no change, 11 had slight, and one moderate fatty metamorphosis. Of the 84 others without cholesterol gallstones, most of the non-lithogenic diet-fed animals had little or no fatty metamorphosis in their livers.

The nine animals with fatty livers included five on the α-starch butter diet, two on the glucose butter diet, one on the β-starch butter diet, and one on the glucose fat free diet. Two of these had slight hepatic fibrosis with cholesterol gallstones and two had vacuolated nuclei with cholesterol gallstones. All four were in the α-starch butter diet group. On the whole, fatty metamorphosis was more common in the animals fed lithogenic diets than in the others (Figure 3 and 4). In most of the fatty livers, fat droplets were distributed in the peripheral portions of the liver lobules (Table 5 and Figure 8).

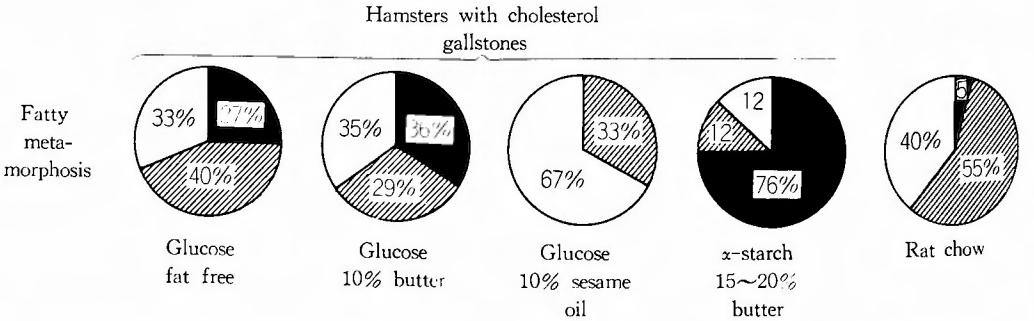

Fig. 3 Relationship between cholesterol gallstone formation and fatty metamorphosis in liver sections

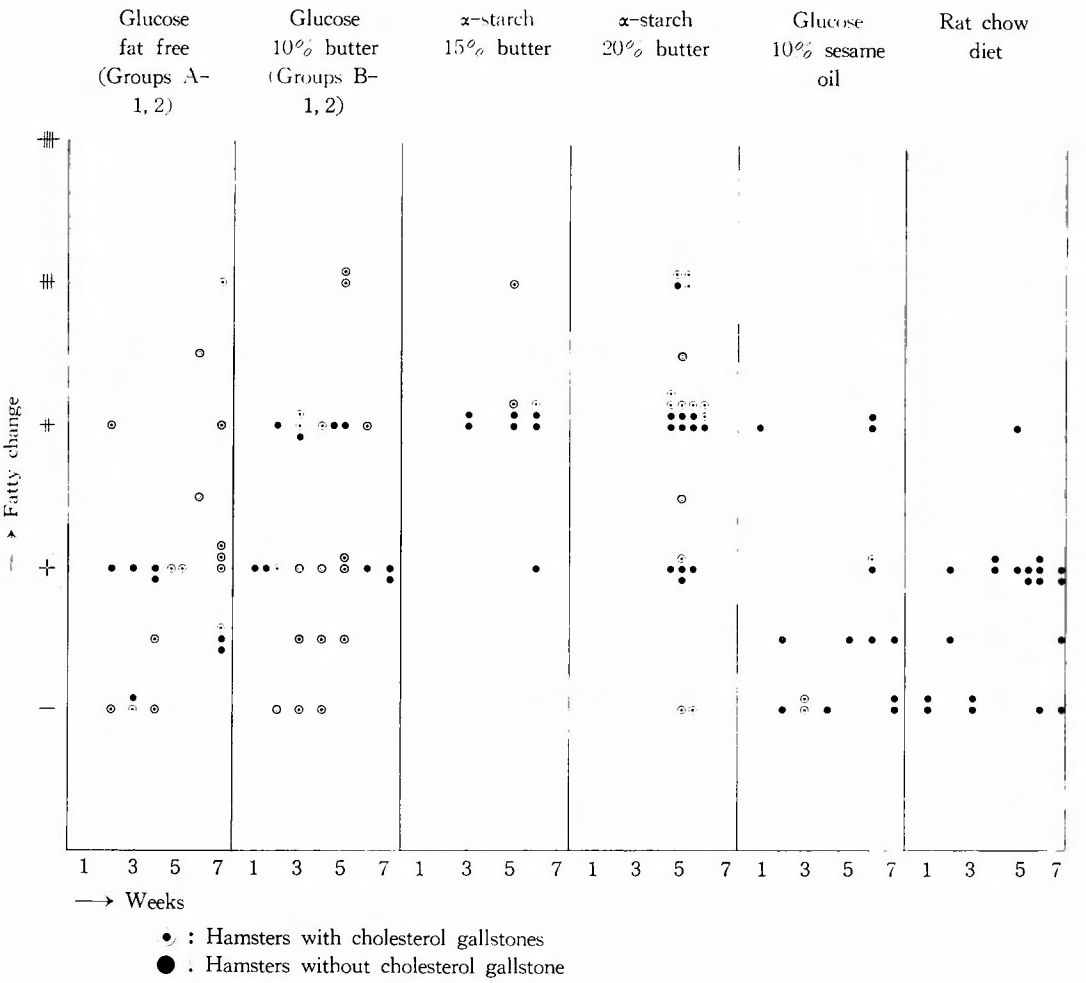

Fig. 4 Cholesterol gallstone formation and fatty change

Table 5 Classification of fatty liver type

Type of diet \ Type of fatty liver	Peripheral type	Central type	Diffuse type
Glucose fat free	1		
Glucose 10% butter		1	1
β-starch 20% butter	1		
α-starch 15% butter	1		
α-starch 20% butter	4		

5) Hemosiderin Deposits in Liver Sections

Hemosiderin deposits were found in the liver sections of one of the glucose butter diet group and one of the glucose sesame oil group. In the former, significant amounts of iron containing pigments were noted chiefly in the liver cells, but no fibrotic or fatty changes were present. In the latter, the liver sections showed a moderate inflammatory response and a small amount of iron pigment was present chiefly in the connective tissues.

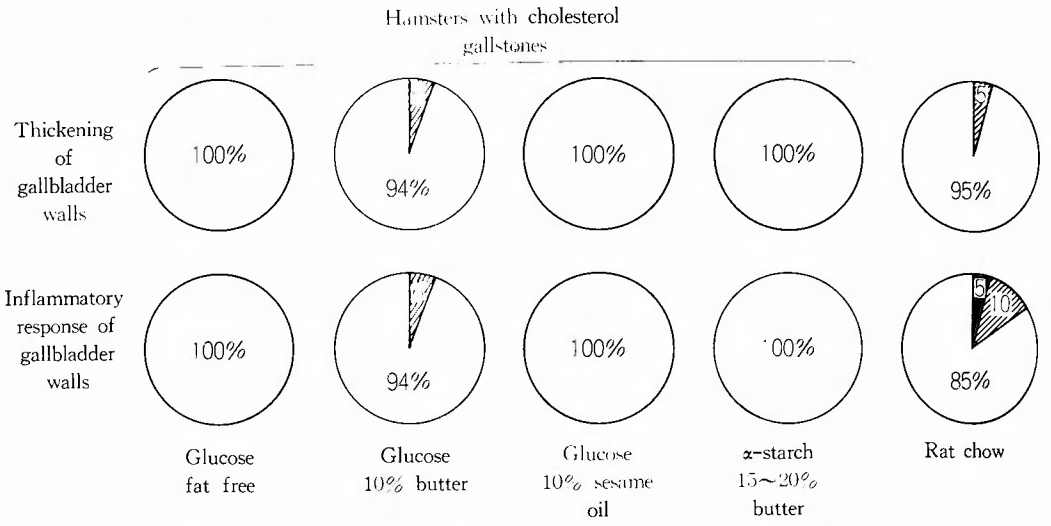

Fig. 5 Relationship between cholesterol gallstone formation and histological findings in gallbladders

The glucose butter diet fed animal had cholesterol gallstones.

6) Relationship between Cholesterol Gallstone Formation and Microscopic Appearance of Gallbladders

Gallbladder sections obtained from the animals fed the various diets were examined after one to eight weeks. The 53 animals with pure cholesterol gallstones showed almost no inflammatory response or thickening of the walls (Figure 5 and 6). Of the 84 without cholesterol gallstones, some showed a slight and some a moderate to marked inflammatory

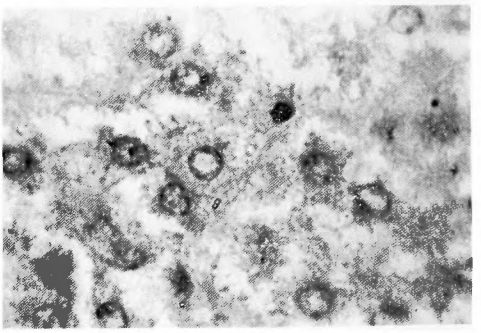

Fig. 7 Vacuolated nuclei in the liver cells of a hamster fed an α-starch butter fat diet for five weeks and had cholesterol gallstones.
(H & E ×400)

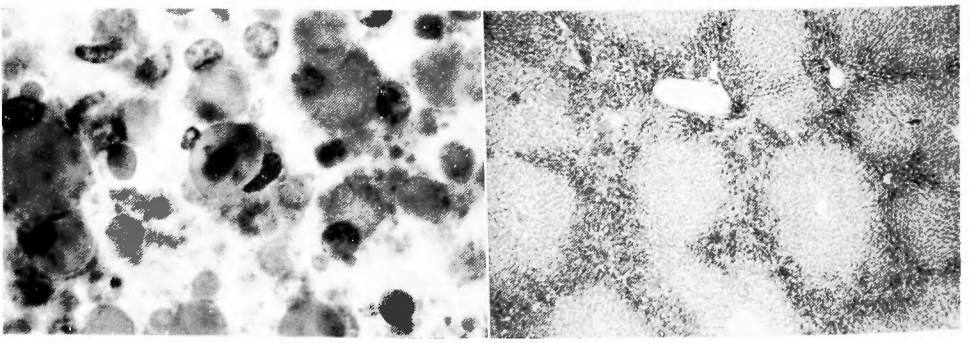

Fig. 8 The liver of a hamster fed the α-starch fat diet for five weeks and had cholesterol gallstones. There was moderate fatty change (grade 3). The fatty areas appear black.
(Sudan III, ×40 and ×400)

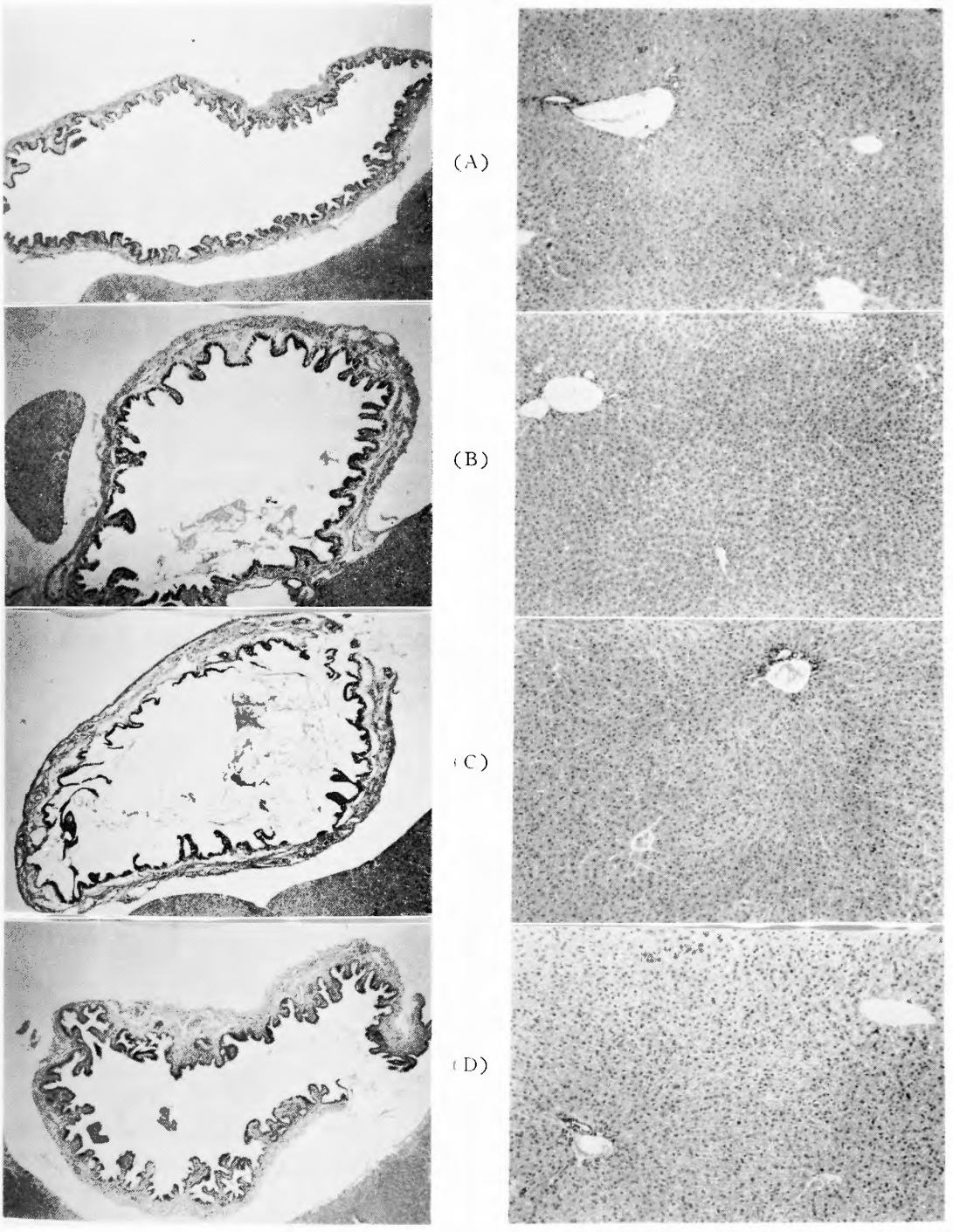

Fig. 6 Microscopic appearance of the livers and gallbladders of hamsters with cholesterol gallstones: (A) showed the liver and gallbladder of a hamster fed a glucose butter-fat diet for two weeks, (B) for three weeks, (C) for four weeks, and (D) for five weeks. None showed any inflammatory response.
H & E ; ×40 (gallbladders) and ×100 (livers)

response. However, this inflammation was probably not associated with diet but due to incidental cholecystitis or other factors.

DISCUSSION

SINCE DAM and CHRISTENSEN[5] first described pure cholesterol gallstones produced experimentally in golden hamsters, many authors[8)-16)] have studied these stones in the same animals. SHIODA[13] showed that unsaturated fatty acids, cod liver oil, and sesame oil (especially the latter, which containes much FFA) protected hamsters against cholesterol gallstones. TANIMURA[14] found that the higher the ratio of FFA to saturated fatty acids plus oleic acid, the lower was the incidence of cholesterol gallstones. HASHIMOTO[13] first observed that there was a high incidence of cholesterol gallstones in hamsters fed excessive amounts of lower saturated fatty acids with mashed potatoes, a source of easily digestible polysaccharide. In the present study, cholesterol gallstone formation was correlated with the histological findings in the liver and gallbladder.

The liver sections of only a few of the animals with cholesterol gallstones showed a slight or moderate inflammatory response in the parenchymal or portal areas. Most of the gallbladder sections also were free of inflammatory response or thickening of the walls, while some of the liver and gallbladder sections of animals without gallstones, showed a slight to marked degree of inflammatory response. Although many animals on the glucose fat free diet or glucose butter fat diet had cholesterol gallstones not only in their gallbladders but also in their cystic or bile ducts and had acholic feces, most of their livers and gallbladders showed no inflammatory response. It is generally said that a gallstone in the bile duct predisposes to inflammation in the liver due to ascending infection. If the animals with gallstones in the present study had been maintained on their diets for a longer period, they might have developed secondary inflammation of the liver and gallbladder.

The degree of hypertrophy of the Kupffer cells closely paralleled the degree of inflammatory response frequently found in the liver sections of the animals without gallstones. These lesions are most probably due to factors other than gallstone formation or type of diet.

The liver sections of only a few of the animals with cholesterol gallstones showed slight hepatic fibrosis, and there was no particular correlation between diet or cholesterol gallstones and hepatic fibrosis. In the liver sections of some animals without gallstones, however, marked hepatic fibrosis closely paralleled the degree of inflammatory response. As mentioned above, this hepatic fibrosis with inflammation was probably due to factors other than diet.

LARGE[3] stated that minimal damage to the gallbladder wall, which is most likely due to infection, is the initiating mechanism in gallstone formation. MIYAKE[2] reported that the decreased cholesterol holding capacity of the bile, induced by infection with Cl. Welchii etc., might be the initiating factor in cholesterol gallstone formation. The present study produced no evidence to support the concept that infection is primarily associated with the development of cholesterol gallstones.

It is of interest that abnormal nuclei with slight or moderate vacuolization were frequently noted in the liver cells. In the animals fed α-starch with much butter fat, vacuoli-

zation of the liver cell nuclei was most frequent. In the glucose fat free and the glucose butter fat diet groups, most of the animals with vacuolated nuclei in their liver cells had cholesterol gallstones. As is shown in Table 4, these vacuolated nuclei occurred more frequently in the lithogenic diet groups than in the other groups.

EHRLICH (1883)[17] first noted large glycogen-filled vacuoles in the liver cells in cases of diabetes mellitus. Of course, glycogen is not found normally in the nuclei of the liver cells at any time. Its presence there is not limited to diabetes mellitus, but is also frequently noted in other diseases. Since the detailed report by ZIMMERMAN[18], however, several authors[19)~22)] have demonstrated an appreciably greater incidence of vacuolated nuclei in the liver cells of diabetics than in those of non-diabetic patients. So it seems most likely that these findings are relatively peculiar to the liver cells of diabetics. CHIPPS and DUFF[23], and BOGOCH et al[19]. suggested that glycogen infiltration of the liver cell nuclei is closely associated with the amount of glycogen in the cytoplasm. However, BONDY et al[24]. and TAKEUCHI et al[21]. found no particular correlation. ZIMMERMAN[18] suggested that these lesion were associated with hyperglycemia, but this was not supported by the findings of CHIPPS and DUFF. CHIPPS and DUFF[23], and KOSAKA et al[20]. reported that the vacuolated nuclei in liver cells are distributed chiefly in the periphery of the lobules in the portal areas. In the present study, however, they were distributed in the central or mid-zonal portions of the lobules. SHELDON[25] demonstrated by electron micrographs that the particles of glycogen within the nucleus were uniformly smaller than those in the cytoplasm, so it may be that small amounts of cytoplasmic glycogen can pass through the nuclear pores and be deposited in the nucleus. It remains unknown, however, why the small particles develop. It is interesting to note that several authors[21)26)] have pointed out the possibility of making a diagnosis of prediabetes on the basis of histological findings such as vacuolated nuclei and fatty change in the liver. It is generally accepted not only experimentally but also clinically that a high fat high protein diet predisposes to diabetes mellitus. Thus, in endeavoring to correlate the presence of vacuoles in the hepatic nuclei with the lithogenic diet, it was hypothesized that the animals with cholesterol gallstones might be in a probable-diabetic state, but this is only a suggestion, since those vacuolated nuclei were never numerous in the present study.

Another point of interest is the relationship between fatty metamorphosis in the liver sections and the occurrence of cholesterol gallstones. In the α-starch butter diet group the incience of fatty metamorphosis was higher than in the α-starch fat free diet group or the control chow diet group. On the other hand, in both the glucose butter fat and the glucose fat free diet groups, there was a slight increase in the incidence of fatty metamorphosis. Among the 157 animals, nine had fatty livers. Six of these, or 67% had choleaterol gallstones. On the whole, fatty metamorphosis was more common in the lithogenic diet groups, especially in the butter fat diet groups, than in the others as shown in Figure 4.

KOTAKE and INADA[27] discsovered that tryptophan given together with high fat induced a diabetic state because of excessive xanthurenic acid, a metabolite of tryptophan, in animals with vit. B_6-deficiency. It is interesting that both tryptophan and fat are present in our daily diets, especially the more expensive ones. Thus, it is interesting to speculate that the animals in the glucose fat free diet, the glucose butter fat diet, and the α-starch butter

fat diet groups, might be in a prediabetic state with markedly increased cholesterol synthesis in the liver.

Since MEADE[28] first noted in 1748 the occurrence of fatty metamorphosis of the liver in patients with diabetes mellitus, many authors[18)~22)29)~33)] have confirmed this observation by autopsies or biopsies. Fatty livers have been found more frequently in diabetics than in non-diabetics. CAZAL[34] stated that fatty metamorphosis in the central portions of the liver lobules, vacuolization of liver cell nuclei, and increased intracellular glycogen in the liver were characteristic of pancreatic diabetes, while fatty metamorphosis in the periphery of the liver lobules was characteristic of pituitary diabetes. KALK[33] reported that most of the fatty metamorphosis in livers of diabetics was peripheral in type. However, TAKEUCHI et al[21]. noted it was mostly central. KOSAKA et al[20]. and KUZUYA et al[22]. reported that it was usually central or diffuse but occasionally peripheral. In the present study, most of the fatty metamorphosis was peripheral in type, as shown in Table 5. Several authors[21)26)33)] have suggested that it is possible to diagnose or predict probable diabetes by the histological findings of fatty metamorphosis and vacuolization of nuclei in the liver cells.

Hemosiderin deposits were found in the livers sections of animals on the glucose butter diet and the glucose sesame oil diet. Much research[35)~38)] on hemosiderin deposition, especially hemochromatosis, or bronze diabetes, had been reported, but the exact cause remains a mystery. GUBLER et al[36]. reported that iron was significantly increated in pyridoxine deficient rats. This observation is very interesting because the occurrence of cholesterol gallstones may be closely associated with pyridoxine deficiency.

An accidental interesting finding was that when straw was spread on the floors of the cages of animals on glucose diets, there was a marked decrease in the incidence of cholesterol gallstones. TANIMURA[14] observed that in hamsters fed the sucrose fat free diet to which indigestible ingredients such as carboxyl methyl cellulose (CMC) or agar-agar were added, the development of gallstones was prevented or markedly delayed. This phenomenon may be attributed to the increased synthesis of pyridoxine by intestinal flora the proliferation of which is enhanced by the feeding of straw, CMC, or agar-agar[39].

WITTEN and HOLMAN[40] reported that the conversion of linoleate to arachidonate was stimulated by pyridoxal phosphate. HIRANO[41], YOSHINAGA[42] and MARUYAMA[43] demonstrated that cholesterol esterified with EFA might be the direct precursor of bile acid and that pyridoxal phosphate might be involved in the production of bile acid from cholesterol. NAKAHARA et al.[44] reported that rats fed a high fat diet revealed a decrease in the pyridoxal phosphate content and pyridoxine phosphate oxidase activity.

The incidence of cholesterol gallstones in association with a probable-diabetic state is of some interest. The positive correlation between gallstones and diabetes mellitus has been demonstrated statically by various authors[45)~50)]. Most authors have reported on the increased incidence of cholelithiasis in diabetics. On the other hand, HOTTA and CHAIKOFF[51] observed a definit increase in cholesterol formation in the diabetic liver. BRAUNSTEINER et al.[52] reported the average fasting blood glucose was higher in patients with gallstones than in normal persons and many of these patients were in a probable-diabetic state. In the present study, it is probable that the animals fed the lithognic diets were in a probable-diabetic state. HIKASA et al.[7] showed by glucose tolerance tests that not only patients

with cholesterol gallstones but also animals fed various kinds of lithogenic diets might be in a probable-diabetic state. Furthermore, they observed that in animals fed a high fat diet with much lower saturated fatty acids, there was a higher incidence of alloxan diabetes than in animals fed other fats. From the results of the present study the following conclusions were drawn on the occurrence of cholesterol gallstones.

1) Glucose-fed animals can absorb glucose almost completely and rapidly in the upper intestine, so they may develop pyridoxine deficiency as a result of bacterial imbalance.

2) In animals fed the α-starch butter fat diet, inactivation of pyridoxine phosphate oxidase may occur due to excessive fat intake. Thus, the deficiency of pyridoxine due to the occurrence of bacterial imbalance or the deficiency of pyridoxal phosphate due to decreased activity of pyridoxine phosphate oxidase results in not only a probable-diabetic state but also a disturbance of the conversion of cholesterol to bile acid. On the basis of these changes, there may be a marked increase in the biosyntheis of cholesterol in the liver which may then precipitate the formation of cholesterol gallstones.

SUMMARY AND CONCLUSION

Cholesterol gallstones were produced in golden hamsters by feeding them various kinds of diets for 5—55 days. The histological findings in the livers and the gallbladders were correlated with the incidence of gallstones.

1) In animals fed a high butter fat diet with glucose or α-starch as carbohydrate, there was an appreciably increased incidence of cholesterol gallstones, especially in those on the glucose butter fat diets, all of which developed cholesterol gallstones.

2) An accidental interesting finding was that when straw was spread on the floor of the cages of animals fed the the glucose fat free and the glucose butter fat diets, there was a marked decrease in the incidence of cholesterol gallstones.

3) There was no particular correlation between the kind of diet or the presence of cholesterol gallstones and hepatic fibrosis.

4) Most of the liver sections of the animals with cholesterol gallstones showed almost no inflammatory response in the parenchymal or portal areas, and the gallbladders of these animals also showed almost no evidence of inflammatory response or thickening of the walls.

5) There was no evidence to support the concept that infection is primarily associated with the occurrence of cholesterol gallstones.

6) Slight or moderate vacuolization of the liver cell nuclei was often noted. These vacuolated nuclei occurred more often in the lithogenic diet-fed animals than in the others.

7) Fatty metamorphosis of the liver was more common in the animals fed lithogenic diets, especially butter fat diets, than in the others and fatty livers were frequent. In most of these fatty livers, fat droplets were distributed in the peripheral portions of the liver lobules.

8) Hemosiderin deposits in the liver were noted in one animal on the glucose butter diet and in one on the glucose sesame oil diet ; the former animal had cholesterol gallstones.

ACKNOWLEDGEMENT

I wish to express appreciation to Dr. Y. Yamori, Dept. of Pathology, for his advice regarding various problems in histopathology, and to Dr. H. TANIMURA, the 2nd Surgical Division, Faculty of Surgery, for his suggestions.

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和文抄録

ハムスターに於けるコレステロール系結石の実験的作成とその肝臓及び胆嚢の組織学的研究に就いて

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胆石の成因についての説はNaunynの炎症説以来幾多の変遷を経て今日に至つたが，胆汁鬱滞，炎症，新陳代謝障害等の条件下で，胆汁の膠質化学的性状の変化が如何にして起こるか，なお不明な点が多い．

我々の教室では日笠等の胆石症，特にコレステロール系結石に関する一連の研究により，その成因として次の様な結論に達した．即ち日常に於ける美食は必然的に高脂質，高蛋白食の過量摂取と，それに伴なう糖質摂取の相対的減少をもたらし，遂にはProbab'e diabetic stateを招来せしめ，その結果肝臓に於けるコレステロール合成能の異常な増大を来たす一方，他方ではピリドキサール燐酸の欠乏は肝臓に於けるコレステロールから胆汁酸への分解過程の障害をもたらし，このことより胆汁中のコレステロール量／総胆汁酸量の比は一層増大することとなり，又，胆汁酸組成の質的変化と相まつて，遂には胆嚢中に於いてコレステロールの折出，沈澱，結石形成へと発展せしめることが判明した．

高脂質，高蛋白食は糖尿病を誘発し易いと言う事実は，高脂質摂取によるピリドキサール燐酸の体内欠乏（中原）により，トリプトフアン代謝の異常を来たし，そのためキサントウレン酸の生成能を異常に亢進せしめて，その結果インシュリン活性の低下を招来し，遂には膵β-細胞の緩徐なる崩壊のため糖尿病となる事が証明されている（古武）．

本実験ではハムスターを用いて種々の飼料投与によりコレステロール系結石を作成せしめ，得られた全例の肝臓及び胆嚢について種々の染色法による組織標本を比較検討し次の如き結果を得た．

1)　ブドウ糖群に於いても又α-型澱粉群に於いてもバターを添加すると高率にコレステロール系結石の発生が認められた．特に前者に於いては全例に認められた．

2)　偶然観察されたことであるが，ブドウ糖群及びブドウ糖バター群に於いて飼育期間中，籠に藁を敷いたところ，それらの群のみ，藁を敷かない群に比べ，コレステロール系結石の発生は著明に抑制され，しかも全身状態及び発育も良好であつた．これは藁が多量のセルローズの供給源として利用され，Dysbacteriaの発生が防止されたものと思われる．

3)　胆石形成群に於いて特に肝線維症の発生は認められなかつた．又，飼料の種類と肝線維症の発生との相関関係も全く認められなかつた．

4)　コレステロール系結石を有するハムスターの肝臓組織の炎症反応は大多数に於いて全く認められなかつた．同様に胆嚢の肥厚や炎症反応は殆んどの例で認められなかつた．

5)　3),4)の結果よりコレステロール系結石の成因として，まず第一に感染による炎症が先行すると言う事実は全く認められない．

6)　肝細胞の核空胞化が時々認められたが，その発生の有無を各群毎に調べて見ると，コレステロール系結石を生じやすい飼料群，特にα-型澱粉バター群に於いて最も発生頻度が高かつた．

7)　脂質変性の発生頻度及びその程度もコレステロール系結石を生じやすい飼料群，特にそのバター添加群に於いて最も多く見られた．又，脂肝はα-型澱粉バター群に最も多く認められた．

8)　6),7)の所見は糖尿病時の肝臓組織の所見と一見類似性を有する．

9)　肝臓に於けるヘモジデリン沈着がブドウ糖バター群及びブドウ糖ゴマ油群中より各一例づつ認められ，前者に於いてはコレステロール系結石も認められた．