plasma Epo concentration stays at a constant and low level, feeding new erythroblasts into the erythroid compartment at a fairly constant rate, a locally acting agent(s) may be responsible for the fine quantitative regulation of erythrocytic production. The so-called hematopoietic-inductive-microenvironment (HIM)[17], possibly involving cell to cell interactions as well as intercellular communications mediated by short-distance-acting metabolites, may well be part of the local regulating factors proposed here.

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Effect of pectin and cellulose on formation and regression of gallstones in hamsters[1]

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Summary. Male Syrian hamsters were fed a lithogenic diet containing 7% cellulose or 4.2% pectin. After 50 days, pectin was 76% and cellulose 64% less lithogenic than the control diet. Hamsters fed the control diet for 50 days were maintained on that diet for another 50 days or fed diets containing cellulose or pectin. There was a 52% increase in gallstone incidence in hamsters continued on the control diet and a 9% increase in those on cellulose. Pectin promoted regression of gallstones (by 52%).

Bile is a finely balanced solution of cholesterol, bile salts and phospholipids. Oversecretion of cholesterol or undersecretion of bile salts will result in bile which is supersaturated with cholesterol and gallstones will result. Epidemiologically, gallstones are considered to be a disease of highly developed societies[3,4]. One dietary difference between developed, gallstone-prone societies and underdeveloped, gallstone-free societies lies in the fact that the former ingest a diet higher in refined carbohydrate and poorer in fiber than the latter. It has also been shown[5,6] that wheat bran will increase the size of bile salt pools in man, a condition which would inhibit gallstone formation. No direct studies on the effects of dietary fiber on gallstone dissolution in man have been carried out.

Dam and Christensen[7] demonstrated in 1952 that gallstones could be produced in hamsters by feeding a diet containing 74.3% sucrose, 20% casein, 5% salt mix, 0.5% vitamin mix and 0.2% choline. Although many variations of this diet have been suggested[8,9], it remains the standard means of inducing gallstones in hamsters. Heaton[3] has summarized the dietary means of causing gallstones in experimental animals and all of them involve feeding large amounts of refined carbohydrate and no fiber. Gallstone formation can be inhibited by replacing the sucrose by whole wheat, rolled oats or sorghum[10] and by replacing or diluting the casein with soy protein[11,12]. Bergman and van der Linden[13] and Rotstein et al.[14] have shown that gallstone formation can be partially inhibited by addition of 5% pectin to the diet and almost completely inhibited by adding 5% lignin. We thought it of interest to compare the effects of pectin and cellulose on gallstone formation, liver cholesterol levels and biliary lipids. Pectin has been shown to reduce cholesterol levels in rats[15] and might be expected to affect the level of biliary cholesterol. We also studied the effects of pectin and cellulose on pre-established gallstones on the premise that readjustment of bile composition might lead to dissolution of existing gallstones.

Materials and methods. Male Syrian hamsters weighing 60±5 g were maintained 5 to a cage in an air-conditioned (21 °C) animal room and subjected to a 12-h light/dark cycle. Control hamsters were fed the lithogenic diet of Dam and Christensen[7]: 74.3% sucrose, 20% casein, 5% salt mix, 0.5% vitamin mix and 0.2% choline chloride. The composition of the salt and vitamin mixes has been detailed elsewhere[11].

Hamsters on the cellulose-containing diet received 67.3% sucrose and 7% cellulose, the other components of the diet being unchanged. The pectin group was fed 7% of a pectin formulation[16] containing 60% apple pectin (NF XV, methoxyl content 9.5%), 37% sucrose and 3% excipient. Thus they received 69.9% sucrose and 4.2% pectin. A group of 100 hamsters was fed the control lithogenic diet for 50 days and groups of 25 hamsters each were fed the cellulose and pectin diets for the same period. At 50 days all the hamsters on the test diets and one-quarter of those on the control diet were killed and their gallbladders examined for stones. The remaining hamsters were either maintained on the control diet or fed one of the fiber-containing diets for 50 days more. Livers were removed and aliquots assayed for total and free cholesterol content[17]. Bile was aspirated

and pooled to give 0.03 ml of material (3-6 hamsters). The bile was diluted with 5 times its volume of 0.9% saline and analyzed for cholesterol[18], phospholipid[19] and the bile acid[20] content.

Results and discussion. Table 1 summarizes the findings in hamsters maintained on the diets for 50 days. Pectin and cellulose inhibited lithogenesis by 76 and 64%, respectively. Biliary phospholipid was considerably higher in the control hamsters. Hamsters fed the fiber-free diet exhibit significantly higher liver cholesterol levels.

The results of the 'regression' experiment (100-day feeding) are summarized in table 2. When the lithogenic diet was fed for another 50 days, incidence of gallstones rose from 33 to 50%. Cellulose prevented an increase in gallstone incidence, whereas pectin caused significant regression. At 50 or 100 days pectin was significantly less lithogenic than the control diet ($p < 0.03$ and < 0.025, respectively). Hamsters maintained on the control diet for 100 days exhibited significantly more gallstones ($p < 0.05$) and those placed on the pectin diet for 50 days, after 50 days on the control diet, showed significantly fewer gallstones ($p < 0.05$). Statistical analyses were done by the χ^2 test. Biliary cholesterol was highest in the control hamsters. Biliary cholesterol in the hamsters fed pectin was 31% lower than in those fed cellulose. Biliary phospholipid levels were significantly elevated in the control hamsters and levels in the pectin-fed group were 40% lower than in that fed cellulose. Biliary cholesterol in the control hamsters was about 20% higher than that seen in the other 2 groups.

It is not possible to compare the 2 experiments directly. In the first study (table 1) we compared the effects of pectin and cellulose on gallstone formation in hamsters fed the various diets for 50 days. The second experiment (table 2) was 100 days in duration and was designed to test the effects of pectin or cellulose on progression of gallstone formation or regression of stones existing when the fiber regimen was instituted (after 50 days). Hamsters fed the lithogenic diet for 100 days exhibit doubled biliary cholesterol, a 50% increase in biliary phospholipid and a 36% increase in biliary bile acid content. There is a 52% increase in gallstone incidence. When fiber is added to the diet after 50 days of exposure to the lithogenic diet, gallstone formation appears to cease (cellulose) or gallstones are dissolved (pectin).

Liver cholesterol levels in all 3 groups were significantly higher in animals fed for 100 days than in those fed for 50 days. Liver cholesterol levels in hamsters fed the cellulose-containing diet were significantly higher than those observed in the other 2 groups. Liver cholesterol levels in the pectin-fed hamsters were significantly lower than in the controls. Cellulose has been found to be more hepatocholesterolic than a fiber-free control diet in rats fed 0.5-1.0% cholesterol[21-23]. The level of esterified cholesterol in the livers of the hamsters fed pectin was significantly lower than in those fed cellulose or the fiber-free diet. The data suggest that cellulose exerts a slight anti-lithogenic effect but has no influence on pre-established gallstones. Pectin significantly reduces gallstone formation and leads to dissolution of pre-established stones as well.

Table 1. Influence of cellulose (7%) or pectin (4.2%) on lithogenesis in hamsters[a] (diets fed 50 days)

	Regimen		
	Control	Pectin	Cellulose
Survival (%)	96	100	100
Gallstones			
Incidence	8/24[c]	2/25[c]	3/25
%	33	8	12
Biliary lipids (mM/dl)[b]			
Cholesterol	0.93 ± 0.16	1.03 ± 0.37	0.98 ± 0.14
Phospholipid	4.24 ± 1.31	2.18 ± 0.42	2.41 ± 0.29
Bile acid	18.77 ± 0.86	18.68 ± 2.61	20.23 ± 2.79
Liver cholesterol (mg/100 g)	248 ± 12[c,d]	155 ± 7[c]	151 ± 5[d]

[a] Diets described in text; [b] Pools used, control 2, pectin 4, cellulose 5; [c,d] Significant difference ($p \leq 0.05$).

Table 2. Influence of cellulose (7%) and pectin (4.2%) on pre-established gallstones in hamsters[a] (control diet fed 50 days; control, pectin or cellulose diet fed for 50 days more)

	Regimen		
	Control	Pectin	Cellulose
Survival (%)	88	76	100
Gallstones			
Incidence	11/22[c]	3/19[c]	9/25
%	50	16	36
Biliary lipids (mM/dl)[b]			
Cholesterol	2.10 ± 0.67	0.46 ± 0.33	0.67 ± 0.12
Phospholipid	6.89 ± 1.40[c,d]	1.55 ± 0.21[c]	2.58 ± 0.61[d]
Bile acid	25.55 ± 6.88	20.02	20.98 ± 8.08
Liver cholesterol (mg/100 g)			
Total	322 ± 15[c,d]	247 ± 10[c,e]	367 ± 16[d,e]
% Ester	21.1 ± 1.7[c]	9.5 ± 1.4[c,d]	26.2 ± 2.5[c]

[a] Diets described in text; [b] Three pods per group. 2 pods from pectin group lost to bile acid analysis; [c,d,e] Significant difference ($p \leq 0.05$).

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