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Effects of β-Sitosterol and Ferric Chloride On Accumulation of Cholesterol in Mouse Liver.* (21989)

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Since repeated experimental evidence has associated high cholesterol levels with atherosclerosis, various attempts have been made to lower blood and organ cholesterol content. The effects of several substances on cholesterol absorption have been studied using various experimental animals. Soybean sterols, consisting mainly of β-sitosterol, have been shown to decrease the accumulation of dietary cholesterol(1-5). Studies on dihydrocholesterol treated animals have shown that this substance is also effective in reducing cholesterol absorption(6-8). Moreover, it has been found that, in the cockerel, precipitation of bile acids by ferric chloride results in a reduction of blood cholesterol(9,10). On the other hand, in the case of the mouse and rat, evidence has been produced that soybean sterols fail to lower organ and blood cholesterol(11,12).

The object of this experiment is to study: first, the effectiveness of β-sitosterol in prevention of liver cholesterol accumulation; and second, the effectiveness of ferric chloride in a mammalian species.

Methods. Exp. I. Dietary β-sitosterol. Webster strain female albino mice weighing 15 to 20 g were used. Throughout the experiment all mice were fed *ad lib.* a basal fat-free, cholesterol-free diet, described in a previous paper(7), supplemented by adequate amounts of vit. A and D. After a 2-week preliminary period on this basal diet, the mice were divided into 7 groups. The controls, Group I, were continued on the basal diet. For the other 6 groups the basal diet was supplemented with 1% cholesterol. Cholic acid was also added to the various diets as follows: Groups II and V received 0.25%; Groups III and VI, 0.5%; and Groups IV and VII, 1%. In addition, diets for Groups V, VI, and VII contained

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TABLE I. Effect of β-Sitosterol and Cholic Acid on Total Mouse Liver Cholesterol.

Group and diet	No. of mice	Additions to basal fat-free diet (%)			Total cholesterol in liver (mg)	
		Cholesterol	Cholic acid	β-sitosterol	Per organ†	Per g body wt‡
I	12	—	—	—	10.6 ± 2.1*	.53 ± .11*
II	10	1	.25	—	36.1 ± 6.3	1.88 ± .28
III	9	1	.5	—	47.1 ± 9.1	2.40 ± .52
IV	10	1	1.0	—	48.0 ±13.3	2.20 ± .60
V	10	1	.25	2.5	30.0 ± 6.1	1.37 ± .23
VI	11	1	.5	"	24.9 ± 6.4	1.27 ± .33
VII	9	1	1.0	"	34.2 ± 6.8	2.04 ± .41

* Stand. dev.

† Significance of the difference in results of the following groups: (a) II & V, $P \cong 0.04$; (b) III & VI, $P < 0.01$; (c) IV & VII, $P < 0.01$.

‡ Significance of the difference in results of the following groups: (a) II & V, $P < 0.01$; (b) III & VI, $P < 0.01$; (c) IV & VII, $P \cong 0.5$.

2.5% β-sitosterol. All diets were fed *ad lib.* for a 3-week experimental period, during which food consumption for the 7 groups varied from 2.9 g to 3.4 g per mouse per day. After the experimental feeding, the mice were sacrificed, and the livers removed, weighed, and quick frozen. Total liver cholesterol was assayed by a procedure similar to the one used in our previous study(7).

Exp. II. Dietary ferric chloride. National Institute of Health strain female albino mice weighing 15 to 20 g were used. As in Exp. I, all animals were fed *ad lib.* a basal fat-free, cholesterol-free diet for an initial 2-week period. The mice were then divided into 5 groups. For all groups, the basal diet was supplemented with 1% cholesterol and 0.5% cholic acid. In addition iron, as ferric chloride, was added to the diets as follows: Group I received no iron; Group II received 0.05%; Group III, 0.5%; Group IV, 1%; and Group V, 2%. After being fed *ad lib.* on these experimental diets for 3 weeks, the mice were sacrificed, and the livers quickly removed, weighed and frozen. Total cholesterol determinations were made as in Exp. I.

Results. Table I presents the data on the effect of β-sitosterol and cholic acid on the accumulation of mouse liver cholesterol. As can be seen, increasing the dietary cholic acid increased the concentration of liver cholesterol up to a maximum value attained in Group III. Comparison of Groups II and V, III and VI, and IV and VII shows the effectiveness of β-sitosterol in preventing liver cholesterol build-up. It is to be noted that β-sitosterol is most effective in the presence of 0.5% cholic acid. However, in none of these groups did total cholesterol concentration approach the control values.

Results of simultaneous feeding of cholesterol and ferric chloride are shown in Table II. The data indicate that ferric chloride failed to decrease cholesterol accumulation. In fact, increasing the concentration of ferric chloride seemed to increase the cholesterol level. Even though comparison of Groups I and II may suggest a slight decrease, the significance of this difference is doubtful in view of the large standard deviation.

Discussion. From the data in Table I, it can be seen that β-sitosterol is effective in decreasing liver cholesterol accumulation under certain conditions. However, comparison of the present results with those obtained in an identical experiment(7) with dihydrocholesterol, shows that, in the presence of equal amounts of cholic acid, dihydrocholesterol is more effective than β-sitosterol.

Data on the ferric chloride experiment indicate that this compound does not reduce dietary cholesterol accumulation in liver. This finding does not coincide with that of Siperstein *et al.*(9), who postulated that ferric chloride binds the bile acids in the intestinal tract, thereby suppressing cholesterol absorption. However, it must be noted that two major differences exist between their experiment and the present one: First, Siperstein *et al.* experimented on cockerels, while in our experiment, mice were used; and second, they studied blood cholesterol levels, while our data

TABLE II. Effect of Ferric Chloride on Total Mouse Liver Cholesterol.

Group and diet	No. of mice	Additions to basal fat-free diet (%)			Total cholesterol in liver (mg)	
		Cholesterol	Cholic acid	Iron as $FeCl_3$	Per organ	Per g body wt
I	7	1	.5	—	56.3 ± 11.7*	2.81 ± .65*
II	8	1	.5	.05	43.9 ± 10.4	2.22 ± .38
III	7	1	.5	.5	53.5 ± 11.5	2.54 ± .46
IV	7	1	.5	1.0	68.6 ± 17.4	3.36 ± .65
V	7	1	.5	2.0	67.6 ± 7.6	3.76 ± .50

* Stand. dev.

are measurements of liver cholesterol concentrations. The differences found in these experiments may be explained by the hypothesis that ferric ions have a toxic action on liver cells, which prevents the normal distribution of cholesterol between the blood stream and the liver. In agreement with Siperstein *et al.*, we found that ferric chloride, fed in concentrations of 2% or more, proved to be highly toxic and, in some cases, fatal after a few days. The possible physiological after-effects of even small amounts of this compound cannot be overlooked.

Summary. 1. Increasing the cholic acid content of a diet rich in cholesterol, significantly increased total liver cholesterol. 2. The addition of β-sitosterol to diets containing cholic acid and cholesterol, decreased total liver cholesterol. 3. Addition of ferric chloride, in varying amounts, to diets rich in cholesterol and cholic acid, resulted in practically no change in total cholesterol content of mouse liver.

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