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Influence of Dietary Bile Salts on Blood Cholesterol Levels. (20669)

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Mueller(1) carried out the first critical experiments on the role of bile in absorption of cholesterol. When a cholesterol or cholesterol oleate emulsion was fed to dogs in which the bile was diverted from the intestinal tract there was no significant rise in the total cholesterol of the lymph as compared to normal dogs. Schoenheimer(2) also observed that the serum cholesterol of rabbits was increased more after feeding cholesterol with desoxycholic acid than after cholesterol alone. Kim and Ivy(3) found that the serum cholesterol of rats was significantly raised when cholic acid was added to a diet containing cholesterol and oleic acid. When bile acid was added to a cholesterol-free diet no significant increase in blood cholesterol was observed. Recently Siperstein *et al.*,(4) reported that when labeled cholesterol was fed to bile fistula rats none of the labeled compound could be recovered in the lymph. It was suggested that bile was obligatory for the absorption of cholesterol. In addition to bile, pancreatic secretions and fat have been shown to be intimately related to cholesterol absorption(5,6). There is good evidence that esterification of cholesterol is an important step in its absorption and that this process takes place in the intestinal wall(7,8) under the influence of pancreatic cholesterol esterase. In connection with studies (9-11) on this enzyme, it has been demonstrated that it will synthesize and hydrolyze the long-chain fatty acid esters of cholesterol only in the presence of bile salts. In the preceding paper(11) the effect of different bile salts on cholesterol esterase activity *in vitro* was demonstrated. The type and amount of bile salt present in substrate mixture is important in the activity of the enzyme. Bile salts having 3, 2, and 1 hydroxyl groups respectively showed a decreasing order of activity while dehydrocholate with no hydroxyl groups was found to be inactive in promoting either synthesis or hydrolysis. Also the conjugated bile salts, taurocholate and glycocholate showed differences in activity when compared to cholate.

The purpose of the present investigation was to determine, using the blood cholesterol level as an indicator of cholesterol absorption, if different bile salts had different effects on cholesterol absorption comparable to the relations between bile salt structure and cholesterol esterase activity *in vitro* demonstrated in the preceding paper(11). If comparable effects could be demonstrated this would be added evidence that the requirement of bile salts for the absorption of cholesterol is due to its being necessary for cholesterol esterase activity and that cholesterol esterase is directly involved in cholesterol absorption.

Methods and materials. Forty-five rats from our stock colony and raised on Purina pellet chow were used. The animals were housed in separate cages and fed the experimental diets and water *ad libitum.* Following a control period on pellet chow during which the blood cholesterol fractions were determined, the rats were divided into two series.

TABLE I. Feeding Regimen and Dietary Intake of Cholesterol, Bile Salts, and Olive Oil.

Series	No. rats	Group	Days on diet	Bile salt	Dietary intake/day/rat* Cholesterol, mg	Bile salt, mg	Olive oil, cc
I	5	E	21	0	200–250	0	2.5–3.
	5	F	21	Cholate	"	100–150	"
	5	G	21	Taurocholate	"	"	"
	5	H	21	Desoxycholate	"	"	"
	5	I	21	Dehydrocholate	"	"	"
II	10	K	7	Dehydrocholate	150–200	75–100	2.0–2.5
	8	K	7	Taurocholate	"	"	"
	8	K	5	Dehydrocholate	"	"	"
	10	L	21	0	"	0	"

* Range of wt of rats in Series I was 225-300 g and in Series II, 125-175 g.

In Series I four groups were fed a basal diet containing 2% cholesterol plus 1% of different bile salts. One group served as a control and received the basal diet with no bile salts. In Series II, two groups (K and L) were fed a basal diet containing 2% cholesterol, with group K receiving in addition 1% of bile salts in the diet successively for 7, 7, and 5 day periods. The basal diet had the following composition: 20% casein, 23% starch, 23% glucose, 25% olive oil, 5% Hubbel-Mendel-Wakeman salt mixture, 2% ruffex, 2% cholesterol, and adequate amounts of the crystalline vitamins. The bile salts were added to the basal diet at the expense of the starch and glucose. Table I shows the feeding regimen and the approximate dietary intake of cholesterol, bile salts and olive oil per rat in Series I and II. The rats were bled from the tail. The blood samples were collected directly into .2 cc pipettes and placed in 1:1 acetone alcohol for cholesterol analysis. Free and total cholesterol were determined on the whole blood by the method of Schoenheimer-Sperry(12) with modifications(13).

Results. Series I. The results are shown in Fig. 1. The total blood cholesterol increased significantly in all groups after one week on the diets. This increase was predominantly in the ester fraction. In comparing the effect of the different bile salts on the blood cholesterol during this initial period, it can be seen that the blood chcolesterol in the group receiving sodium dehydrocholate did not rise significantly above that of the control group not receiving bile salts. The groups receiving cholate, taurocholate, and desoxycholate showed increases in total blood cholesterol of 41.1, 36.9 and 16.5% respectively when compared to the group not receiving bile salts in their diet. During the remainder of the feeding period, the total, the ester and to a lesser extent the free cholesterol fractions continued to rise in the blood of the cholate, taurocholate, and desoxycholate groups. The blood cholesterol of the dehydrocholate and control groups remained about the same. At the end of the feeding period (21 days) the total blood cholesterol levels in the bile salt groups ranged from 280 to 320 mg% and the ester fraction from 170 to 210 mg%. The dehydrocholate and control groups had total cholesterol levels of 145 and 137 mg% and ester levels of 58.6 and 67.6 mg% respectively. It is also interesting to note that the ratio of ester to total cholesterol rose from an average pre-diet value of 26 to 63% in the cholate, taurocholate, and desoxycholate groups. During the same period, the dehydrocholate and control groups showed an increase from 26 to 47% in that ratio. After 21 days the animals were taken off the experimental diets and returned to pellet chow. Within 5 days the blood cholesterol in all groups dropped very markedly, approaching the pre-diet level.

Series II. The effects of different bile salts demonstrated in Series I, especially after 7 days, exhibited a pattern in general agreement with the results obtained in the study on the *in vitro* effect of bile salts in cholesterol esterase activity. It was especially significant that dehydrocholate did not have any effects on the blood cholesterol. In Series I the bile salts were fed to different animals.

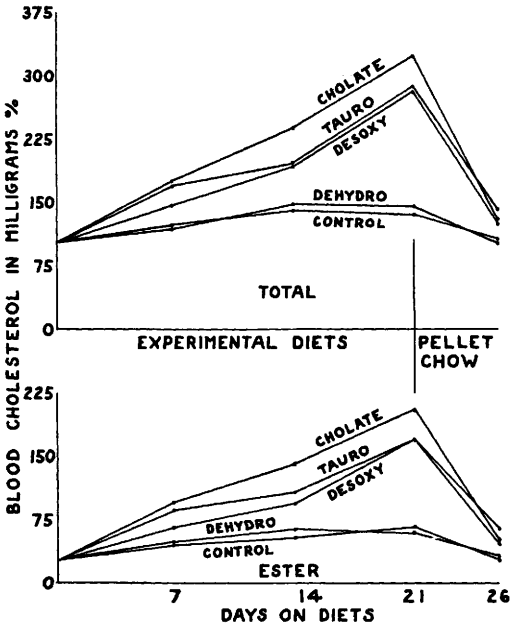

FIG. 1. Total and ester blood cholesterol levels of rats fed different bile salts. Each point represents avg of 5 animals.

It was of further interest to determine if similar differences between different bile salts could be demonstrated in the same animal. The experiment was also carried out to determine if the animals would respond to changes in the bile salt composition of the diet thereby providing evidence that the changes in the blood cholesterol observed in Series I were readily reversible and not due to a pathologic effect of the bile salts. The results are shown in Fig. 2. After 7 days both control and bile salt groups showed significant increases in total and ester blood cholesterol. However, both groups showed the same degree of elevation. This confirms the findings in the previous experiment, namely, that dehydrocholate is ineffective in raising the blood cholesterol level above that of the control group. The bile salt group was then switched to a diet containing sodium taurocholate. After 7 days on this diet, the total blood cholesterol level increased markedly from 131 to 300 mg%. During the same period, the total blood cholesterol of the control group increased from 127 to 160 mg%. It is also interesting to note that the blood cholesterol increased much more rapidly than in the previous group fed sodium taurocholate. Since the animals were younger in the present group, age may be an important factor.

In the final feeding period the bile salt group was returned from the diet containing sodium taurocholate to the diet containing sodium dehydrocholate. After 5 days on that diet both the total and ester blood cholesterol decreased sharply. The total cholesterol decreased from 300 to 215 mg%. The cholesterol fractions in the control group remained at about the same levels. The decline in bood cholesterol observed when the bile salt group was returned to the dehydrocholate diet demonstrates that the effect of bile salts is reversible and that it is unlikely that permanent damage to the animals had taken place. It is also of interest to mention that the feeding periods were of such a short duration that toxic effects of the bile salts were not indicated by examination of the livers. No difference in weight or gross appearance of the livers of the bile salt and control groups was noted in the animals in Series I and II.

Discussion. Previous work relating to the influence of bile on the absorption of cholesterol has been carried out on animals in

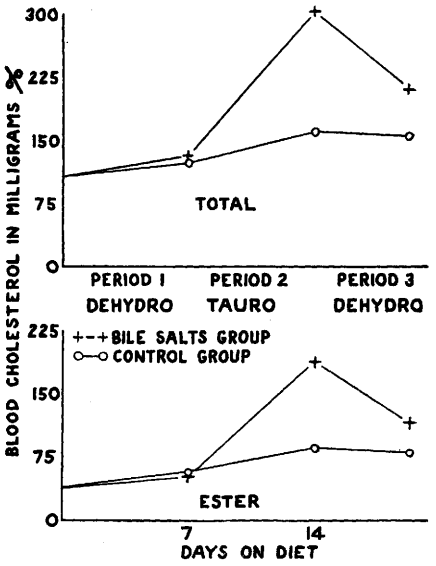

FIG. 2. Total and ester blood cholesterol levels of rats fed sodium dehydrocholate, taurocholate, and dehydrocholate successively. Each point represents avg of 8-10 animals.

which bile has been diverted from the intestinal tract(1,4). However, in studying this process, 4 factors must be considered, namely, pancreatic cholesterol esterase, cholesterol, fat, and bile. It seems likely that each of these components must be present and in certain optimum proportions to each other for maximum absorption of cholesterol. Thus, if there is an excessive amount of cholesterol ingested, sufficient fat and bile must be present for complete absorption. In this series of experiments we have used the intact animal and supplied high levels of cholesterol and fat in the diet. There was a slight increase in the blood cholesterol level during the experimental periods in the animals on the control diet which, according to the above, could be due to the effect of the rats own bile salt supply on the absorption of the dietary cholesterol. In the animals receiving the bile salt diets the markedly higher blood cholesterol levels were due to the effect of the dietary bile salts on the absorption of the dietary cholesterol. This view, which is in accord with the available data, leads to the interesting possibility that the level of the blood cholesterol is controlled, in part, by the amount of bile salts excreted into the intestine.

The present findings indicate that the structure of a bile salt has an important relationship to its effect on the blood cholesterol. It is especially striking that the feeding of dehydrocholate had no effect on the blood cholesterol. This correlates with the finding in the previous paper(11) that dehydrocholic acid, which has no hydroxyl groups, has no effect on cholesterol esterase activity, *in vitro*. It provides further evidence that esterification of cholesterol is required for its absorption and that bile salts are essential for that esterification.

Summary. Different bile salts were fed to rats on high cholesterol-fat diets. The blood cholesterol increased very markedly with the feeding of sodium cholate and taurocholate, somewhat less with the feeding of desoxycholate and no increase was observed in those fed dehydrocholate. When sodium dehydrocholate, taurocholate, and dehydrocholate were fed successively to the same animal, taurocholate increased the blood cholesterol very markedly while dehydrocholate first had no effect and later lowered it. The results of this study agree in general with the findings of the preceding study on the *in vitro* effect of bile salts on cholesterol esterase activity, in that a comparable pattern of activity was observed in relation to the effect of bile salts on the blood cholesterol. It is suggested that the bile salts are necessary for cholesterol absorption because they are a necessary co-factor for cholesterol esterase activity.

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