crease in aldolase activity in primaquine-sensitive erythrocytes could not explain the increase in their average DPN^+ content (Reactions 3 and 4). However, glutathione reductase can utilize DPNH as coenzyme in hemolyzates of human erythrocytes, though less effectively than TPNH(17). Whether the increased activity of glutathione reductase in primaquine-sensitive erythrocytes accounts for the increase in average DPN^+ content, can not, however, be substantiated by our present data.

Summary. TPN^+ content of erythrocytes of primaquine-sensitive males is greater than that of erythrocytes of normal males. The increase in TPN^+ may be explained by two known factors: 1) decrease in glucose-6-phosphate dehydrogenase activity, and 2) increased glutathione reductase activity in primaquine-sensitive erythrocytes. The mean values of DPN^+ content of erythrocytes in the two populations studied were different, but the values showed considerable overlap. Our current studies concern the effect of drug administration on both oxidized and reduced coenzymes in normal and primaquine-sensitive erythrocytes from both males and females.

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Received July 1, 1958. P.S.E.B.M., 1958, v99.

Liver Cholesterol Mobilization in Mice as Effected by Dietary β-Sitosterol and Cholic Acid.* (24349)

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The value of β-sitosterol as agent for lowering tissue cholesterol has been the basis of numerous investigations. A number of these reports have indicated that β-sitosterol fed together with cholesterol effectively prevents accumulation of tissue cholesterol in several species(1,2,3). This effect has been shown by Hernandez *et al.*(2) to be due to blocking of cholesterol absorption in the intestinal lymph. Since β-sitosterol is effective in preventing cholesterol absorption, one could assume that this substance would prevent absorption of recirculating cholesterol, and thus speed up the mobilization of accumulated tissue cholesterol. In a previous study on mobilization rate of accumulated plasma cholesterol in the rabbit (4), β-sitosterol had a slight, initial accelerating effect. Alfin-Slater *et al.*(5) have reported that soybean sterols, composed mainly of β-sitosterol and stigmasterol, have little ef-

* This investigation was supported in part by research grant from Michigan Heart Assn.

fect in preventing blood cholesterol accumulation but have considerable effect in reducing cholesterol deposition in liver. Therefore, it was of interest to study the effect of β-sitosterol on rate of mobilization of accumulated liver cholesterol. Recent reports on cholesterol metabolism have shown that bile acids are important agents affecting cholesterol mobilization. Our studies(6) have demonstrated that cholic acid blocks liver cholesterol mobilization in mice. Since cholic acid apparently alters the pathway of cholesterol metabolism, it seemed worthwhile to investigate the effect of β-sitosterol on mobilization of accumulated liver cholesterol in animals fed cholic acid.

Procedure. 225 N.I.H. strain female albino mice, weighing 15-20 g, were placed on synthetic basal cholesterol free diet (CFD)(7) for 2 weeks, then divided into 2 groups. Group A. consisting of 50 mice, was continued on CFD, while Group B, 175 mice, was placed on CFD plus 0.5% cholic acid and 1.0% cholesterol. At the end of 3 weeks, 10 mice from Group A (normal) and 10 mice from Group B (elevated cholesterol) were sacrificed. The remaining 40 normal mice in Group A were continued on CFD. The mice with elevated cholesterol (Group B) were divided into 4 groups of 40-45 mice each, and placed on the following diets: Group C, the regression controls, were returned to the CFD; Group D received CFD plus 2.5% β-sitosterol† Group E, CFD plus 0.5% cholic acid; and Group F, CFD plus 0.5% cholic acid and 2.5% β-sitosterol. At weekly intervals, approximately one-fourth of the mice from each of the groups, *viz.* A, C, D, E, and F, were sacrificed. The livers were removed, drained of excess blood, weighed, and analyzed. *Sterol determination.* Digitonin precipitable Lieberman-Burchard positive sterols were determined by a method based on modified Sperry-Webb(8) and Abell *et al.*(9) methods. The present method has the advantage of reducing the number of aliquot transfers. The steps were as follows: whole livers, 0.8-2.2 g, were placed in 25 cc screw-cap vials, pre-calibrated to 20 cc volume. To these were added 5 cc of freshly prepared alcoholic-KOH (containing 50 mg KOH/cc). The livers were incubated at 37°C for approximately 16-18 hours, and then placed in a Dubnoff shaker for 2 hours at 80-90°C. At half-hour intervals the samples were removed and vigorously shaken for a few moments. On completion of the hydrolysis, the samples were allowed to cool, and made up to 20 cc with absolute ethanol. Five cc aliquots of these samples, 5 cc of water, and 10 cc of petroleum ether were mixed together in non-calibrated screw cap vials and vigorously shaken one minute, after which the aqueous and ether fractions were allowed to separate. Aliquots of the ether fraction were withdrawn and allowed to evaporate in 15 ml centrifuge tubes over a warm water bath. The residue was dissolved by the addition of 1:1 ethanol-acetone. Digitonin solution was added and cholesterol digitonide allowed to precipitate overnight. The washing and color determination were those described by Sperry-Webb(8).

Results. Table I summarizes cholesterol values for the normal (Group A) and elevated cholesterol (Group B) mouse livers, at the end of the 3-week initial build-up period.

Liver cholesterol values at the end of each week of the regression period are summarized in Table II. It can be seen that: 1. Rates of mobilization of accumulated liver cholesterol in the β-sitosterol group (D) and control group (C) were similar. 2. Dietary cholic acid (Group E) prevented mobilization of accumulated liver cholesterol. 3. The liver cholesterol levels of mice fed both cholic acid and β-sitosterol (Group F) regressed at approximately the same rate as those of both the control mice (C) and the mice fed β-sitosterol (D), but regressed more rapidly than those of mice fed cholic acid (Group E).

TABLE I. Accumulated Liver Cholesterol in Mice after 3 Week Build-up Period.

Group	Total cholesterol in liver mg/g liver	mg/g body wt
A. Normal—CFD*	4.7 ± 1.0	.41 ± .06
B. Elevated cholesterol—CFD* + 0.5% cholic acid & 1.0% cholesterol	22.7 ± 9.4	2.02 ± .72

† Recrystallized from methanol.

* CFD = cholesterol free diet.

TABLE II. Mobilization of Accumulated Liver Cholesterol in Mice Fed β-Sitosterol and Cholic Acid.

Group	Time, wk	Total cholesterol in liver mg/g liver	mg/g body wt
A. Normal—CFD*	1	5.0 ± 1.5	.44 ± .12
	2	5.8 ± 1.4	.52 ± .09
	3	7.4 ± 2.2	.67 ± .22
	4	6.2 ± 1.1	.53 ± .08
C. Regression controls —CFD	1	20.1 ± 5.1	1.82 ± .36
	2	11.0 ± 2.7	1.17 ± .37
	3	9.4 ± 4.1	.84 ± .57
	4	8.3 ± 4.0	.63 ± .29
D. CFD + 2.5% β-sitosterol	1	14.1 ± 3.0	1.32 ± .27
	2	13.3 ± 3.7	1.43 ± .48
	3	9.0 ± 2.9	.78 ± .23
	4	6.5 ± 2.9	.55 ± .28
E. CFD + 0.5% cholic acid	1	14.7 ± 2.3	1.37 ± .28
	2	16.1 ± 3.0	1.49 ± .23
	3	18.9 ± 3.8	1.96 ± .41
	4	18.1 ± 2.6	1.98 ± .47
F. CFD + 0.5% cholic acid & 2.5% β-sitosterol	1	12.8 ± .8	1.29 ± .35
	2	11.8 ± 3.0	1.30 ± .31
	3	10.6 ± 3.1	1.11 ± .29
	4	9.6 ± 2.3	.95 ± .21

Statistical comparison of values:

Groups	1st wk	4th wk
C and D	$P = .33$	$P = .21$
C and E	.32	$P < .01$
C and F	.28	$P = .51$
D and F	.35	$P = .02$
E and F	.08	$P < .01$

* CFD = cholesterol free diet.

Discussion. Results of this experiment provide evidence that β-sitosterol does not increase rate of accumulated cholesterol mobilization in comparison with that of control mice (Groups C and D). If accumulated liver cholesterol were mobilized as cholesterol, one would expect β-sitosterol to be effective in increasing its rate of regression, since β-sitosterol is highly effective in preventing cholesterol absorption when fed together with dietary cholesterol. In view of our results, we can speculate that cholesterol is not excreted as such but is metabolized to bile acid prior to excretion, as in the case of the rat.

The fact that β-sitosterol reduces the effect of cholic acid in preventing mobilization of accumulated mouse liver cholesterol, may be explained in 2 possible ways: (a) β-sitosterol may prevent cholic acid absorption, or (b) the cholesterol mobilization mechanism may be altered by addition of dietary cholic acid.

The first possibility (a) is untenable in view of the fact that β-sitosterol alone did not increase rate of cholesterol mobilization (Group D). The second possibility (b) seems more probable because of the findings of Wells(10), who has shown that addition of bile acids to the diet increases level of cholesterol in the feces. This could mean that under these conditions cholesterol is excreted as cholesterol.

Summary. 1. Cholic acid prevents mobilization of elevated liver cholesterol. 2. β-sitosterol did not increase rate of mobilization of accumulated mouse liver cholesterol. 3. β-sitosterol reduces in large measure the effect of cholic acid on liver cholesterol mobilization.

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Received July 9, 1958. P.S.E.B.M., 1958, v99.