Bile Acid and Cholesterol Metabolism in the Mouse as Affected by Cholestyramine.* (31278)

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One important factor in the homeostatic control of tissue cholesterol concentrations is the rate of elimination of fecal end products of sterol metabolism. For example, the hypophysectomized rat, because of a slow rate of elimination of fecal bile acids and sterols, accumulates high concentrations of tissue cholesterol when fed diets containing concentrations of cholesterol easily handled by normal rats(1,2). Thus, any substance effective in altering the rate of reabsorption of gastrointestinal bile acids or sterols from their recirculating pools should be effective in controlling the accumulation and elimination of tissue sterols which are in equilibrium with these pools.

Cholestyramine (MK 135), a non-absorbable(3), bile acid-binding, anionic exchange resin, has been shown to lower blood cholesterol levels and increase the rate of elimination of fecal bile acids and sterols in a number of species(4,5). Therefore this substance should be effective in accelerating the mobilization of accumulated blood and liver cholesterol. This seems probable because it has been shown that dietary cholic acid effectively blocks tissue sterol mobilization(6). In the present experiments, the effects of MK 135 on rates of elimination of accumulated blood and liver cholesterol in the mouse have been studied, and detailed observations made of its effects on sterol excretion and on bile acid pool size, spectrum, turnover and synthesis.

Methods. A. Regression studies. One hundred Webster strain female albino mice, weighing approximately 25 g, were used in this study. Ten mice were maintained on an unsupplemented commercial mouse diet; the other 90 received this diet supplemented with 1% cholesterol and 0.5% cholic acid to effect accumulation of blood and liver cholesterol. After 14 days, the 10 mice on the unsupplemented diet were killed along with 10 mice from the supplemented group. Analyses of blood and liver from these 2 groups established basal and peak cholesterol concentrations.

The remaining 80 mice with elevated cholesterol were divided into 2 groups: (a) controls, fed unsupplemented mouse rations; and (b) treated mice, fed mouse rations supplemented with 1% MK 135.

At 3-, 6-, 9-, 13- and 17-day intervals, 8 mice from each group (a and b) were killed, and blood and livers removed for analyses. Liver cholesterol was extracted and determined according to Abell(7); total blood cholesterol by the method of Sperry and Webb (8).

B. Bile acid metabolism and fecal β-sterol excretion studies. Forty-eight Webster strain female albino mice weighing 25 g were used. Each mouse received an intraperitoneal injection containing 1 μc (4.03 mc/mM) of cholic acid-24-C^{14}, dissolved in isotonic sodium bicarbonate. The mice were placed in individual metabolism cages and divided into 2 groups: (a) fed unsupplemented mouse ration; and (b) fed mouse ration supplemented with 1% MK 135.

Feces were collected daily for 9 days. The mice were then killed and the livers removed. The caecums, large and small intestines, and gall bladders were excised with their contents. Since most of the bile acid pool would be present in the gall bladder and small intestine, analysis was simplified by combining these tissues from each mouse. Feces and tissue samples were frozen at collection and dried by lyophilization.

Total lipids were exhaustively extracted with boiling ethanol in Bailey-Walker extractors. Fractions of these extracts were plated in cup planchets, and counted to determine the total bile acid-24-C^{14} activity in each sample. The accumulated amount of C^{14} activity in the daily fecal collections at any time t is designated u^t; the sum of the counts

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in the feces for the 9 days plus the total tissue counts is designated u^{max}. From these data, the half-life of the bile acid pool can be determined(9).

To determine the bile acid pool size, ethanol extracts from the small intestine plus gall bladder samples were evaporated to dryness, and autoclaved in the presence of 7 N sodium hydroxide for 3 hours at 15 lb pressure to hydrolyze the conjugated bile acids. Petroleum ether extraction of the hydrolysate removed the unsaponifiable fraction. The alkaline residue was acidified and extracted with petroleum ether to remove fatty acids. The acidic residue was exhaustively extracted with ethyl ether to isolate the "acidic fraction," containing the bile acids. Fractions of these extracts were plated and counted. Comparison of these counts with those obtained for the fecal ethanol extracts revealed any losses incurred during the extractions, and made it possible to correct for these losses in the quantitative bile acid determinations.

Cholic acid was determined by scanning thin-layer chromatograms of the "acidic fraction" with an automatic recording transmission densitometer(2,10). This gave the size of the small intestine plus gall bladder pool, which comprises 90-95% of the total pool. The total pool was calculated from the small intestine plus gall bladder bile acid pool size and C^{14} activity by assuming that the pool components were in a state of equilibrium. This view has been defended in detail by Strand(11).

Total digitonin precipitable sterols were determined in the ethanolic feces extracts by evaporation, saponification, petroleum ether extraction, and precipitation with digitonin. The digitonides were filtered, dried and weighed. β-Sterols were calculated assuming a sterol molecular mass of 386.4 and 1:1 digitonin:β-sterol complex. Flow gas counting was used to determine C^{14} activities.

Results and discussion. Fig. 1 shows the regression of accumulated blood and liver cholesterol in the mouse as a function of time. It is clear that there is an increase in rate of mobilization of both blood and liver cholesterol in the MK 135-treated mice. At no time during the experiment, however, did cholesterol levels go significantly below basal concentrations, indicated by the dotted line. It is interesting that in the rat(5) and pigeon (12) cholestyramine does not lower normal blood cholesterol levels, presumably because the rate of sterol synthesis increases sufficiently(5) to counteract the drain caused by increased bile acid and sterol excretion.

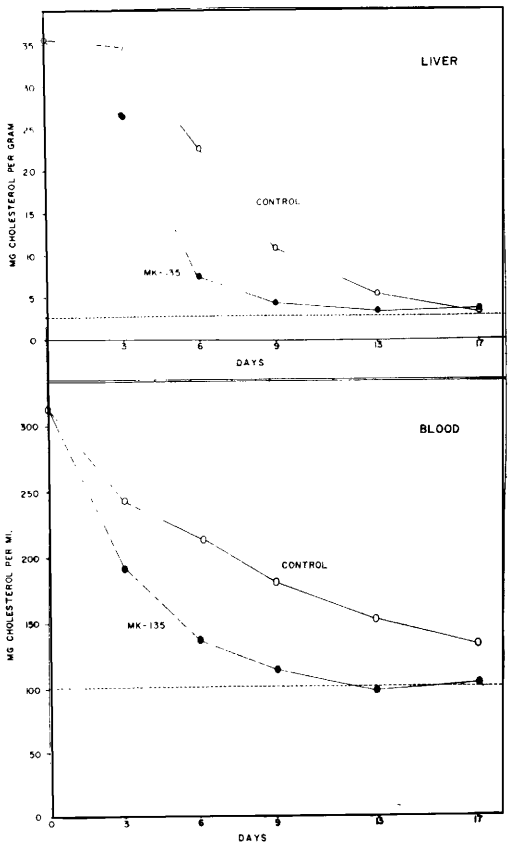

FIG. 1. Effect of cholestyramine (MK 135) on mobilization of accumulated liver and blood cholesterol in the mouse. Dotted lines represent cholesterol concentrations of normal mice.

Since in the mouse cholestyramine greatly increases the rate of mobilization of blood and liver cholesterol, the effect of this substance on the major pathways of cholesterol elimination was of interest. If bile acid elimination follows first order kinetics, we would get a straight line when the $-\text{Log}\left(1 - \frac{u^t}{u^{max}}\right)$ is plotted against time, and when $u^t/^{max} = 0.5$, half of the bile acid-24-

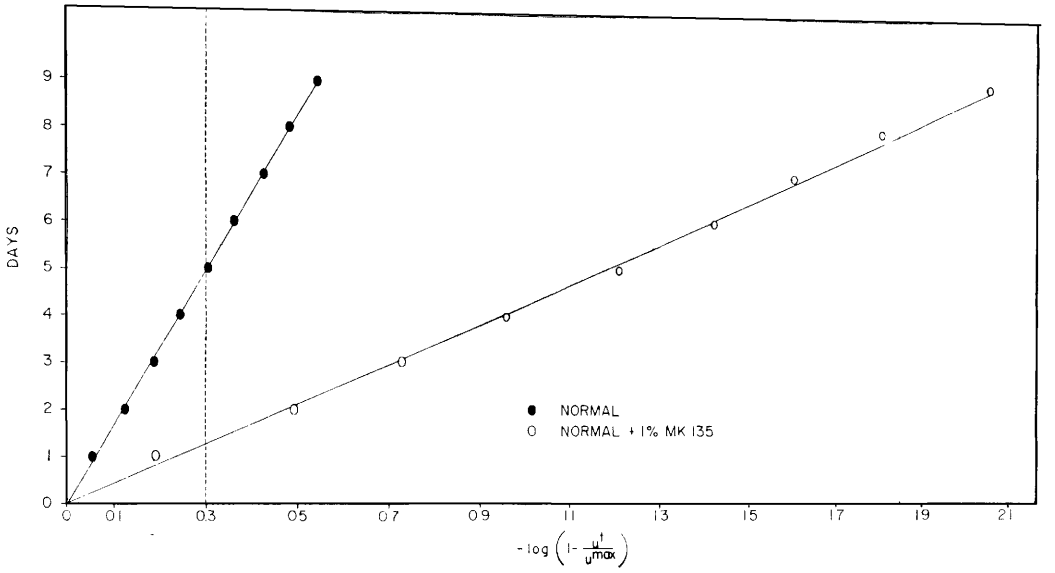

FIG. 2. Fecal bile acid-24-C^{14} excretion in normal and cholestyramine-treated mice following cholic acid-24-C^{14} injection. Dotted vertical line was drawn from the point on the abscissa at which $u^t/u^{max} = 0.5$, and thus intersects the curves at the half-life of the bile acid pool.

C^{14} in the pool would be excreted. Since the bile acid-24-C^{14} molecule is handled in the same manner as the unlabeled molecule, the 0.5 point would then represent the half-life or turnover rate of the pool. The straight line plot in Fig. 2 makes it obvious that the bile acid-24-C^{14} excretion did follow first order kinetics for both control and treated mice. The half-life of the bile acid pool in normal mice averaged 5 days, and in mice treated with 1% cholestyramine, 1¼ days. While this is a striking acceleration, its effect on bile acid synthesis or excretion depends on the effect of cholestyramine on the bile acid pool size.

Fig. 3 is a photograph of a thin-layer chromatogram of the acidic fraction isolated from small intestine and gall bladder plus contents in normal and cholestyramine-treated mice. In contrast to the rat(2,11), the mouse bile acid pool contains very little chenodeoxycholic acid. It can be seen that cholestyramine did not alter the qualitative composition of the bile acid pool.

Quantitative determinations of cholic acid in the pools showed that the bile acid pool size in normal mice was 5.62 ± 1.09 mg; this was reduced to 4.58 ± 0.65 mg in mice treated with 1% cholestyramine. This reduction is not large but it is significant. Since MK 135 in larger doses might reduce the pool to a larger extent, especially in animals exhibiting a slow rate of bile acid synthesis, it could lead to instability of gall bladder bile and deposition of biliary concretions.

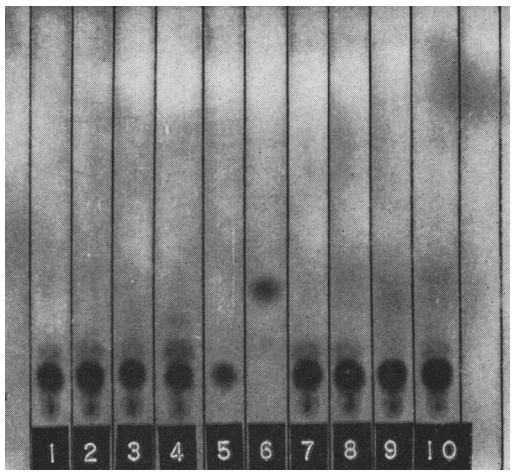

FIG. 3. Separation of bile acids in the "acidic fraction" isolated from small intestine and gall bladder (plus contents) of normal and cholestyramine-treated mice. Columns 1-4, normal mice; column 5, standard cholic acid; column 6, standard chenodeoxycholic acid; columns 7-10, cholestyramine-treated mice. Chromatography plates coated with silica gel G and developed with isooctane:ethyl acetate:acetic acid, 10:10:2 (v/v). (See references 2 and 10.)

The rates of bile acid synthesis were calculated from bile acid pool size and turnover rates in the 2 groups. Daily synthesis of bile acids in normal mice was 0.56 mg; in cholestyramine-treated animals, 1.84 mg. Thus the diet supplemented with 1% cholestyramine caused a 3- to 4-fold increase in bile acid synthesis or excretion. Determination of fecal digitonide-precipitable sterols showed that normal mice excreted 5.54 ± 0.65 mg/day; cholestyramine-treated mice, 7.58 ± 1.14 mg/day.

These increases in bile acid and sterol excretion account for the increased mobilization rate of accumulated blood and liver cholesterol in the mouse. However, it is not possible to tell whether excretion of just one or of both fractions is responsible for the increase in cholesterol mobilization. Although there is evidence that accumulated liver cholesterol is eliminated only after conversion to bile acid(6,13), under certain circumstances accumulated liver cholesterol can be eliminated in the sterol fraction(6).

Summary. Dietary cholestyramine (MK 135) increased the rate of mobilization of blood and liver cholesterol in mice. The bile acid turnover rate in normal mice was 5 days, in mice treated with 1% cholestyramine, 1¼ days, or 4 times as fast. Bile acid pool size was 5.62 ± 1.09 mg in normals, 4.58 ± 0.65 in cholestyramine-treated mice. The pool was shown to consist almost exclusively of cholic acid and this spectrum was not altered by cholestyramine. The daily rate of synthesis of bile acids in normal mice was 0.56 mg, 1.84 mg in cholestyramine-treated mice. Digitonin precipitable fecal sterol excretion was 5.54 mg/day in normal mice, 7.58 mg/day in mice treated with 1% MK 135. No conclusion could be reached as to whether the increased rate of mobilization of accumulated cholesterol affected by MK 135 was due to the increased excretion of either the fecal bile acid or sterol fraction exclusively, or was attributable to the excretion of both these fractions.

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1. Beher, W. T., Baker, G. D., Beher, M. E., Vulpetti, A., Semenuk, G., Proc. Soc. Exp. Biol. and Med., 1964, v117, 738.
2. Beher, W. T., Beher, M. E., Semenuk, G., Metabolism, 1966, v15, 181.
3. Gallo, D. G., Bailey, K. R., Sheffner, A. L., Proc. Soc. Exp. Biol. and Med., 1965, v120, 60.
4. Tennent, D. M., Hashim, S. A., Van Itallie, T. B., Fed. Proc., 1962, v21, 77.
5. Huff, J. W., Gilfillan, J. L., Hunt, V. M., Proc. Soc. Exp. Biol. and Med., 1963, v114, 352.
6. Beher, W. T., Baker, G. D., Anthony, W. L., Am. J. Physiol., 1960, v199, 736.
7. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., J. Biol. Chem., 1954, v208, 307.
8. Sperry, W. M., Webb, M., ibid., 1950, v187, 97.
9. Lindstedt, S., Norman, A., Acta Physiol. Scand., 1956, v38, 121.
10. Semenuk, G., Beher, W. T., J. Chromatog., 1966, v21, 27.
11. Strand, O., J. Lipid Res., 1963, v4, 305.
12. Kottke, B. A., Juergens, J. L., Bolmann, J. L., J. Atherosclerosis Res., 1963, v3, 129.
13. Danielsson, H., Acta Physiol. Scand., 1960, v48, 364.

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Effect of Sodium Salicylate on Magnesium Metabolism in the Rabbit.* (31279)

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Mg^{28} administered parenterally in a tracer dose is rapidly concentrated in tissues(1). Tissue uptake of this isotope is facilitated by

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