# Cholesterol Metabolism in Rabbits with Oleic Acid-Induced Cholelithiasis (36887)

PATRICIA A. KYD AND IAN A. D. BOUCHIER
(Introduced by S. Sherlock)
*Medical Unit, The Royal Free Hospital, London WCIX 8LF, United Kingdom*

Rabbits fed a high oleic acid diet for 8 wk developed gallstones composed of a mixture of glycodeoxycholic acid and glycoallodeoxycholic acid (1). The raised concentration of allodeoxycholate in bile is accompanied by a 2/3-fold increase in the hepatic concentration of its precursor, cholestanol (expressed as a percentage of total sterols). Since the high oleic acid diet contained no supplementary cholestanol, a possible source of increased tissue cholestanol is endogenous synthesis from cholesterol (2, 3). Cholesterol metabolism was therefore investigated in rabbits fed the gallstone inducing diet and compared with cholesterol metabolism in control rabbits.

*Methods.* Male New Zealand white rabbits were fed a diet of 40% casein, 15% oleic acid, 6.8% sucrose, 6.5% glucose and 31.7% S.G.1 pellets for 8 or 16 wk (1). Control rabbits were fed S.G. 1 laboratory pellets.

Plasma cholesterol concentrations were measured by the Boehringer test combination method (4). Blood was collected from the marginal ear vein at the beginning of the dietary regime and subsequently every 2 wk.

The rabbits were sacrificed by an overdose of Nembutal and the right lobe of the liver was removed immediately. For the measurement of hepatic cholesterol synthesis, slices approximately 0.5 mm in thickness were cut freehand (5). Cholesterol synthesis was measured at several points along the small intestine, because of the reported variation along the small intestine (6). The entire small intestine was removed and divided into three segments of equal length. Approximately 5 cm of the middle portion of each segment was removed, together with the terminal 5 cm of small intestine. After rinsing well with cold Krebs–Ringer bicarbonate buffer, slices 0.5 mm thick were prepared from each segment using a McIwain tissue slicer.

Duplicate 400 and 200 mg liver slices were used for incubation, together with 400 and 200 mg slices for blank incubations. Intestinal cholesterol synthesis was measured using 200 mg slices of each segment in duplicate, together with 200 mg for the blank. The slices were placed in the outer compartment of a 50 ml Erlenmeyer flask with a center well, and incubated in 5 ml Krebs–Ringer bicarbonate buffer (pH 7.4), previously gassed with 95% $O_2$, 5% $CO_2$, containing 2.5 m*M* 2-$^{14}$C-acetate (1 $\mu$Ci). One-half milliliter of 10 *N* $H_2SO_4$ was added to the blank flasks. Each flask was gassed for 30 sec with 95% $O_2$, 5% $CO_2$, sealed with a rubber cap and incubated in a metabolic shaker at 100 oscillations/min at 37° for exactly 2 hr. The reaction was stopped by injection of 0.5 ml 10 *N* $H_2SO_4$ through the rubber cap. One-half milliliter of 1 *M* Hyamine hydroxide solution in methanol was injected through the cap into the center well and $CO_2$ produced was absorbed by keeping the flasks at 4° for 1 hr with occasional shaking. The Hyamine solution was transferred to a counting vial and counted in toluene scintillation fluid (7) in a Packard Tricarb liquid scintillation spectrometer. The incubation mixture was transferred to 50 ml stoppered tubes containing 1 mg carrier cholesterol, 5 ml ethanol and 2 ml 40 g/100 ml aqueous potassium hydroxide solution. After saponification at 70° for 1 hr, nonsaponifiable lipids were extracted and sterols were precipitated as the digitonide (8). The digitonides were dissolved in methanol and counted in toluene scintillation fluid. After acidification of the incubation mixture with 2 ml 10 *N* $H_2SO_4$, fatty acids were extracted with 2 × 20 ml petroleum

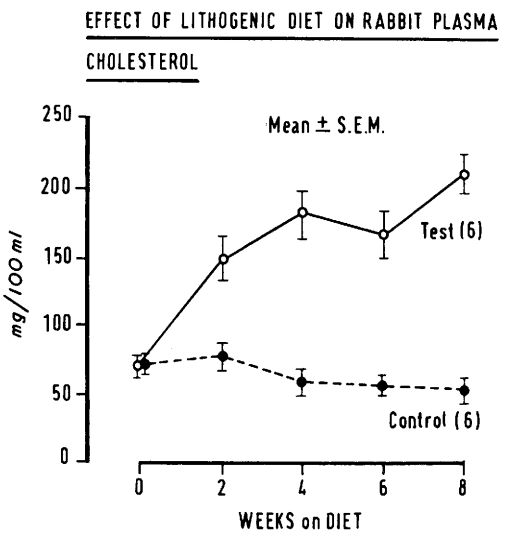


FIG. 1. Mean plasma cholesterol concentrations of a group of control rabbits and a group of test (lithogenic diet-fed) rabbits.

ether (bp 40–60°). The petroleum extracts were washed with distilled water to remove any remaining $^{14}$C-acetate, evaporated, and the fatty acids were dissolved in toluene scintillation fluid for counting.

The data are expressed as nmoles of 2-$^{14}$C-acetate incorporated into cholesterol, fatty acids and $CO_2$ per gram wet weight of tissue per hour of incubation. In preliminary experiments it was demonstrated that under these conditions the rates of incorporation of 2-$^{14}$C-acetate into cholesterol and fatty acids were linear with respect to time of incubation and tissue weight.

Liver total and free cholesterol concentrations were measured in duplicate portions of the right lobe (9).

*Results.* After 2 wk on the lithogenic diet, the test rabbits had significantly higher plasma cholesterol concentrations (Student's $t$ test $p < .01$) than the control rabbits (Fig. 1). These values continued to rise until after 8 wk the plasma cholesterol concentrations of test rabbits were approximately four times the control values ($p < .001$). There was a striking increase in liver cholesterol concentrations, mainly in the ester fraction (Table I). The Wilcoxon rank sum test (10) was used to assess the significance of the increases because of the small numbers in each group. After 16 wk on the lithogenic diet the major increase in liver cholesterol was again in the ester fraction, but there was also a marked increase in free cholesterol concentrations.

The marked hypercholesterolemia was not due to a higher content of cholesterol in the lithogenic diet. The cholesterol content of the control and lithogenic diets was measured by gas–liquid chromatography, and it was found that lithogenic rabbits received less cholesterol per day (6.7 mg) compared with control rabbits (11.0 mg/day).

Hepatic cholesterol synthesis in lithogenic rabbits was markedly inhibited (significant at 1% level) compared with control rabbits (Fig. 2). The results of two experiments have been combined. The wide range of control values was due to individual variation between rabbits, since the results of duplicate incubations agreed well (coefficient of variation 4.3%). Lithogenic rabbits showed a striking inhibition of fatty acid synthesis also, probably due to feedback inhibition by the 15% oleic acid content of the diet. However, there was no difference in $CO_2$ production,

TABLE I. Effect of Lithogenic Diet on Liver Cholesterol Concentrations.

| Diet ($n$) | Duration (wk) | Cholesterol Mean (mg/g wet wt ± SEM) | |
|---|---|---|---|
| | | Free | Ester |
| Control (6) | 8 | 2.34 ± 0.13 | 0.57 ± 0.13 |
| Lithogenic (6) | | 2.86 ± 0.08 | 6.08 ± 1.04 |
| Significance (Wilcoxon) | | 1% | 1% |
| Control (5) | 16 | 1.89 ± 0.07 | 0.25 ± 0.11 |
| Lithogenic (4) | | 4.49 ± 1.35 | 5.46 ± 0.82 |
| Significance (Wilcoxon) | | 1% | 1% |

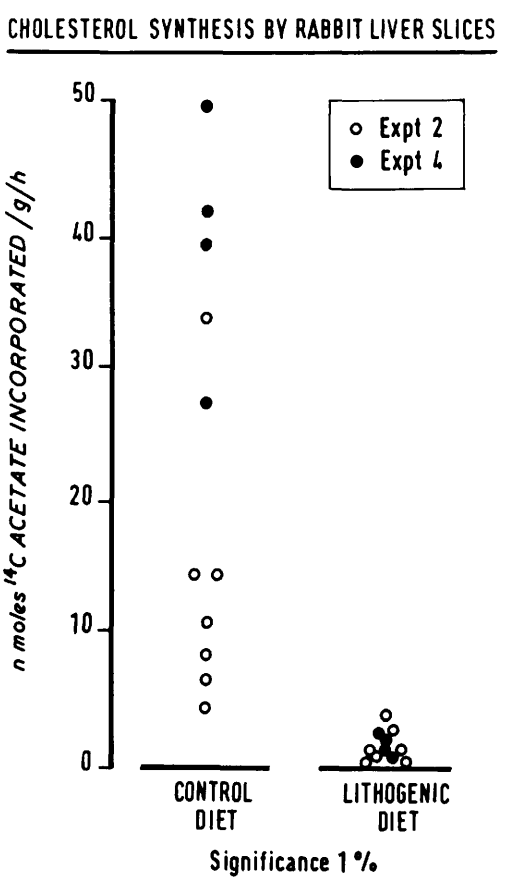


FIG. 2. Incorporation of acetate-2-$^{14}$C into cholesterol (digitonin precipitable sterols) by liver slices of rabbits fed the control or the lithogenic diet. The results of two experiments have been combined.

showing that the inhibition of cholesterol synthesis was not due to a nonspecific effect of the diet in depressing overall hepatic metabolism.

Intestinal cholesterol synthesis in lithogenic rabbits was within the control range at most points along the small intestine (Fig. 3). The higher rates of synthesis observed in the middle segment of the intestine of lithogenic rabbits cannot be considered significant because of the small number of animals in each group. In both control and lithogenic rabbits, there was a trend towards increased synthesis rates of cholesterol, fatty acids and $CO_2$ from proximal to distal small intestine, suggesting that the distal portion of rabbit small intestine is metabolically the most active.

*Discussion.* Marked hypercholesterolemia and increased liver cholesterol concentrations have been observed in rabbits fed a low cholesterol diet with a reduced roughage content (laboratory chow contributing only 31.7% of the diet). These findings agree with previous reports of the production of hypercholesterolemia and liver cholesterol accumulation in rabbits fed low cholesterol semisynthetic diets (11, 12). As reported by Carroll (11), increased hepatic cholesterol synthesis did not contribute to the raised cholesterol levels, since hepatic cholesterogenesis was markedly inhibited.

It is possible that the low roughage content of the diet reduced the fecal elimination and daily production of bile acids, thus reducing the disposal of cholesterol. Hellstrom, Sjovall and Wigand (13) observed a reduction in the daily synthesis of bile acids and an increase in the half-life of deoxycholic acid in rabbits fed a semisynthetic diet, which had associated hypercholesterolemia. However, these authors found no correlation between changes in rabbit serum cholesterol concentration induced by different dietary fats and the turnover of deoxycholic acid.

From present knowledge of the control of hepatic cholesterol synthesis (14) the

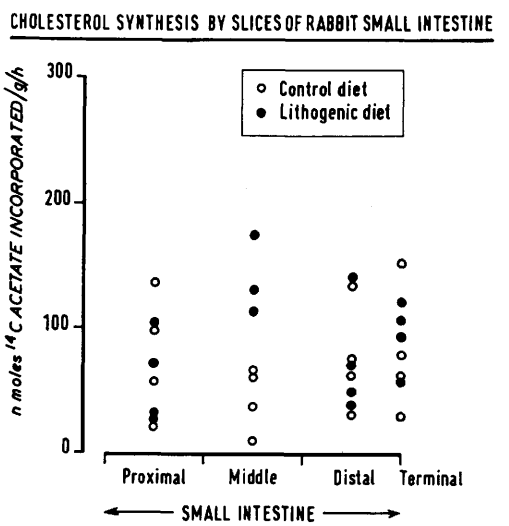


FIG. 3. Incorporation of acetate-2-$^{14}$C into cholesterol (digitonin precipitable sterols) by intestinal slices of control and lithogenic rabbits, taken at various points along the small intestine.

marked inhibition of hepatic cholesterol synthesis observed in rabbits fed the lithogenic diet suggests that more cholesterol entered the liver in the form of chylomicrons from the intestine. The rate of cholesterol synthesis in rabbit intestine was higher than cholesterol synthesis in the liver. This finding supports the conclusion of Popjak and Beeckmans (15) that the intestine is the most active site of cholesterol synthesis in the rabbit. Since intestinal cholesterol synthesis was unchanged in lithogenic rabbits, it is suggested that cholesterol absorption was enhanced due to the high fat content of the lithogenic diet (15% oleic acid). High levels of dietary fat are known to facilitate intestinal uptake and lymphatic transport of cholesterol, oleic acid having the greatest effect of the fats and fatty acids investigated.

*Summary.* Cholesterol metabolism has been studied in rabbits with bile acid gallstones induced by feeding a high oleic acid diet. Lithogenic rabbits showed hypercholesterolemia, compared with control rabbits, which was accompanied by a striking increase in the liver cholesterol concentration. The lithogenic diet contained no supplementary cholesterol and hepatic cholesterol synthesis was markedly inhibited. Since intestinal cholesterol synthesis was unchanged in lithogenic rabbits, it is suggested that the increase in plasma and liver cholesterol concentrations was due to increased absorption of cholesterol by the presence of 15% oleic acid in the lithogenic diet. The significance of the raised plasma and liver cholesterol concentrations in relation to the 2–3 fold increase in cholestanol concentration in the livers of lithogenic rabbits remains to be elucidated.

The authors are grateful to Mr. A. Shaw for technical assistance.

1. Kyd, P. A., and Bouchier, I. A. D., Biochem. J. **128**, 169 (1972).

2. Shefer, S., Milch, S., and Mosbach, E. H., J. Biol. Chem. **239**, 1731 (1964).

3. Shefer, S., Milch, S., and Mosbach, E. H., J. Lipid Res. **6**, 33 (1965).

4. Watson, D., Clin. Chim. Acta **5**, 637 (1960).

5. Umbreit, W. W., Burris, R. H., and Stauffer, J. S., *in* "Manometric Techniques," 4th ed. Burgess, Minneapolis (1964).

6. Dietschy, J. M., and Wilson, J. D., N. Engl. J. Med. **282**, 1128 (1970).

7. Kyd, P. A., and Bouchier, I. A. D., Gastroenterology **61**, 723 (1971).

8. Siperstein, M. D., and Guest, M. J., J. Clin. Invest. **39**, 642 (1960).

9. Sperry, W. M., and Webb, M., J. Biol. Chem. **187**, 97 (1950).

10. Wilcoxon, F., and Wilcox, R. A., "Some Rapid Approximate Statistical Procedures." Lederle Laboratories Division, Pearl River, NY (1964).

11. Kritchevsky, D., and Tepper, S. A., J. Atheroscler. Res. **8**, 357 (1968).

12. Carroll, K. K., Atherosclerosis **13**, 67 (1971).

13. Hellstrom, K., Sjovall, J., and Wigand, G., J. Lipid Res. **3**, 405 (1962).

14. Weis, H. J., and Dietschy, J. M., J. Clin. Invest. **48**, 2398 (1969).

15. Popjak, G., and Beeckmans, M. L., Biochem. J. **47**, 233 (1950).

16. Swell, L., Flick, D. F., Field, H., and Treadwell, C. R., Amer. J. Physiol. **8**, 124 (1955).

17. Sylven, C., and Borgstrom, B., J. Lipid Res. **9**, 596 (1968).

Received July 17, 1972. P.S.E.B.M., 1972, Vol. 141.