The rate of biliary cholesterol secretion in high- and low-responding rhesus monkeys[1]

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Summary. The rates of secretion of cholesterol in bile measured by an isotope ratio method were found similar in cholesterol-fed high- and low-responding rhesus monkeys. The results indicate that the failure on the part of the high-responders to increase proportionately the fecal excretion of neutral steroids to compensate for the higher absorption of cholesterol than the low-responders, as suggested earlier, is not due to a difference in the rate of biliary cholesterol secretion but must lie in some other aspect of cholesterol metabolism.

Key words. Cholesterol; biliary secretion; rhesus monkeys.

We have been studying two groups of rhesus monkeys (high-responders and low-responders) that differ markedly in response of plasma cholesterol concentration when fed a high fat, high cholesterol diet. We have observed consistently that the high-responders absorb significantly higher amounts of cholesterol than the low-responders[2-4]. We have also observed that upon feeding the high cholesterol diet, fecal excretion of neutral steroids and bile acids increased in both groups[2]. The increase in fecal excretion of neutral steroids was similar in the two groups, whereas the increase in fecal excretion of bile acids was greater in the high- than in the low-responders[2]. This indicated that in contrast to the low-responders, the high-responding animals failed to increase neutral steroids excretion in feces sufficiently to compensate for the greater absorption of cholesterol[2-4]. It has also been shown that while fed cholesterol the high-responders had much lower concentration of cholesterol in the serum high-density lipoproteins (HDL) than the low-responders[5]. In view of the suggestion that free cholesterol in HDL appears more rapidly as biliary cholesterol than from any other cholesterol carrying lipoproteins[6], we hypothesized that the decreased HDL-cholesterol in plasma of cholesterol-fed high-responders causes a decrease in the secretion of cholesterol in bile which, in turn, results in a decrease in the excretion of cholesterol as neutral steroids in the feces. The present study was, therefore, designed to test this hypothesis.

Materials and methods. Eight (3 high-responders and 5 low-responders) adult, male rhesus monkeys *(Macaca mulatta)* weighing between 7 and 12 kg were used in the study. The monkeys were identified as high- or low-responders from a group of 36 young adult monkeys on the basis of the response of plasma cholesterol to an atherogenic diet fed for 12 weeks[2]. The animals were fed a diet which provided fat at 38% and proteins at 15% of calories and cholesterol at a level of 0.75 mg/kcal. The basic composition of the diet has been reported[3]. The animals were fed once daily approximately 150 g diet or 600 cal/day, an amount sufficient to maintain body weight. The monkeys were fed the diet for nearly 5 years and therefore were in a steady state. The rate of biliary cholesterol secretion was measured by the method of Crouse et al.[7]. Briefly, about 50 μCi of [1,2-^{3}H]-cholesterol, purified by thin layer chromatography (TLC)[4], was dissolved in 0.5 ml ethanol, suspended in 20 ml saline and injected i.v. to each animal 6 weeks before feeding the ^{14}C-cholesterol. A known amount (0.1 μCi) of ^{14}C-cholesterol, purified by TLC[4], and homogenized with about 20 g of the regular diet was fed by gavage daily for 10 days. On the last 5 days of the ^{14}C-cholesterol feeding, the ^{3}H/^{14}C ratio in the feces (fecal isotope ratio) and plasma ^{3}H-cholesterol specific activity were determined. The rate of biliary cholesterol secretion was calculated as described by Crouse et al.[7].

Feces collected daily during the last 5 days of the feeding period was homogenized with water (1:1). An aliquot of the homogenized feces was saponified with alcoholic KOH and extracted with hexane by the method of Miettinen et al.[8]. The extract was made up to volume and ^{3}H and ^{14}C radioactivity determined in an aliquot using liquid scintillation counter equipped with external standardization. The plasma ^{3}H-cholesterol specific activity was determined in an aliquot of plasma after saponification and extraction by the method of Abell et al.[9]. An aliquot of the extract was taken for radioactivity counting as above and another aliquot was used to measure cholesterol by the method of Abell et al.[9]. Data were analyzed for statistical significance by the Student t-test.

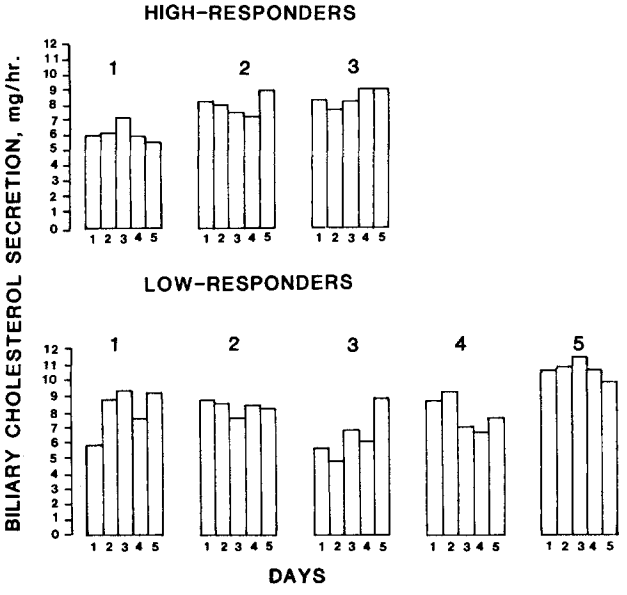
Results. The rate of biliary cholesterol secretion in mg/h for the last 5 days of the radiocholesterol feeding in each of the 8 animals is shown in the figure. Two low-responding animals (3 and 4) show increasing and decreasing trends respectively in biliary cholesterol secretion rate. However, regression analysis for time trends showed no statistically significant trend in these two or in any other animals. In the table, the mean values (± SD) of biliary cholesterol secretion are presented. The small difference in the rate of biliary cholesterol secretion between the high- and low-responding rhesus monkeys was not statistically significant (> 0.5).

Discussion. The study demonstrated that the rate of biliary cholesterol secretion in the cholesterol-fed high-responding rhesus monkeys was no different from that in the low-responding rhesus monkeys. The results did not, therefore, sustain our hypothesis that the failure on the part of the cholesterol-fed high-responders to increase proportionately the excretion of neutral steroids in feces to compensate for the greater absorption of cholesterol is due to a lower rate of biliary cholesterol secretion resulting form decreased HDL-cholesterol in plasma. The decrease in HDL-cholesterol in plasma of the high-responders upon feeding cholesterol was reported previously[5]. We have also observed consistently that the high-responders absorb significantly higher amounts of cholesterol that the low-responders[2-4]. We also reported that upon feeding cholesterol, the high-responders excrete higher amounts of bile acids in the feces than the low-responders[2]. Our earlier studies have also shown that the high-responders have a lower rate of cholesterol biosynthesis than the low-responders[3]. Further, we found that the increase in the total body pool of cholesterol that occurred upon cholesterol

Rate of biliary cholesterol secretion in rhesus monkeys

Animal number	Body weight (kg)	Biliary cholesterol secretion mg/h	mg/h/kg b.wt
High-responders			
1	10.5	6.18 ± 0.82*	0.59
2	11.3	8.08 ± 0.67	0.72
3	10.3	8.50 ± 0.58	0.83
Mean ± SD	10.7 ± 0.53	7.59 ± 1.24	0.71 ± 0.12
Low-responders			
1	11.5	8.24 ± 1.47	0.72
2	12.4	8.14 ± 0.51	0.66
3	10.6	6.49 ± 1.57	0.61
4	7.1	7.88 ± 1.16	1.11
5	8.6	10.76 ± 0.58	1.25
Mean ± SD	10.0 ± 2.16	8.30 ± 1.54	0.87 ± 0.29
All monkeys			
Mean ± SD	10.3 ± 1.69	8.03 ± 1.39	0.81 ± 0.24

*Values are mean of 5 days ± SD.



Biliary cholesterol secretion (mg/h) for days 1–5 in each of the 3 high- and 5 low-responding rhesus monkeys measured by the isotope ratio method.

feeding was distributed similarly in the various exchangeable cholesterol pools in the body of both groups of monkeys[10]. Possibly then the failure on the part of the cholesterol-fed high-responders to increase proportionately the excretion of neutral steroids in feces to compensate for the greater absorption of cholesterol might be related to the number or affinity of hepatic lipoprotein receptors which might determine the ability of the animal to cope with the burden of the excess absorbed cholesterol. The excess absorbed cholesterol might cause a greater 'down regulation' of the hepatic lipoprotein receptors because of a higher accumulation of cholesterol in the liver cells in the high-responders than in the low-responders. Studies are currently underway to answer these questions.

Schwartz et al.[6] have shown that free cholesterol on HDL appear more rapidly as biliary cholesterol than that from any other cholesterol containing lipoproteins. In the earlier study[5] in which it was shown that HDL-cholesterol concentration decreased in the high-responders but not in the low-responders upon feeding cholesterol, the free and esterified cholesterol concentrations in HDL were not determined. It is possible, then, that though the HDL-total cholesterol concentraiton decreased in the cholesterol-fed high-responders, the HDL still contained enough free cholesterol to provide for biliary secretion. It may also be possible that other cholesterol carrying lipoproteins provided enough cholesterol for secretion into bile so as to maintain the rate of secretion of biliary cholesterol. Further, newly synthesized cholesterol in the liver may also contribute to the biliary cholesterol secretion. Further studies are needed to address these questions.

1 Acknowledgments. The study was financed by the research grant HL-08974 from the National Heart, Lung, Blood Institute, National Institutes of Health, Bethesda, Maryland.
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0014-4754/86/11/121227-02$1.50 + 0.20/0

Changes in plasma enzyme concentrations in response to blood substitution with perfluorocarbon emulsion in the conscious rat[1]

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Summary. The effects of near total blood replacement with the proprietary perfluorocarbon (PFC)-based emulsion, Fluosol-DA 20%, on plasma concentrations of 2 enzymes, lactate dehydrogeanse (LDH) and alkaline phosphatase (ALP), have been examined in conscious, chronically catheterized rats. A pronounced fall in both plasma LDH ($p < 0.05$) an ALP ($p < 0.01$) occurred in response to exchange-transfusion. However, at 6 h following blood replacement, plasma concentrations of both enzymes had risen to values significantly greater than those measured immediately before perfusion. The observed changes in plasma LDH and ALP after blood replacement with Fluosol-DA indicated alterations in normal functioning of tissues from which these enzymes originate.
Key words. Blood replacement; perfluorocarbon emulsion; Fluosol-DA; lactate dehydrogenase; alkaline phosphatase; rats.

Totally synthetic, oxygen-carrying blood substitutes are likely to play an increasingly important part in medical science. The availability of such substitutes would have very profound clinical implications and solve a number of problems associated with conventional transfusion[3–5]. Previous work has shown that emulsified perfluorocarbons (PFC) may have value as substitutes for red blood cells and the effects of transfusion with such materials have been examined in a number of mammalian species[5–7]. Moreover, one proprietary preparation, Fluosol-DA 20% – an emulsion containing perfluorodecalin and perfluorotripropylamine and manufactured by the Green Cross Corporation, Japan – has already been tested in preliminary clinical trials in Japan, Canada and the USA with generally encouraging results[8–11].

Using a method for continuous, isovolaemic, exchange-transfusion of conscious rats, it has been possible to analyse in detail some of the acute physiological responses to blood replacement with Fluosol-DA[12–14]. This work was an important advance over previous similar studies in which the perfusion procedure was performed on anaesthetised animals[3,15–17] and demonstrated that near-total blood replacement with Fluosol-DA could be achieved in conscious rats with no immediate disruption of normal cardiovascular and respiratory functions.

A clearer understanding of the body's responses to varying de-