Cholesterogenesis During Acute Infection in Chronically Hypercholesterolemic Rhesus Monkeys[1] (36448)

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Cholesterol concentrations in plasma have been said to either increase, decrease, or remain unchanged during a wide variety of infectious illnesses in man or laboratory animals (1). A similar variability has been reported in the direction and magnitude of infection-induced changes in other plasma lipids (1). Gallin, Kaye, and O'Leary (2) suggested that the varying patterns of lipid response during infection were related to the specific causative organism and to the presence or absence of circulating bacterial endotoxins. However, their subsequent findings in experimental infections of rabbits did not fully substantiate this concept (3). Our earlier studies (4–6) indicated that clinical and experimental data could best be explained in terms of interrelated alterations in the rates of synthesis and utilization of each host lipid moiety. These individual factors appeared to vary with each experimental infection and during the sequential progression of a single infection.

The relationship between the antecedent diet of a host and the metabolic response to infectious illness is not well defined, especially when the intake of certain nutrients has been excessive (7). The present studies were designed to determine if a preexisting diet-induced abnormality in host cholesterol metabolism might influence the patterns of metabolic response by host lipids during an acute infection.

Materials and Methods. Chronic hypercholesterolemia was induced experimentally in rhesus monkeys, *Macaca mulatta*, by extended feeding of the cholesterologenic diet described by Armstrong, Conner, and Warner (8). Two groups of 8 healthy rhesus monkeys weighing 3–4 kg, paired by sex and weight, were maintained for 7 months under optimal animal colony conditions on either a normal control diet or a cholesterologenic diet. Both diets were obtained from General Biochemical, Chagrin Falls, OH, and fed as identically flavored pellets. The control monkey diet contained 20% of the calories as protein, 76% as carbohydrate and 4% as fat (corn oil). The cholesterologenic diet contained 15% of the calories as protein, 44% as carbohydrate and 41% as egg yolk fat; the cholesterol sources provided 1.2% of the diet as cholesterol, 0.8% from egg yolk and 0.4 from crystalline cholesterol. Measurements of plasma lipids, lipoproteins, serum glutamic oxaloacetic transaminase (SGOT), total leukocyte counts and blood cultures were obtained serially by previously described procedures (4, 5). After an overnight 12 hr fast, monkeys were inoculated intravenously with 10^8 virulent Type I *Diplococcus pneumoniae* to initiate a generalized infectious

[1] In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council. The facilities are fully accredited by the American Association of Accreditation of Laboratory Animal Care.

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TABLE I. Plasma Lipid Concentrations.

Dietary group	Base line control (mg/100 ml)	24 hr postinfection		
		Infected (mg/100 ml)	Change	p
Group I, normal diet				
Cholesterol	123 ± 4[a]	77 ± 3	−46	.001
Phospholipid	368 ± 32	278 ± 41	−90	.05
FFA	20 ± 1	20 ± 3	0	NS[b]
Triglyceride	85 ± 12	42 ± 8	−43	.05
Group II, high cholesterol diet				
Cholesterol	412 ± 38	305 ± 37	−107	.001
Phospholipid	582 ± 42	450 ± 54	−132	.01
FFA	25 ± 2	23 ± 3	−2	NS
Triglyceride	34 ± 5	70 ± 8	+36	.05

[a] Values shown are mg/100 ml: mean ± SE 8 monkeys/group.
[b] Not significant.

process and fasting was continued (4). Twenty-four hours later, cholesterogenesis was studied using ^{14}C-mevalonate (DL-mevalonic-2-^{14}C, 5 mCi/mm, obtained from New England Nuclear, Boston, MA). A pulse dose of 30 μCi in 1 ml neutral bicarbonate solution was given rapidly with a saline flush through a femoral venous catheter (5). At 15, 30, 60, 120 and 180 min after injection of the labeled mevalonate, 2.5 ml of blood were withdrawn and placed in tubes containing ethylenediaminetetraacetic acid. Plasma was separated immediately by centrifugation for 10 min at 12,000 rpm at 4°.

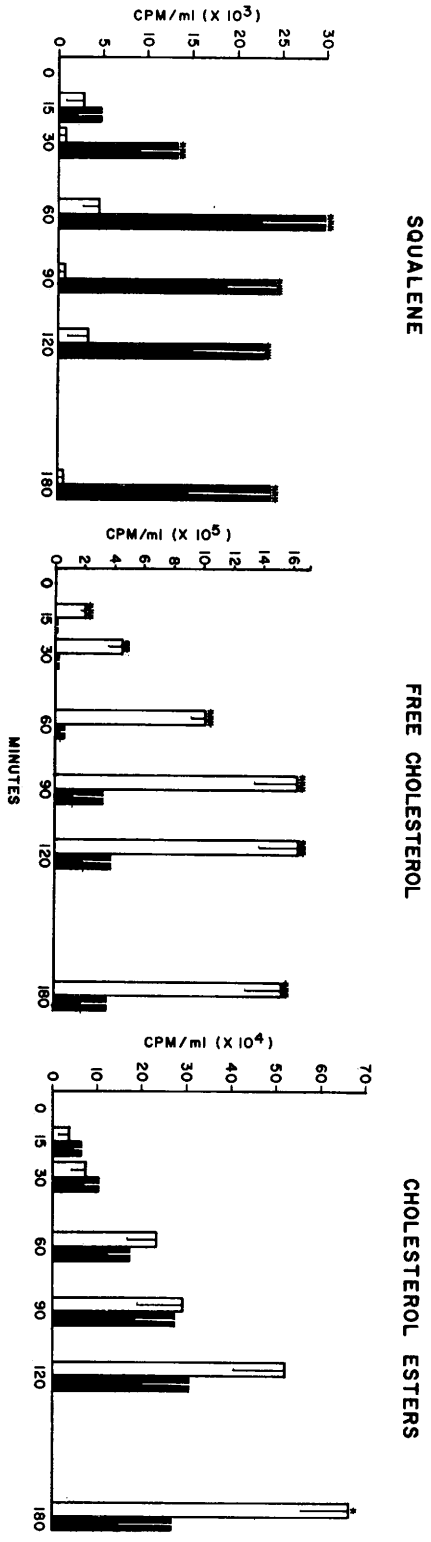
At the end of the 180 min period, the monkeys were killed with Lethane (A. J. Buck and Son, Baltimore, MD). As in earlier studies (5), the livers were removed immediately, quick-frozen in an acetone–dry ice bath, and stored at −60° until analysis. Student's t test was used to compare laboratory findings in control and hypercholesterolemic groups.

Results. When compared with the control group, the monkeys receiving the cholesterologenic diet developed an elevation of plasma cholesterol, and phospholipids but a depression of triglyceride concentrations. These changes became evident within 1 month on the diet and stabilized at the values shown in Table I. No differences in mean body weights were observed between the 2 groups during the course of the study.

Fever, lethargy and polydipsia were noted within 12 hr after the intravenous inoculation of *D. pneumoniae.* At the time of ^{14}C-mevalonate injection, 24 hr postinoculation, body temperatures were elevated (102.5 to 104°), pneumococcal bacteremia was present, and a 2- to 4-fold increase in white blood cells was observed in all monkeys. Values for SGOT did not become elevated. During the 27 hr study period, the 2 groups did not differ clinically or pathologically in the apparent severity of infection. No sites of pneumococcal localization were evident in the bacteremic monkeys; atheromatous lesions were not present in the hypercholesterolemic monkeys.

When studied 24 hr after inoculation with pneumococci (after 36 hr of fasting), both the control and hypercholesterolemic monkeys had developed a significant depression of both plasma cholesterol and phospholipid values below their respective preinfection base lines (Table I). Although the absolute magnitude of these depressions was greater in the hypercholesterolemic group, relative changes were greater during infection in the monkeys with normal base line values. The percentage of cholesterol in the ester fraction was not significantly different in the 2 groups of monkeys.

Triglyceride values of the hypercholesterolemic group increased whereas those of monkeys on the control diet decreased during in-



fection. No appreciable change in free fatty acid (FFA) values of either group was observed. Squalene concentrations in plasma were consistently less than 50 μg/100 ml and could not be quantitated with accuracy.

Figure 1 depicts the incorporation of the ^{14}C label into plasma squalene, free cholesterol and cholesterol ester fractions of both experimental groups. A significant increase in radioactivity was noted throughout the sampling period in the squalene fraction of the hypercholesterolemic monkeys when compared with values in the control group of infected monkeys.

Initial appearance of ^{14}C in the free cholesterol fraction of the infected control monkeys occurred within 15 min and was followed by a marked increase. In contrast, radioactivity in the plasma free cholesterol was barely detectable for the first 60 min in the hypercholesterolemic group. At 90 min, control group values were 4-fold greater than those of hypercholesterolemic monkeys. This difference persisted throughout the study period.

Initially the rate of appearance of ^{14}C radioactivity into cholesterol esters was equivalent in both groups, but by 180 min values for normal diet monkeys were approximately 2-fold greater than for hypercholesterolemic monkeys.

Total cholesterol content of the liver was significantly ($p < .01$) increased in hypercholesterolemic monkeys (7.5 $\pm$ 0.8 mg cholesterol/g liver vs 4.8 $\pm$ 0.2 in control monkeys) while hepatic specific activity appeared to be slightly depressed.

Discussion. Although plasma cholesterol concentrations were significantly depressed during pneumococcal infection in both control and hypercholesterolemic monkeys, this study revealed marked differences between groups in the dynamic response of cholesterogenesis. The rates at which labeled meva-

FIG. 1. Sequential patterns of appearance of radioactivity in plasma lipid fractions are shown as the mean and standard error values for normal (□) and and hypercholesterolemic (■) monkeys. ^{14}C-Mevalonate was given at time 0, 24 hr after pneumococcal infection was initiated. p:* $< .05$; ** $< .01$; *** $< .001$.

lonate was incorporated into the free and esterified fractions of plasma cholesterol were increased during pneumococcal infection in the present group of control monkeys as they were in monkeys studied earlier (4). During these studies in monkeys with normal baseline-period lipid metabolism, acute infection appeared to increase the rate of both synthesis and peripheral utilization of cholesterol (4).

In comparison, there was a marked slowing in the timing and quantitative appearance of ^{14}C in the free cholesterol fraction of plasma following infection of hypercholesterolemic monkeys. A part of this difference may be ascribed to a greater dilution of newly formed ^{14}C-cholesterol within the combined hepatic and plasma pools of nonlabeled cholesterol which were approximately twice the control value in the hypercholesterolemic monkeys. But other observations suggest that the between-group differences were far greater than could be accounted for by a discrepancy in pool size.

Unlike the findings for free cholesterol, appearance of ^{14}C in the cholesterol ester fraction of plasma was equivalent in both control and hypercholesterolemic monkeys during the first 90 min period after mevalonate injection. Only during the second 90 min period did cholesterol ester values of the 2 groups become divergent. An even greater difference was manifested by the marked accumulation of ^{14}C in the squalene fraction of plasma in infected hypercholesterolemic monkeys. These observations suggested that infection in the hypercholesterolemic group was associated with: (a) some degree of blockade in the cholesterogenic process, and (b) a greater than normal rate of conversion of newly synthesized cholesterol to an esterified form.

Increased radioactivity in the squalene fraction of infected hypercholesterolemic monkeys and its stability over a 180 min period suggest partial inhibition of cholesterogenesis at the metabolic step immediately after squalene synthesis. This finding was in contrast to published observations suggesting that variables such as starvation or the feeding of excess cholesterol may inhibit cholesterol synthesis at a step prior to the formation of mevalonate (9). The observation in infected hypercholesterolemic monkeys was in keeping, however, with the report of Gould and Swyryd (10) who emphasized that prolonged cholesterol feeding could lead to a progressive inhibition of cholesterol synthesis beyond the mevalonate step. Alternatively, increased radioactivity in the squalene fraction may have been due to enhanced production by other tissues such as kidney, whose importance in the metabolism of mevalonate with subsequent failure of conversion to cholesterol was recently shown by Raskin and Siperstein (11).

Gallin, O'Leary, and Kay (12) reported that plasma squalene concentrations were increased in patients with influenza. In the present study, however, the altered status of lipid metabolism prior to infection, rather than the causitive organism, could be singled out as the factor which led to an atypical response of squalene metabolism during illness. Similarly, between-group differences in responses of cholesterol synthesis and triglyceride utilization could be explained by differences in the preinfection metabolic status of the two host groups since all monkeys were inoculated with identical infecting organisms.

An understanding of infection-related metabolic responses must take into consideration the prior dietary intake of the host with respect to increased as well as decreased alimentation. Differences in cholesterol intake of individual patients and the incidence of hypercholesteremia in our population may contribute to the often conflicting reports of variation in plasma cholesterol concentrations during infection (1).

Summary. The presence of chronic, experimentally induced hypercholesterolemia in rhesus monkeys led to atypical patterns of lipid response during acute pneumococcal infection. When compared to infection in previously normal monkeys, hypercholesterolemic monkeys showed an impaired synthesis of cholesterol with accumulation of squalene in the plasma, an increased conversion of newly formed cholesterol to an esterified form, and an impaired utilization of triglycerides.

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