Effects of Acute Infection on Cholesterogenesis in the Rhesus Monkey[1] (35951)

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(Introduced by Robert W. Wannemacher, Jr.)
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During a variety of infectious illnesses, values for the concentration of cholesterol in plasma have been reported to increase, decline, or remain unchanged (1). These differences in the response of cholesterol metabolism to infection have been ascribed in part to possible organism-specific effects (2) or to a possible block in cholesterol synthesis at the squalene step (3, 4).

Interpretation of earlier reports, however, has been hampered by the fact that they document only the alterations in plasma concentrations and accordingly, provide little insight into the dynamics involved in cholesterol synthesis, transport, or utilization. This report describes the initial studies on cholesterogenesis during experimentally induced acute infection of rhesus monkeys with either *Diplococcus pneumoniae* or *Salmonella typhimurium*.

Materials and Methods. Twenty-four healthy rhesus monkeys, weighing 3–4 kg, were maintained on a commercial diet (Purina Monkey Chow, Ralston Purina Co., St. Louis, MO) for a minimum of 4 months under optimal animal colony conditions and then held for 1 week in individual restraint chairs. Monkeys were studied in pairs with each infected monkey being matched with a sham-inoculated control of similar weight and sex. Six monkeys were infected with *D. pneumoniae* and 6 with *S. typhimurium*.

Stock cultures (10^8 organisms/ml) of Type I *D. pneumoniae* were stored at −60°. Bacterial virulence was maintained by mouse passage and identity was verified by gram stain, growth on differential media, and the presence of a Quellung reaction with Type I antiserum. For use as an inoculum, 0.2 ml of stock culture was incubated for 18 hr in heart infusion broth containing normal rabbit serum and defibrinated sheep red blood cells. Organisms were recovered by centrifugation, resuspended in normal saline, and inoculated as a 1 ml inoculum containing 10^8 cells.

Stock cultures (10^8 organisms/ml) of *S. typhimurium* (MIT strain) containing 17% by volume of glycerin were also stored at −60%. Inocula were prepared by streaking 0.2 ml of the stock culture on short nutrient agar slants and incubating at 37° for 18 hr. The nutrient slants were then washed with 3 ml of phosphate buffered saline containing 1% normal rabbit serum. Organisms from this wash were resuspended in 1 ml of saline (10^8 cells) for inoculation.

Measurement of total and differential white blood count, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) (Sigma Test Kit No. 505, a product of Sigma Chemical Co., St. Louis, MO), and blood cultures were made serially during the postinoculation period. Body temperatures were obtained with rectal probes.

Cholesterogenesis was studied through the use of ^{3}H-mevalonate, (DL-mevalonic-5-^{3}H, 380 mCi/mmole; obtained from New England Nuclear, Boston, MA). By use of this metabolite, the effects of fasting or prior dietary intake, which are believed to influence cholesterol synthesis at the enzymatic steps

[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. Presented: Fed. Amer. Soc. Exp. Biol., Chicago, IL, April, 12–17, 1971.

prior to mevalonate formation, were minimized (5). Cholesterogenesis was measured 48 hr after inoculation with *D. pneumoniae* or 96 hr after *S. tryphimurium*. Indwelling femoral arterial and venous catheters were inserted 36 hr prior to mevalonate injection in each pair of monkeys; their access to food (but not water) was stopped at that time.

At the time of study, 100 μCi of mevalonic acid was given in a pulse dose through the venous catheter in a neutral bicarbonate solution.

At 30, 60, 120, and 180 min after injection of the labeled mevalonate, 2.5 ml of blood were withdrawn and placed in tubes containing EDTA. Plasma was removed from cells by centrifugation for 10 min at 12,000 rpm at 4°.

The lipids in 1 ml of plasma were extracted with chloroform–methanol by the method of Sperry and Brand (6). Fifty micrograms of lipid was chromatographed on thin-layer silica gel, 0.25 mm (Brinkman Instruments, Inc., Westbury, NY), in an *n*-heptane solvent system that separates squalene from other lipoidal material and in petroleum ether–diethyl ether–acetic acid (80:20:1) which separates the major lipid classes (7). Lipid fractions on thin-layer plates were detected by spraying with 50% sulfuric acid followed by heating at 150° and identified by comparison with reference standards. Thin-layer densitometry techniques were used to quantitate the proportions of free and esterified cholesterol (8).

Another portion, 50 μg of lipid was subjected to identical chromatographic techniques and identified by comparison with reference standards. The cholesterol, cholesterol esters, and squalene areas were scraped and eluted from the thin-layer plates with 10 ml of counting cocktail, (Scintolute, Isolab Inc., Elkhart, Ind.) and 0.5 ml of methanol into glass counting vials. Tritium content was assayed in a liquid scintillation counter (Nuclear Chicago, Des Plaines, IL).

Total cholesterol, phospholipid, triglycerides, and free fatty acids were analyzed by automated techniques (9).

At the end of the 180 min study period, the monkeys were killed with Lethane (A. J. Buck and Son, Baltimore, MD). The livers were immediately removed, quick frozen in an acetone–Dry Ice bath, and stored at −60° until analysis. Total hepatic digitonide precipitable sterol content and radioactivity were determined by the method of Baruch and Chaikoff (10).

Gross and microscopic pathology were studied at the end of the study period. Student's *t* tests were used to compared control and experimental test values of each group.

Results. Fever, lethargy, and polydypsia were noted 12 hr or less after the intravenous inoculation with pneumococcus and reached a peak (103.5–105.4° F range) at 48 hr, the time of study. Bacteremia and a 2- to 4-fold increase in polymorphonuclear cells were demonstrated at 48 hr in all monkeys. The SGPT and SGOT values did not become elevated. Histologic lesions consisted of acute or subacute interstitial pneumonia, splenitis, and focal areas of hepatic necrosis.

The *S. typhimurium* infection ran a slower clinical course. An abrupt "endotoxic" type fever was noted in 2 monkeys within 6 hr of inoculation, but disappeared by 24 hr. Fever developed in all monkeys between 48–72 hr with a peak response (103.8–105.4° F) at 96 hr. Anorexia and lethargy closely followed the febrile state. *Salmonella* bacteremia was demonstrated in 5 of the 6 monkeys through 96 hr. Leukopenia (25–90% decrease from base line) typically developed within 72 hr of inoculation. A slight, but not significant, increase in SGOT and SGPT values was noted at 72 and 96 hr after inoculation in each infected monkey. The histologic lesions included acute diffuse hepatitis, splenitis with vasculitis, adrenal inflammation, and leukocyte sludging in the vessels of the liver, spleen, and lung.

Table I presents total cholesterol, triglyceride, phospholipid, and free fatty acid levels for both infections. Free fatty acid values were depressed during the *Salmonella* infection while triglycerides were markedly increased. Phospholipid and cholesterol values remained unchanged. The percentage of cholesterol in the ester fraction was not significantly different from the control group during either infection. Concentrations of

TABLE I. Plasma Lipid Concentrations.
Values shown are (mg/100 ml; mean ± standard error) of 6 monkeys/group.

Group	Control	Infected	p
Pneumococcus			
Cholesterol	111 ± 3	109 ± 3	NS
Phospholipid	326 ± 12	354 ± 15	NS
FFA	27 ± 2	29 ± 1	NS
Triglyceride	48 ± 3	55 ± 3	NS
Salmonella			
Cholesterol	112 ± 8	114 ± 4	NS
Phospholipid	365 ± 9	319 ± 21	NS
FFA	19 ± 1	15 ± 1	<0.05
Triglyceride	55 ± 5	179 ± 10	<0.001

squalene were less than 50 μg/100 ml and could not be quantitated with accuracy.

Figure 1 depicts the incorporation of tritium derived from labeled mevalonic acid into the plasma squalene, free cholesterol, and cholesterol ester fraction. Squalene values were highest at 30 min and declined thereafter. At no point studied did significant differences of squalene radioactivity exist between the infected and control groups during either pneumococcal or *Samonella* infection.

Initial appearance of radioactivity in the free cholesterol fraction was seen within 30 min with a significantly increased appearance of the label in the free cholesterol fraction ($p<0.05$) at 1 hr after mevalonate injection during the pneumococcal infection, and at 2 hr during the *Salmonella* infection ($p<0.05$). More marked differences were seen at the 3 hr interval during both infections with an approximate 2-fold increase noted in the infected monkeys ($p<.01$).

During pneumococcal infection, the rate of appearance of the radioactive tag into the plasma cholesterol ester fraction was not enhanced, and no significant differences were seen between control and infected monkeys. In contrast, monkeys with *Salmonella* infection showed a significant inhibition of incorporation of tritium into the ester fraction which was evident within 30 min after the mevalonate injection.

The total cholesterol content of the liver and its specific activity 3 hr after mevalonate injection were not different when pneumococcus-infected monkeys were compared with their matched controls. Monkeys infected with *Salmonella* showed a significant ($p<0.01$) reduction in hepatic cholesterol content to 81% of that found in their control pairs, but the specific activity was similar to that of the controls.

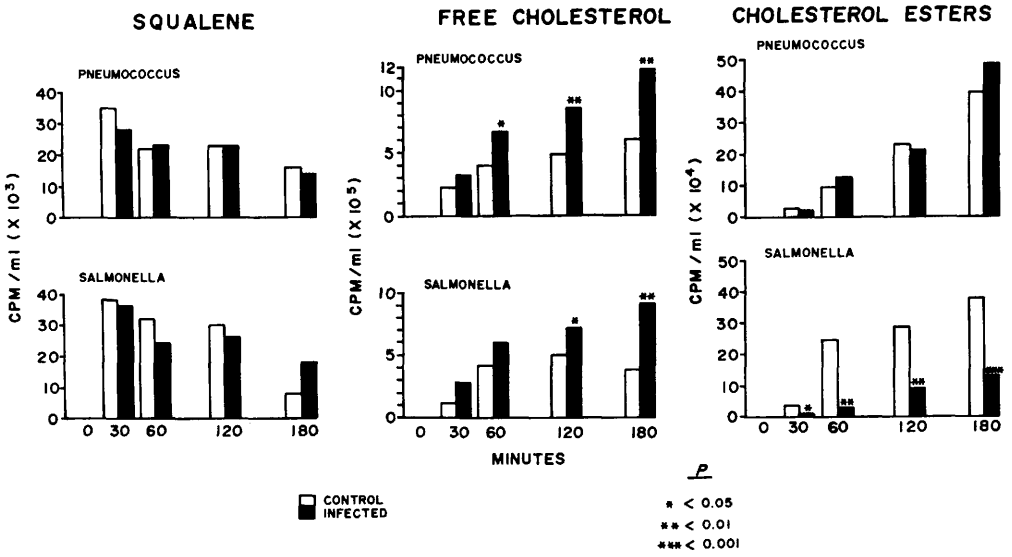

FIG. 1. Incorporation of ^{3}H-mevalonic acid into plasma squalene, free cholesterol and cholesterol esters during *D. pneumoniae* and *S. typhimurium* infections in rhesus monkeys. Values shown are the mean of 6 monkeys/group.

Discussion. Despite the maintenance of normal concentrations of free and esterified cholesterol in plasma, the dynamic aspects of cholesterogenesis were markedly altered in monkeys studied early in an acute infectious process due to either gram-negative or gram-positive pathogens.

A measurement of plasma concentration represents the algebraic summation at a given point in time of cholesterol influx and outflow from the plasma pool. Assuming that pool size has remained essentially unchanged, the present data suggest an infection-related increase in the rate of influx of newly synthesized free cholesterol into plasma. Actual measurements by the use of the Evans blue dye test indicate a normal or slightly expanded plasma volume in monkeys with infections of similar etiology, severity, and duration (G. L. Bilbrey, personal communication). Only a diminished plasma pool size would invalidate our interpretation that cholesterogenesis was enhanced.

Increased rates of influx of cholesterol into plasma together with unchanged values for its concentration provide indirect evidence suggesting that the rates at which cholesterol disappears from plasma are increased similarly. Falling plasma cholesterol values, seen in more advanced stages of these infections in monkeys (11), as well as during acute sandfly fever in man (12), would indicate that rates of cholesterol removal from plasma eventually exceeded those of input.

Precise measurements of squalene concentration or its specific activity in plasma could not be obtained. However, the rapid appearance and subsequent disappearance of radioactivity in the squaline fraction, as well as the close similarities of total counts between infected and control monkeys, provided indirect evidence that the production and utilization of squalene was neither impaired nor enhanced.

Despite evidence for increased rates of cholesterol synthesis and flux through the plasma pool, the esterification of free cholesterol was not enhanced in the pneumococcal infection and was actually impaired in monkeys with salmonellosis. These findings may indicate a greater need or utilization by host tissues for cholesterol in the nonesterified form.

The mechanism responsible for the reduction in the cholesterol ester fraction during the *Salmonella* infection remains unclear. This may be partly explained by hepatocellular damage, a mechanism known to depress the ester fraction (13). However, the sites of esterification are not well defined and liver enzyme elevations (SGOT and SGPT) were similar during both infections. Depressed content of liver cholesterol in *Samonella*-infected monkeys may be explained by either an organism-specific effect or an increased degradation rate during infection. The interval after inoculation may also be an influencing factor.

The febrile state and blood flow to the various organs could be contributing factors to this enhanced turnover. However, no direct correlation with body temperature could be obtained, and one might expect a possibly diminished blood flow to the splanchnic areas at the height of the infectious state.

Depressed free fatty acid (FFA) and heightened triglyceride levels in plasma of *Salmonella*-infected monkeys are in keeping with other studies which suggest an increased uptake of tagged FFA by the liver followed by an increased hepatic release of labeled triglycerides into plasma (11). The present studies also serve to indicate that *Samonella* infection has a more profound influence on lipid metabolism that does infection with the pneumococcus.

These studies provide data relative to cholesterogenesis during acute infections. The acute nature of the illnesses precluded the inclusion of measurements during steady state conditions which are necessary for a complete evaluation of cholesterol turnover and degradation. The present studies, however, point to possible organism-specific effects on metabolic pathways responsible for synthesis of both free cholesterol and cholesterol esters and indirectly of their degradation. These findings, as well as those we have previously found (11, 12), concerning increased free fatty acid turnover and triglyceride synthesis, are thus suggestive of a marked enhancement of lipid turnover during an infec-

tious illness.

Summary. Rhesus monkeys acutely infected with *D. pneumoniae* or *S. typhimurium* exhibited an increased rate of ^{3}H-mevalonic acid incorporation into free cholesterol in comparison with values observed in control monkeys. When studied early in either infection, there was no evidence for an inhibition of squalene synthesis or its conversion to cholesterol. The estimated volume of plasma cholesterol did not change. Total hepatic cholesterol was decreased and entry of radioactivity into plasma cholesterol esters was reduced in the *Samonella*-infected monkeys.

The authors are grateful to Miss Karen Bostian, Sp 5 Terry D. Shultz and Sp 4 John Drummond for expert technical assistance and to Mrs. Phylis Rinehart and Mrs. Phebe Summers in the preparation of this manuscript.

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Received June 18, 1971. P.S.E.B.M., 1971, Vol. 138.