THE EFFECT OF EGG YOLK CHOLESTEROL ON SERUM LIPID PATTERNS AND SERUM CHOLESTEROL SPECIFIC ACTIVITY OF HEALTHY YOUNG MEN

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

A metabolic study was undertaken to examine the response of serum lipids and serum cholesterol specific activity in healthy young men to cholesterol intakes of 113 to 1819 per day. Five diets, a 0-Egg, 1-Egg, 2-Egg, 3-Egg and 6-Egg diet were designed to differ in the content of egg yolk cholesterol only. A mixture of fats, formulated to simulate the fatty acid composition of the average Canadian diet, provided three-quarters of the calories from fat in each of the experimental diets. This mixture was prepared from lard, tallow, corn oil, butter and hydrogenated soybean oil. The remaining one-quarter of the calories from fat was provided by egg yolk lipid, simulated yolk fat prepared by combining Palm oil and hydrogenated soybean oil in a 2:1 mixture, or a combination of these depending upon the number of whole eggs in the diet. Using these fat mixtures ensured that fat provided 40% of the calories in the 3000 kcal diets and that the fatty acid composition of the five diets was similar.

The subjects were 10 young men 18-29 years of age. They resided in their own homes and maintained their usual activities throughout the study, but were required to eat their meals in the Home Economics facility on the university campus. Approximately 25 days prior to commencing the study, subjects were infused with 50 uc. of tritium labelled cholesterol. The 42 day metabolic trial was divided into 2 three-week diet periods. Two subjects were randomly assigned to each of the 5 diets for the first three weeks.

At the end of this diet period subjects were reassigned to different diets. This Incomplete Latin Square Design provided 4 replicate observations for each diet. Fasting venous blood samples were taken at the beginning of the study and at weekly intervals thereafter.

The level of cholesterol in the diet had a significant effect ($P<0.05$) on serum cholesterol levels. The relationship between dietary cholesterol and serum cholesterol was linear ($P<0.01$) and highly correlated ($r^2 = 0.90$). The relationship between the number of whole eggs consumed and serum cholesterol concentration is defined by the equation: serum cholesterol (mg/dl) = 147.7 + 11.9 (the number of whole eggs per day). Thus, for each additional egg in the diet (300 mg cholesterol) serum cholesterol increased by approximately 12 mg/dl. The effect of dietary cholesterol on serum phospholipids and triglycerides was not significant. The pattern of response in serum phospholipids was similar to that of serum cholesterol butthe magnitude of response was more variable. With an increase in egg consumption the proportion of cholesteryl arachidonate in the cholesteryl esters increased slightly, less than 5%, but consistently and this appeared to be primarily at the expense of cholesteryl linoleate. The proportion of saturated, monounsaturated and polyunsaturated fatty acids of cholesteryl esters was not altered by a change in egg consumption.

There was no significant change in the slope of the plasma specific activity - time curve for any subject when the level of cholesterol in the diet was changed. However, for several of the

subjects the change in serum cholesterol, consequent to a change in dietary cholesterol intake, was accompanied by a small change in the slope of the plasma specific activity - time curve. These changes are likely to be a result of an increase or decrease in absorption of cholesterol and thus an increase or decrease in the dilution of the plasma label, respectively. The transient nature of these changes suggests that in the human compensation for an increase or decrease in absorption of cholesterol occurs rapidly.

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INTRODUCTION

Death as a consequence of a compromised circulation to the heart, brain, kidneys and limbs is the most prominent cause of mortality operating in affluent societies today (Kannel, 1974). There is much evidence to suggest that modification of our life-style by modern technology - which has replaced manpower with machines thus diminishing the need for physical exercise while at the same time providing a surfiet of rich food and drink - has contributed to the increased toll by cardiovascular disease. That atherosclerosis is the leading cause of death in North America and that alteration of our environment may be promoting it demands some kind of action.

Atherosclerosis is a disease characterized by the accumulation of lipid substances in the walls of large blood vessels (Gresham, 1972). The disease is manifest as myocardial infarction or angina pectoris when the coronary arteries are involved and as stroke when the cerebral arteries are involved. It is a disease that evolves under the influence of multiple contributors and to date no single essential factor, without which the disease fails to occur, has been identified. While the general tendency to deposit lipid in the arterial walls is largely determined by the level of blood lipids and blood pressure, dynamics of blood flow, arterial calibre and the integrity of the vascular intima also influence whether and where atheromata will form (Altshule, 1974).

These parameters are under the influence of genetic, cultural and environmental factors. Of the cultural and environmental variables known to increase the risk of atherosclerosis many are under the control

of the individual; dietary habits, sedentary living habits, smoking habits and emotional stress. Genetic influences regarded as predisposing factors in atherosclerosis are generally not perceived as responsive to preventative intervention; racial influences, sex differences, familial history of vascular disease and diabetes mellitus.

The concept of multiple continuous interrelated variables has confounded delineation of the degree to which each of these contributes to the atherosclerotic process. Abnormalities considered atherogenic are more a matter of degree than kind - blood lipids, blood pressure and glucose tolerance. These biological factors are graded characteristics of normal body constituents, continuously distributed within the population with no discernible critical values separating diseased fron non-diseased individuals. As well, the complex tissue histology and haemodynamics influencing the evolution of atherosclerotic lesions, the inability at present to accurately assess the presence and extent of the disease in living persons and the lack of a satisfactory experimental animal model for study of the disease process have further complicated efforts to identify and isolate these predisposing factors.

Although cardiovascular disease has been correlated with a number of risk factors the one that stands out more than any other in clinical tests, animal experimentation and epidemiological observations is the association of coronary disease with blood lipid elevations. All serum lipids have been incriminated in atherogenesis: cholesterol, triglycerides, phospholipids and fatty acids as well as the associated lipid-protein structures which exist for lipid transport.

A disproportionate amount of coronary heart disease in the

general population develops among individuals with serum cholesterol values greater than 250 mg/ dl. (Dawber, 1962; Kannel, 1971; Schrimshaw and Gunzman, 1968). And, hypercholesterolemia appears to be a factor common to all forms of coronary heart disease, hence interest has focused on serum cholesterol. Epidemiological studies such as the Framingham Enquiry and the National Diet Heart Study have shown that diseases associated with hypercholesterolemia are also associated with premature atherosclerosis and that the risk of developing coronary heart disease is proportional to the elevation of blood cholesterol (Gordon, 1971; National Diet Heart Study Research Group, 1968). Countries with high average serum cholesterol values among their inhabitants report high coronary death rates, those with low values report lower rates (Epstein, 1965; Carlson and Bottiger, 1972). This association between blood cholesterol and atherogenesis has also been substantiated by clinical tests and animal experimentation: persons with inborn errors of cholesterol metabolism exhibit precocious development of atherosclerotic disease (Kannel, 1974); atherosclerotic deposits have abnormally high cholesterol concentrations and concentrations as high as 500% of those in normal aorta have been reported in fatty streaks of human aorta (Insull and Bartsch, 1966); movement of cholesterol from blood into atherosclerotic deposits has been demonstrated by Jagannathan et al (1974); inducing hypercholesterolemia in experimental animals produces atherosclerotic deposits and studies with dogs, fowl and primates indicate that these deposits can be made to regress by lowering serum cholesterol (Wissler et al, 1968; Armstrong et al, 1970).

Although the most intense focus has been directed toward serum

cholesterol and its relationship to atherosclerosis several other blood lipids have also undergone scrutiny in this regard. Turpeinen et al (1968) have stated that the risk for coronary disease can be shown to rise in relation to the serum phospholipid level. The significance of this is difficult to establish as phospholipid levels have been found to parallel those of serum cholesterol (Connor et al, 1961; Erickson et al, 1964; and Anderson et al, 1976). Insull and Bartsch (1966) have also reported phospholipid values in atherosclerotic deposits which are 150% of those in adjacent normal intimae. Since 1959 when Albrink and Mann suggested that elevated serum triglycerides might be related to the pathogenesis of heart disease several investigators (Brown, 1959; Albrink et al, 1961; Allard and Goulet, 1967; and Albrink, 1973) have demonstrated a firm relationship between elevated serum triglycerides and coronary heart disease. Carlson and Bottiger (1972) and Allard and Goulet (1967) have reported that coelevations of serum triglycerides and cholesterol are more critical in producing morbidity than an increase in either lipid component alone. And, Ho et al (1974) has observed that serum phospholipid and triglyceride levels of US whites are linearly related to serum cholesterol levels even in the presence of elevations in serum cholesterol.

Results of numerous controlled clinical trials have established that dietary lipids have an effect on serum lipid patterns in man. Diets high in saturated fats, containing high proportions of C12:0, C14:0 and C16:0 fatty acids have been shown to elevate serum cholesterol (Grande et al, 1972; Cobden, 1975). Diets containing high proportions of poly-unsaturated fats are generally conceived of as having a cholesterol

depressing effect (Sodhi et al, 1967; Nestel et al, 1975). Studies have also shown that dietary cholesterol has a cholesterol elevating effect (Mattson et al, 1972; Anderson et al, 1976). The changes induced in serum cholesterol by a change in dietary lipids are generally paralleled by changes in the serum phospholipid concentration (Connor et al, 1964; Grande et al, 1965; Cobden, 1975). It has also been suggested that the serum triglyceride concentration is responsive to changes in dietary fat and dietary cholesterol intake (Connor et al, 1964; Grande et al, 1972) and that the serum cholesteryl esters, phospholipid fatty acid esters and triglyceride fatty acid esters can be altered by changing the fat component of the diet (Ahrens, 1957; Nestel et al, 1965).

To counteract the present incidence and mortality from atherosclerosis in North America, the diet should be modified to minimize elevations of serum lipids. Epidemiological studies have shown that populations who consume diets low in fat, such as those which characterize tribal African and rural Japanese populations, have a much lower incidence of atherosclerotic disease (Wynder et al, 1972). Metabolic studies have indicated that dietary alterations should include, besides a decrease in total fat, replacement of animal fats with vegetable oils in order to increase the proportion of polyunsaturated fatty acids and to decrease the proportion of saturated fatty acids and cholesterol in the diet. But generally these diets tend to be less acceptable to the North American population at large.

Many of the early studies which implicated various fats in the diet as being atherogenic were conducted with institutionalized subjects who were fed formula diets or with subjects with manifest hyper-

lipidemia and/or atherosclerosis. It now seems appropriate to investigate the effects of these dietary fats on serum lipid patterns in healthy free living individuals in order to determine which dietary alterations are truly beneficial with respect to serum lipid levels. At the same time it is imperative that it be determined by what mechanism the dietary alteration effects these changes in serum lipids. That is, it is unacceptable if a decrease in serum lipids is brought about by deposition of lipid in tissues as it is likely that localized accretion of lipid in arterial walls is critical in the development of atherosclerosis. With these facts resolved it will be possible to make realistic recommendations with respect to preventative measures that will be beneficial to the North American society at large.

A very important food, in terms of the Canadian economy as well as the nutrient needs of Canadians, which contains a high level of dietary cholesterol is the egg. Since the general recommendation to Canadians is to decrease their egg consumption to 3 eggs per week the effect of egg yolk cholesterol on serum lipids of healthy free living individuals needs to be better defined to justify these practices. The present study investigated the effect of egg yolk cholesterol on serum lipids in healthy young men consuming a mixed diet and the mechanism whereby dietary cholesterol effects a change in serum cholesterol levels.

OBJECTIVES

The primary objective of this research was to investigate the effect of dietary cholesterol from egg yolk on cholesterol metabolism of healthy young men. Five levels of cholesterol intake were achieved by incorporating 0, 1, 2, 3 and 6 whole eggs into diets consisting of natural foods in which 40% of calories were supplied by egg yolk lipid and a mixture of fats similar to those in the Canadian diet. The diets resembled the average Canadian diet with respect to proportion of calories from fat, protein and carbohydrate and fatty acid composition but provided five levels of dietary cholesterol (113, 441, 781, 1058 and 1819 mg/day). The effects on cholesterol metabolism were assessed by determining serum cholesterol levels and changes in the specific activity of serum cholesterol. Secondary objectives included examination of the response of serum triglycerides, serum phospholipids and fatty acid patterns of serum cholesteryl esters to egg cholesterol.

PART I

THE EFFECT OF DIETARY CHOLESTEROL ON SERUM LIPID PATTERNS

REVIEW OF LITERATURE

A. SERUM CHOLESTEROL

The experiments of Anitschow in 1933, in which diets high in cholesterol caused increased plasma lipid levels and produced in rabbits and chickens lesions analogous to atherosclerotic lesions seen in humans, made this substance suspect as a factor in human food of etiological importance in human atherosclerosis. And, until cholesterol was shown to be synthesized in the body by Rittenberg and Shonheimer in 1937 it was considered that the cholesterol of the diet was the primary determinant of serum cholesterol levels. In fact, in 1933 Shonheimer had demonstrated that a cholesterol free plant fat diet caused a marked decrease in the serum cholesterol concentration of one hypercholesterolemic female patient. Further investigation of vegetarian diets by Groen in 1952 and Hardinge in 1954 indicated that strict vegetarians had lower serum cholesterol values than partial vegetarians who ate eggs and dairy products and that non-vegetarians had the highest serum cholesterol values. Kinsell, in 1952, reported that diets high in vegetable fat produced dramatic decreases in serum cholesterol levels, whereas isocaloric substitution of animal fats in these diets caused the levels to rise promptly. Kinsell also observed that adding cholesterol to the vegetable fat diets did not reverse these effects (Ahrens, 1957). These

findings were confirmed by Ahrens et al (1954) and about this time the emphasis shifted from the effect of dietary cholesterol on serum cholesterol to the effect of animal versus plant fats and subsequently to saturated versus unsaturated fats in the diet.

Prior to 1960 the greatest number of reports in the literature supported the hypothesis that dietary cholesterol was insignificant in the determination of serum cholesterol concentration (Ahrens, 1957). Turner et al (1939) added 10 gm of crystalline cholesterol in milk to the daily diet of a group of patients for six weeks without altering significantly the serum cholesterol level. Ahrens et al (1954) have demonstrated that when serum cholesterol levels were depressed by feeding diets high in corn oil the addition of 2 gm per day of crystalline cholesterol produced no significant elevation in serum cholesterol levels and the administration of 4 and 8 gm per day led to small but significant increases in cholesterol levels. The addition of 600 mg cholesterol per day to formula diets containing 40% of calories as lard also failed to bring about further increases in serum cholesterol. Beveridge et al (1955) noted that when vegetable fat diets, with added cholesterol and without cholesterol added, were ingested the plasma cholesterol fell at approximately the same rates. Bronte-Stewart et al (1956) added 3 gm of cholesterol per day to diets without losing the cholesterol depressant action of unsaturated fats. And, Keys et al (1956) after extensive investigation of the effect of dietary cholesterol on serum cholesterol reported a lack of effect on the basis of (1) long term observations of men eating diets low and high in cholesterol; (2) epidemiological survey data; (3) experiments in which free living men halved or doubled their cholesterol intakes for many months; (4) experiments in which 500 - 600 mg crystalline

cholesterol per day was added to a rice fruit diet; and (5) experiments which tested threefold variations in cholesterol intake in mixed diets containing 66 gm total fat per day.

Studies concerned with the effect of dietary cholesterol on serum cholesterol have taken two forms: crystalline cholesterol has been added directly or a natural food that contains a high concentration of cholesterol, such as, egg yolk or butterfat, has been incorporated into the diet. From the above discussion it can be seen that the ingestion of even large amounts of crystalline cholesterol had regularly failed to induce significant elevations in serum cholesterol.

On the other hand, the feeding of cholesterol in the form of egg yolk has resulted in increased serum values. Okey et al (1933) observed a rise in the mean serum cholesterol level of young women when egg yolks were added to low cholesterol, low fat diets. Steiner et al (1941) fed patients diets containing 2.4 gm cholesterol in the form of egg yolk daily for 6 - 10 weeks and obtained an average increase of 102 mg/dl in serum cholesterol concentration. Messinger et al (1950) found that a diet containing 3.75 gm of cholesterol per day in egg yolk resulted in an increase in serum cholesterol from 27 to 61 mg/dl whereas 30 gm of crystalline cholesterol per day had little effect. Bronte-Stewart et al (1956) reported a pronounced rise in serum cholesterol of 60 mg/dl when 10 eggs replaced a fat with a low iodine value in a cholesterol free diet. The results of these metabolic trials were largely disregarded as addition of egg to the diets generally altered the amount and type of dietary fat as well as dietary cholesterol. And, it was concluded that if crystalline cholesterol in the diet could not effect this change

then some other component of egg yolk was the hypercholesterolemic agent.

A number of reports by Beveridge et al (1959, 1960), Connor et al (1961a, 1961b, 1964), Erickson et al (1964) and Bronte-Stewart et al (1963) which indicated that serum cholesterol was significantly affected by feeding egg yolk, beef steak and butterfat, when only the cholesterol content of the diet was altered, reopened the question of the importance of dietary cholesterol in the determination of serum cholesterol. These studies together with the observations by Bronte-Stewart et al (1963), Steiner et al (1962), Connor et al (1961b) and Grande et al (1965) indicated that the quantitative relationship between dietary and serum cholesterol were dependent upon the degree of solubility of cholesterol in various fats and oils. That is, in order for cholesterol in the diet to exert its full effect it must be incorporated in the diet in a form suitable for digestion - dissolved in or closely associated with enough fat of the right type.

Bronte-Stewart and Wells (1963) and Beveridge and coworkers (1960) not only concluded that dietary cholesterol had a significant effect on serum cholesterol but also that the cholesterol response curve was linear over a range of intakes up to 500 - 600 mg dietary cholesterol per day. Upon reinvestigation of the problem, by feeding crystalline cholesterol to male subjects, Grande and associates (1965) concluded that serum cholesterol was a linear function of the square root of the cholesterol content of the daily diet over a range extending beyond 500 - 600 mg per day. They subsequently extrapolated this relationship to describe the effect of changing the level of cholesterol in the diet,

such that, the change expected in serum cholesterol was expressed by the equation: change in serum cholesterol (mg/dl)=1.5 (Z_2-Z_1) where Z_2 was the square root of the mg cholesterol/1000 kcal in diet 2 and Z_1 was the square root of the cholesterol content of diet 1 in mg/1000 kcal These researchers have also stated that results obtained by Beveridge (1960), Steiner et al (1962), Connor et al (1964) and Erickson et al (1964) were consistent with their prediction equation (Keys et al, 1965).

However, in the same year Hegsted et al (1965) reported that serum cholesterol was a linear function of the absolute level of cholesterol in the daily diet. These investigators concluded that cholesterol fed as a component of egg yolk and butterfat was a significant variable in determination of serum cholesterol. In this study the effect observed was an increase in serum cholesterol of approximately 5 mg/dl for each additional 100 mg of dietary cholesterol. Mattson and coworkers (1972) also demonstrated that dietary cholesterol fed as egg yolk at levels of 0, 126, 212, to 317 mg/1000 kcal had a pronounced effect on serum cholesterol levels and that the relationship was linear. These researchers noted that the change in serum cholesterol was related to a change in dietary cholesterol by the equation: change in serum cholesterol (mg/dl) = 1.60 + 0.118 (dietary cholesterol mg/1000 kcal). Thus, for each additional 100 mg dietary cholesterol/1000 kcal serum cholesterol was increased by approximately 12 mg/dl.

These latter two studies are in close agreement with respect to the nature of the relationship between dietary cholesterol and serum cholesterol. On the other hand, the relationship described by Grande and associates (1965) overestimates the effect of dietary cholesterol on serum cholesterol at low intakes and underestimates this effect at

high intakes of cholesterol compared to the results obtained by Mattson and Hegsted. As regression equations are primarily descriptive of the information from which they are derived, these differences may in part be attributed to the use of crystalline cholesterol in the dietary trials carried out by Grande and associates as opposed to the use of cholesterol in egg yolk and butterfat in the studies by the other two groups.

Several metabolic trials have been undertaken in order to establish if the effects of dietary cholesterol on serum cholesterol are independent of the effects of varying the fatty acid composition of the diet. Hegsted et al (1965), using multiple regression analysis, separated the effect of dietary cholesterol from the effects of total saturated, monounsaturated and polyunsaturated fatty acids as well as specific fatty acids on serum cholesterol. These authors observed that the response to dietary cholesterol was independent of the effects induced by dietary fats. Similar findings have been reported by Grande et al (1965), Connor et al (1964), Erickson et al (1964), Anderson et al (1976) and Nestel et al (1975).

A recent report in the literature by Slater and associates (1976) has challenged the hypothesis that dietary cholesterol has a significant and independent effect on serum cholesterol concentration in healthy free living individuals eating a mixed diet. These authors reported a series of three studies in which the addition of one or two eggs to the usual diets of free living subjects failed to cause significant increases in serum cholesterol concentration. In the first study reported, fifteen healthy male college students, aged 20 - 30 years, were fed 2 eggs per day in addition to their usual diets for six weeks. The plasma cholesterol concentration showed no change as determined by analysis of

variance, however, when each man served as his own control and paired differences were measured, during the third and fourth weeks of eating extra eggs the serum cholesterol values were significantly increased. After three weeks, on extra eggs, the cholesterol concentrations decreased so that by the end of the sixth week serum cholesterol values had returned to pre-experimental levels.

In the second study reported by Slater, a group of older men with a mean age of 51 years ate 1 additional egg per day for five weeks and then 2 additional eggs every second day for a three week period. It was found that 1 additional egg per day resulted in significant increase in serum cholesterol again only when the results were analyzed having each subject serve as his own control. Surprisingly though, while subjects consumed 2 additional eggs every other day serum cholesterol values returned to pre-experimental levels. A comparison of the calculated cholesterol intake from either a protein check list kept by subjects while they ate extra eggs or from a one day diet record kept in the same time period with a two day diet record kept by subjects in the pre-experimental control period indicated that only 7 or 8 of the 21 subjects whose data was reported actually increased their cholesterol consumption by 250 mg per day. The authors assumed the cholesterol content of one egg was 250 mg cholesterol, thus, if egg consumption was actually increased as believed it was at the expense of other cholesterol containing foods.

In the third study of this series a group of college students with a mean age of 24 years ate two additional eggs per day in their diets for eight weeks. The results of this trial suggest that the addition of two eggs per day to the diet caused no significant increase in serum cholesterol values. But, during the fifth, sixth and seventh weeks of

eating extra eggs serum cholesterol values were significantly decreased. By the end of the eighth week cholesterol values approached the pre-experimental control levels. The difficulties with this type of methodology are clearly demonstrated by the observation that during the pre-experimental and post-experimental control periods the serum cholesterol values for subjects were significantly different despite the assumption that their diets were the same in these two periods.

This series of studies strongly suggests that further evaluation of the effect of dietary cholesterol on serum cholesterol levels in healthy free living subjects is required but also that greater control of the dietary variable under investigation must be exercised in subsequent trials.

B. SERUM PHOSPHOLIPIDS AND TRIGLYCERIDES

Connor and associates (1961a; 1961b) reported that a change in dietary cholesterol resulted in a change in serum phospholipids comparable to the change observed in serum cholesterol concentration. They have reported that the addition of 475 mg of egg yolk cholesterol to the diet produced a mean increase in serum cholesterol of 66 mg/dl and in serum phospholipid of 46 mg/dl. The addition of 950 mg of egg yolk cholesterol to the diet produced a mean increase in serum cholesterol of 64 mg/dl and in serum phospholipid of 45 mg/dl while the addition of 1425 mg of egg yolk cholesterol increased serum cholesterol 77 mg/dl and serum phospholipids 34 mg/dl. Results obtained with crystalline cholesterol in the diet, although of considerably lower magnitude, also indicated that changes in serum cholesterol were parallelled by changes in serum phospholipids. On the other hand, these authors reported that changes in serum triglycerides were variable and unrelated to changes in dietary

cholesterol. Again, in 1964, Connor and associates reported that the addition of 729 mg of cholesterol to a diet containing a high proportion of unsaturated fats resulted in a significant increase in serum cholesterol of 28 mg/dl and serum phospholipids of 20 mg/dl. A small increase in the mean serum triglyceride concentration of 14 mg/dl was observed but this was not found to be significant. The removal of cholesterol from diets containing 729 mg cholesterol and a high proportion of saturated fats was found to decrease serum cholesterol 38 mg/dl, serum phospholipids 34 mg/dl and serum triglycerides 16 mg/dl; all decreases were significant.

Erickson and associates (1964) stated that the addition of 742 mg of cholesterol as egg yolk to the diet resulted in a mean serum cholesterol increase of 23.8 mg/dl and an increase in serum phospholipids of 18.4 mg/dl in the presence of diets containing partially hydrogenated soybean oil and 26.6 mg/dl and 22.1 mg/dl, respectively, in the presence of diets containing unhydrogenated fats. These researchers reported that the plasma triglycerides response to the various dietary treatments did not follow a definite pattern.

Grande et al (1965) reported that the addition of crystalline cholesterol and egg yolk cholesterol to mixed diets resulted in significant increases in serum cholesterol and that the changes in serum phospholipids, although they parallelled changes in serum cholesterol, were smaller. However, in most cases the changes in serum phospholipids were significant. Again, no significant changes were observed in the concentration of serum triglycerides in relation to the changes in cholesterol intake. A lack of effect of dietary cholesterol on serum triglycerides has also been noted in the National Diet Heart Study

Report (1968) and by Nestel et al (1975) and Slater et al (1976).

Hegsted and coworkers (1965) observed that changes in serum triglycerides and serum phospholipids to changes in dietary lipids including cholesterol, were in the same direction as changes in serum cholesterol. Although no values were reported for either serum phospholipids or triglycerides, the authors stated that the changes in these lipid fractions were less consistent than the changes in serum cholesterol and that the standard error of the differences were larger, thus, significant differences are more difficult to establish.

Anderson and coworkers (1976) compared the effects of changing the level of dietary cholesterol in the presence of saturated and unsaturated fat diets on serum cholesterol, triglycerides and phospholipids. They reported significant elevations in serum cholesterol when 291 mg crystalline cholesterol was added to both the saturated and unsaturated fat diets. Although the mean increase in serum phospholipids was essentially the same as the mean increase in serum cholesterol, the change was only significant in the presence of the unsaturated fat diet. The lack of significance in the presence of the saturated fat diet was due to the larger standard error for subjects with respect to serum phospholipids as opposed to serum cholesterol. The increase in serum triglycerides, in response to an increase in dietary cholesterol, was also significant in the presence of the unsaturated fat diets only.

Thus, there appears to be general agreement that serum phospholipid levels parallel those of serum cholesterol with respect to direction of change in response to a change in the level of cholesterol in the daily diet. At the same time there is disagreement as to the magnitude of change in phospholipids relative to the magnitude of change

in serum cholesterol as several authors have found the changes to be approximately the same while others have noted smaller changes or less consistent changes. Although there is a discrepancy about the effect of dietary cholesterol on the serum triglyceride concentration, several studies have indicated that changes, although small, have occurred in the same direction as changes in serum cholesterol and phospholipids. It is feasible that the parallel changes which occur in the serum cholesterol, phospholipids and possibly triglycerides reflect a change in a specific plasma lipoprotein fraction in response to dietary cholesterol, such as, the low density lipoproteins as has been found by Nestel et al (1975).

EXPERIMENTAL METHODS

A. DIET COMPOSITION

Five diets were designed to provide approximately 40% of total calories from fat and to differ only in the amount of egg yolk cholesterol; they were designated as the 0-Egg, 1-Egg, 2-Egg, 3-Egg and 6-Egg diets on the basis of the number of whole eggs contained in each diet.

All eggs used in the study were obtained from a single pen of Shaver hens housed in the Animal Science Department at the University of Manitoba. Analysis of a sample of eggs from these hens revealed an average total yolk lipid of 5.75 gm per egg.

To ensure that the lipid and protein components were similar among the five diets, a mixture of fats designed to simulate the fatty acid composition of egg yolk lipid (similated yolk fat) and egg albumin [1] were added to the 0-, 1-, 2-, 3-Egg Diets so that egg lipid and egg protein were equivalent to the amounts present in the 6-Egg diet. Thus, approximately 34 gm or one-quarter of the calories from fat was provided from either egg yolk lipid, simulated yolk fat or a combination of the two depending on the number of whole eggs in a particular diet. The simulated yolk fat (SYF) was prepared by combining hydrogenated soybean oil [2] and palm oil [3] in a 2:1 mixture. The fatty acid composition of the SYF and egg yolk lipid are compared in Table 1.

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[1] Spray dried albumin obtained from Export Packers, Winnipeg, Manitoba.
[2] Crisco, Proctor and Gamble, Toronto, Ontario.
[3] Bleached, deodorized, Canada Packers Ltd., Toronto, Ontario.

TABLE 1

PERCENT FATTY ACID COMPOSITION

OF EGG YOLK LIPID AND SIMULATED YOLK FAT[1]

FATTY ACID	EGG YOLK LIPID	SYF
MYRISTIC, C14:0[2]	tr[3]	tr
MYRISTOLEIC, C14:1	tr	tr
PALMITIC, C16:0	25.0	22.1
PALMITOLEIC, C16:1	3.0	tr
HEXA DECADIENOIC, 16:2	tr	tr
HEPTADECANOIC, C17:0	tr	tr
STEARIC, C18:0	7.6	9.9
OLEIC, C18:1	45.8	40.9
LINOLEIC, C18:2	13.7	21.4
LINOLENIC, C18:3	1.5	2.1
ARACHIDONIC, C20:4	1.3	tr

1. Composition determined by gas-liquid chromatography and reported as a percentage of total methyl esters. All values are means of duplicate analyses.
2. Carbon number: number of double bonds.
3. Represents those values less than 1.0%.

The other three-quarters of the calories from fat (98.5 gm fat) was supplied from a mixture formulated to simulate the fatty acid composition of the average Canadian diet and was the same as that used in previous metabolic studies (Cobden, 1975). The formula used to prepare this fat mixture, which was comprised of butter, corn oil [5] lard, [6] beef tallow [7] and hydrogenated soybean oil [2], was derived from domestic fat disappearance figures provided by Dr. Paul Simms [8]. The fatty acid composition of the fat mix is presented in Table 2. The butter oil and corn oil were used as table spread and salad dressing, respectively. The other fats, lard, beef tallow and hydrogenated soy, were mixed together and incorporated into snacks and menu entrees (for recipes see Fuller, unpublished thesis). A summary of the sources of fat in the experimental diets is presented in Table 3 and the fatty acid composition of the total dietary fats in Table 4.

Substituting SYF and egg albumin for whole eggs insured that the protein and fatty acid composition was qualitatively and quantitatively similar among the five diets while cholesterol content differed. The quantity of cholesterol provided by each diet is presented in Table 5.

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[4] Lucerne, Safeway Brand, Winnipeg, Manitoba.

[5] Mazola, Best Foods Division, Canada Starch Company Ltd., Montreal, Quebec.

[6] Tenderflake, Maple Leaf, Canada Packers Ltd., Winnipeg, Manitoba.

[7] Bleached, clarified, deodorized, Canada Packers Ltd., Winnipeg, Manitoba.

[8] Personal communication, Agriculture Canada, Ottawa, Ontario.

TABLE 2

COMPOSITION OF THE FAT MIX [1,2]

FATTY ACID	PERCENT OF FATTY ACIDS
MYRISTIC, C14:0[3]	3.0
MYRISTOLEIC, C14:1	1.7
PALMITIC, C16:0	20.1
PALMITOLEIC, C16:1	2.4
HEPTADECANOIC, C17:0	0.8
HEPTADECENOIC, C17:1	0.7
STEARIC, C18:0	14.5
OLEIC, C18:1	38.3
LINOLEIC, C18:2	13.4
LINOLENIC, C18:3	0.9

1. Mixture contained by weight: 30% hydrogenated soy, 20% lard, 25% beef tallow, 15% butter oil and 10% corn oil.
2. Composition determined by gas-liquid chromatography, Cobden, 1975.
3. Carbon number: number of double bonds.

TABLE 3

FAT COMPONENT OF THE EXPERIMENTAL DIETS

DIET	EGG YOLK LIPID -gm-	SYF -gm-	CANADIAN FAT MIX -gm-	TOTAL FAT [1] -gm-
0 Egg	0.00	34.50	98.5	133.0
1 Egg	5.75	28.75	98.5	133.0
2 Egg	11.50	23.00	98.5	133.0
3 Egg	17.25	17.25	98.5	133.0
6 Egg	34.50	0.00	98.5	133.0

1. Total fat to provide 40% of daily calories.

TABLE 4

FATTY ACID COMPOSITION OF DIETS [1]

DIETARY REGIMEN	10:0[2]	12:0	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	20:4
0-Egg											
Menu I	tr[3]	0.5	2.5	tr	23.1	0.5	12.0	38.5	19.3	2.0	tr
Menu II	0.5	0.5	2.3	tr	22.6	0.5	12.9	39.3	19.1	1.6	tr
1-Egg											
Menu I	tr	0.5	2.4	tr	22.9	0.5	12.3	38.4	18.9	1.8	0.5
Menu II	0.5	0.5	2.3	tr	21.7	0.9	13.1	38.7	18.9	1.8	tr
2-Egg											
Menu I	tr	0.5	2.4	0.6	22.9	tr	12.1	39.1	18.3	1.7	0.7
Menu II	0.6	0.6	2.4	tr	22.3	0.7	12.8	39.7	18.3	1.4	tr
3-Egg											
Menu I	tr	0.5	2.4	tr	23.9	tr	12.0	39.2	18.6	1.5	tr
Menu II	0.7	0.5	2.3	tr	22.4	1.3	12.6	40.3	17.9	1.3	tr
6-Egg											
Menu I	0.5	0.6	2.5	0.5	22.9	0.5	11.6	39.6	18.2	2.0	0.5
Menu II	0.7	0.6	2.4	0.5	22.0	1.9	12.5	39.9	17.3	1.4	tr

1. Values expressed as percentage of total methyl esters. All values are mean of duplicate analyses.
2. Carbon number: number of double bonds.
3. "tr" represents those values less than 0.5%.

TABLE 5

MEAN CHOLESTEROL CONTENT OF THE 0-, 1-, 2-, 3- AND 6-EGG DIETS [1,2]

DIETARY REGIMEN	CHOLESTEROL (mg/day)
0 EGG DIET	113
1 EGG DIET	441
2 EGG DIET	781
3 EGG DIET	1058
6 EGG DIET	1819

1. Values are the mean of duplicate analyses for Menu I and Menu II.

2. Analyzed by the method of Mietennen et al (1965).

A two day alternating menu (Appendix Table 1) was planned for each of the diets. Each menu included three meals plus between-meal snacks. Subjects were instructed to consume no other foods. Meals were served in the Home Economics Building on the university campus.

Each menu was designed to resemble the customary Canadian diet except that the protein complement was comprised of soy protein (Bontrea [9] and TVP [10]), skim milk and egg albumin. The diets consisted of the same foods except that egg albumin and SYF replaced all of the whole eggs in the 0-Egg diet and some of the whole eggs in the 1-, 2-, and 3-Egg diets. The SYF and egg albumin were incorporated into menu items as shown in Table 6 on the basis of 5.75 gm SYF and 5.0 gm dried egg albumin for each whole egg replaced.

In menu I, three whole eggs were scrambled for breakfast in the 3- and 6-Egg diets. In the 1- and 2-Egg diets egg albumin and SYF were mixed with one and two whole eggs, respectively, and in the 0-Egg diet egg albumin and SYF replaced all whole eggs in the scrambled egg entree. Egg albumin was also incorporated into the orange juice and meringue cookies or a meringue shell in the 0-, 1-, 2- and 3-Egg diets. SYF was incorporated into the macaroni entree, rice and peas as shown in Table 6. One hard boiled whole egg and a Rum and Butter Eggnog, containing two whole eggs, were added to the 6-Egg diet menu.

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[9] Dehydrated soy protein isolate, Registered Trade Name, General Mills Inc., 5000 Plymouth Avenue North, Minneapolis, Minnesota 55427.

[10] Textured Vegetable Protein, Archer Daniels Midland Co., 733 Marquette Avenue, Minneapolis, Minnesota 55440.

In Menu II, scrambled eggs were served at breakfast as described for Menu I. A chocolate eggnog containing two whole eggs and baked custard containing one whole egg were included in the 6-Egg diet. These were replaced with an eggnog and rice pudding prepared with SYF and egg albumin in the remaining diets. SYF also was incorporated into the spaghetti in the 0-Egg diet and mashed potatoes in the other diets.

Bread was included at each meal to utilize the butter and to permit subjects to absorb any visible fat remaining on the serving dishes. Condiments including Worchestershire sauce, HP sauce, ketchup and dill pickles were available for subjects to use at meals in moderation. Diet soft drinks, clear tea and coffee were allowed ad libitum. All food servings were weighed or measured and were prepared according to standardized recipes (Fuller, unpublished thesis).

Each menu was designed to provide 3000 kilocalories daily and to meet the nutrient needs of young men based on the recommendations of the 1975 Revised Canadian Dietary Standard for males 19 - 35 years of age. The calculated nutrient composition of the five diets is compared with the recommended daily intake in Appendix Table 2. The actual daily intakes of fat, protein and energy were determined and the results are compared with the calculated values in Table 7.

Each subject weighed himself daily before breakfast and in order to maintain body weight total calories were adjusted by increasing or decreasing the number of snack items permitted such that fat intake was maintained at 40% of ingested calories.

TABLE 6

THE 6 EGG DIET AND EQUIVALENT LIPID AND PROTEIN REPLACEMENTS [1]

MENU I	6 EGG DIET	3 EGG DIET	2 EGG DIET	1 EGG DIET	0 EGG DIET
Breakfast Orange Juice Eggs scrambled	3 eggs	+10 g albumin[2] 3 eggs +5 g SYF[3]	+10 g albumin 2 eggs +12 g SYF +5 g albumin	+10 g albumin 1 egg +10 g SYF +10 g albumin	+10 g albumin 10 g SYF +15 g albumin
Lunch Macaroni & Cheese Peas Strawberries		+10 g SYF	+10 g SYF Add: 1 meringue shell (5 g albumin)	+10 g SYF Add: 2 meringues (2 g albumin)	+10 g SYF Add: 1 meringue shell (5 g albumin)
Dinner Rice	Add: Rum and Butter Eggnog (2 eggs) 1 hard cooked whole egg	+2.5 g SYF		+9 g SYF	+8.5 g SYF
Snacks		Add: 5 meringues (5 g albumin)		Add: 3 meringues (3 g albumin)	

TABLE 6 continued

MENU II	6 EGG DIET	3 EGG DIET	2 EGG DIET	1 EGG DIET	0 EGG DIET
Breakfast Eggs scrambled	3 eggs	3 eggs	2 eggs +5 g SYF +5 g albumin	1 egg +10 g SYF +10 g albumin	10 g SYF +15 g albumin
Lunch Spaghetti in tomato sauce Chocolate eggnog	2 eggs	5 g SYF +10 g albumin	5 g SYF +10 g albumin	5 g SYF +10 g albumin	+6.5 g SYF 5 g SYF +10 g albumin
Dinner Potato mashed Dessert	Baked Custard (1 egg)	+9.25 g SYF Rice Pudding (5 g albumin + 3 g SYF)	+10 g SYF Rice Pudding (5 g albumin + 3 g SYF)	+10.75 g SYF Rice Pudding (5 g albumin + 3 g SYF)	+10 g SYF Rice Pudding (5 g albumin + 3 g SYF)

1. For complete menus see Appendix Table 1.
2. 5 g dried egg albumin is equivalent to the protein of 1 egg white; used reconstituted 1:6 with water.
3. Simulated yolk fat (SYF); 2:1 hydrogenated soybean oil to palm oil.

TABLE 7

MEAN DAILY FAT, ENERGY AND PROTEIN INTAKES[1]

NUTRIENT	0 Egg Diet		1 Egg Diet		2 Egg Diet		3 Egg Diet		6 Egg Diet	
	Menu		Menu		Menu		Menu		Menu	
	I	II	I	II	I	II	I	II	I	II
Fat gm										
Calculated	133	132	133	132	132	132	133	132	133	132
Analyzed[2]	126	127	130	129	133	131	133	131	139	131
Energy kcal										
Calculated[3]	2975	2976	3002	2992	3015	2998	3003	3009	3026	3035
Analyzed[4]	2920	3136	3139	3176	3253	3179	3157	3333	3207	3276
Protein gm										
Calculated[3]	88	91	86	93	94	96	88	98	106	106
Analyzed[5]	86	94	93	98	101	99	97	112	101	112

1. All analyzed values are mean of duplicate analyses.
2. Analyzed by the method of Bligh and Dyer (1959).
3. Calculated values using USDA Handbook # 8, Composition of Foods (Watt and Merrill, 1963).
4. Values obtained by bomb calorimetry.
5. Analyzed by the Kjeldahl procedure.

B. SUBJECTS

The subjects were eleven healthy young men, age 18 - 29 years ($\bar{x}$ = 24 years) selected from volunteers who responded to notices posted on the university campus. The subjects were chosen on the basis of an interview, a physical examination, expressed cooperativeness and acceptance of the experimental diets as judged by pre-experimental tasting sessions. The subjects were judged to be in good health. Heights, weights and initial serum cholesterol, phospholipid and triglyceride values for each subject are recorded in Table 8. All but one of the subjects (HM) were students at the University of Manitoba; HM was a member of the Technical Staff at the university. During the study the subjects maintained their normal activities and resided in their own homes.

Nine of the ten subjects continued for the duration of the study. One subject had to be replaced on day 29 of the experiment. His replacement, subject BH, completed the entire period II by continuing for one week after the main group had completed the metabolic study.

C. ISOTOPE INFUSION

Approximately 25 days prior to the metabolic study the subjects were infused with 50 microcuries of cholesterol - 1 - 2 - $^{3}H(N)$ [11] (0.36 ug cholesterol in 0.5 ml sterile saline). The infusions were carried out by Ms. Helen Bowan, under the supervision of Dr. John

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[11] Lot No. 787-164 obtained July, 1975 from New England Nuclear, 575 Albany Street, Boston, Massachusetts 02118.

TABLE 8

PHYSICAL DATA OF SUBJECTS

SUBJECT	AGE (years)	HEIGHT (cm)	WEIGHT (kgm) initial	WEIGHT (kgm) average during study	Initial Serum Lipid Values (mg/dl) Cholesterol	Phospholipids	Triglycerides
BH	27	183	78.5	78.0 ± 0.7	189	234	83
JM	29	183	77.3	76.3 ± 1.3	212	264	76
BD	22	183	85.0	82.2 ± 3.1	210	270	142
EC	23	167	78.0	77.4 ± 1.7	195	223	68
HM	23	191	91.7	91.4 ± 2.1	139	200	87
LC	25	185	73.0	71.3 ± 1.9	183	268	97
BS	24	183	75.0	75.2 ± 0.9	192	225	126
DG	18	180	72.2	70.5 ± 1.4	200	224	83
RE	23	186	75.0	74.0 ± 0.9	171	256	77
JB	22	178	65.8	66.2 ± 0.8	184	229	78

1. Mean ± SD for daily weighings.

2. Data for Subject (SD) who was replaced on Day 29 of the study is not included.

Moorhouse, in the metabolic unit at the Health Sciences Centre, Winnipeg, Manitoba. A detailed description of the method for isotope administration has been outlined by Cobden (1975).

D. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The six week metabolic trial was divided into two three-week diet periods designated as Diet Period I and Diet Period II. Two of the ten subjects were randomly assigned to each of the five experimental diets for Diet Period I. At the end of the Diet Period I, the subjects were reassigned to different diets and over Diet Period II, each of the five diets again was consumed by the two subjects. This Incomplete Latin Square Design provided four replicate observations; each replicate consisted of five subjects who consumed the five different diets for three weeks. Hence, each diet period provided two of the four replicates. The experimental design is diagrammed in Figure 1.

The data were subjected to analysis of variance for Extended Incomplete Latin Square Design (Cochran et al, 1957) to determine whether the level of dietary cholesterol fed had any significant effects on serum cholesterol, triglyceride or phospholipid levels. Regression analysis was used to define the relationship between cholesterol intake and serum cholesterol levels. Analysis of covariance of the $\log_{10}$ specific activity was used to test the homogeneity of regression for the rate of decrease of the specific activity of serum cholesterol for the two levels of cholesterol intake for each subject.

E. DIET AND INGREDIENT ANALYSIS

A number of menu items were prepared in advance, frozen and

SUBJECT	DIET PERIOD I	DIET PERIOD II
	REPLICATE I	REPLICATE II
BH	0-Egg	1-Egg
JM	1-Egg	6-Egg
BD	2-Egg	3-Egg
EC	3-Egg	0-Egg
HM	6-Egg	2-Egg
	REPLICATE III	REPLICATE IV
LC	0-Egg	2-Egg
BS	1-Egg	3-Egg
DG	2-Egg	1-Egg
RE	3-Egg	6-Egg
JB	6-Egg	0-Egg

FIGURE 1: EXPERIMENTAL DESIGN AND ALLOTMENT OF SUBJECTS TO DIETS

stored at -10°C for up to four months. Stew, chili and spaghetti in tomato sauce were prepared, stored, heated and served in individual foil containers [12]. Macaroni was frozen and stored in heat sealed polyethylene pouches [13], it was removed from these pouches while frozen and heated on serving dishes in a microwave oven prior to serving.

Fats for the experimental diets were purchased as single lots and stored in sealed containers at 7°C in a refrigerator until required. Eggs were delivered biweekly from the Animal Science Department. Skim milk, fresh fruit and vegetables, and bread were obtained from single local sources on a weekly basis. Other staples and frozen foods were purchased in single lots and stored under appropriate conditions.

Composites were made of each daily menu for each of the five experimental diets. The individual frozen foods were thawed and to these the remaining food items were added, weighed according to menu specifications using a Satorius top-loading balance (Model 2254) [14]. The composites of meals for each day were homogenized with approximately 200 ml of distilled water in a one-gallon Waring commercial blender (Model CB-5) [15]. The homogenate was weighed and an aliquot was lyophilized in a Model 10-140 MR-BA Virtis Freeze Dryer [16].

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[12] ECKO Foil Containers with Lids, Price Wilson Ltd., 830 King Edward Street, Winnipeg, Manitoba.

[13] Scotchpak Kapak Pouches, Kapak Industries Inc., 9809 Logan Ave. South, Livington, Minnesota 55431.

[14] Satorius - Werke AG, Gottingen, Germany.

[15] Waring Products Co., Winsted, Connecticut.

[16] Virtis Co. Inc., Gardiner, New York 12525.

The dried sample was crushed to a fine particle size and stored in Whirl-Pak plastic bags (#8992, 510-30 gm) [17] at -10°C for later analysis.

A chloroform : methanol : water mixture (Bligh and Dyer, 1959) was employed to extract total lipid from lyophilized food samples and from egg yolk. An aliquot of the lipid-containing chloroform layer was dried for total lipid determination and the remaining chloroform layer was transferred to a screw-top vial, flushed with nitrogen and stored at -10°C until required for fatty acid analysis.

For fatty acid analysis the lipid-containing chloroform was evaporated to dryness under a stream of nitrogen and, after saponification with methanolic-NaOH, methyl esters of fatty acids were prepared with BF_3- methanol according to the method of Metcalfe et al (1966). Samples of the SYF were saponified and methylated directly, without prior extraction. Fatty acid analyses were completed with a Varian Aerograph gas chromatograph (Model 1740-1) [18] equipped with dual columns, flame ionization detectors, a Varian Aerograph recorder (Model 20) [18] and a Varian Aerograph digital integrator (Model 477) [18]. The fatty acids were resolved on 2.7m x 3.2 mm steel columns packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q [19]. Flow rates for the gases were: 30 ml/min for helium [20], 25 ml/min for hydrogen [20] and 250 ml/min for air [20]. The columns were operated isothermically at 195°C with injector and detector temperatures of 230°C and 250°C,

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[17] Canlab Laboratory Equipment, Winnipeg, Manitoba.

[18] Varian Aerograph, 6358 Viscount Road, Malton, Ontario.

[19] Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania 16801.

[20] Welder's Supplies, 25 McPhillips Street, Winnipeg, Manitoba.

respectively. Individual fatty acids were identified by comparison with linear-log plots of retention time versus carbon number of fatty acid reference standards [21].

For cholesterol analysis the lipid was extracted from the freeze-dried meal composites by the method described by Folch et al (1957). Extraction efficiency was determined on the basis of recovery of a known amount of ^{14}C-cholesterol [22] added to the samples. Cholesterol was separated from the lipid mixture on Silica Gel H in an ethylether heptane solvent system and silyl ethers of cholesterol were prepared according to the method of Mietennen et al (1965). Cholesterol analysis was completed with a Perkin Elmer gas chromatograph (Model 3920B) [23] equipped with a dual flame ionization detector and a Hewlett Packard reporting integrator (Model 3380-5) [24]. Cholesterol was resolved on a 6 ft. x ¼ in. OD glass column packed with 2% QF-1 on 100/120 mesh Chromasorb W [24]. Flow rates for the gases were: 30 ml/min for helium [20], 35 ml/min for oxygen [20] and 550 ml/min for air [20]. The column was operated at 230°C with injector and detector temperatures of 250°C and 260°C, respectively. The cholesterol concentration was calculated by comparison with the peak area obtained for a known quantity of cholestane [25] added as an internal reference.

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[21] Hormel Institute, Lipids Preparation Laboratory, 801 - 16th Avenue N.E., Austin, Minnesota 55912.

[22] Cholesterol 4 - ^{14}C , obtained from New England Nuclear, 575 Albany Street, Boston, Massachusetts 02118.

[23] Perkin Elmer (Canada) Ltd., 120 Norfinch Drive, Downsview, Ontario.

[24] Hewlett Packard (Canada) Ltd., 1785 Woodward Drive, Ottawa, Ontario.

[25] Applied Science Laboratories Inc., P.O. Box 440, State College, Pennsylvania 16801.

Protein content of the diets was determined by the boric acid modification (AACC, 1962) of the AOAC (1960) Kjeldahl procedure for total nitrogen except that mercuric oxide and potassium sulphate catalysts were replaced by 10.5 gm titanium dioxide. The factor of 6.25 was used to calculate the percentage protein present.

Gross energy of the diets was determined using a Parr Adiabatic Calorimeter (Model U30M) [26] equipped with a Parr # 1241 oxygen bomb calorimeter [26] and a Parr # 1541 water heater [26].

F. BLOOD ANALYSIS

Blood samples were taken on day 1 of the study and at one week intervals thereafter, samples were taken between 7:30 and 8:30 a.m., following a 10 hour fast. From each subject, approximately 50 ml of blood was drawn from the antecubical vein into three 15 ml BD vacutainer tubes (# 4796) [27] and one 7 ml BD vacutainer tube containing 15% EDTA solution (# 4759) [27] which was used for whole blood analysis. Blood used for serum analysis was allowed to clot for one hour at room temperature. After clotting, the three samples were centrifuged [28] at 1400 xg for five minutes. Four 2 - 3 ml and one 5 ml portion of clear sera were pipetted into screw-top glass vials, flushed with nitrogen and stored at -10^{o}C until analyzed.

Prior to analysis, sera were thawed for one-half hour at room temperature. Sera from each subject were analyzed in duplicate for total cholesterol, radioactivity, triglycerides and lipid phosphorus.

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[26] Parr Instrument Co., 211 Fifty Third Street, Moline, Illinois 61625.

[27] Canlab Laboratory Equipment, Winnipeg, Manitoba.

[28] Model HN-2368P-2 Centrifuge, International Equipment Co., Needham Heights, Massachusetts.

Sera samples collected at the start of the study and at the end of Diet Period I and Diet Period II were also analyzed for the fatty acid patterns of the cholesterol esters. Haemoglobin, haematocrit, leucocyte counts and platelet counts of whole blood were monitored at the Haematological Laboratories, Health Sciences Centre, Winnipeg, Manitoba.

G. ANALYSIS OF SERUM

1. Cholesterol: Total cholesterol was determined by the Mann procedure (1961). The optical density of the colour complex which developed by reaction between cholesterol and the ferrous sulphate reagent was measured at 560 nm in a Coleman Junior Spectrophotometer [29]. The micrograms of sterol in the solution were obtained by comparison with a standard curve for cholesterol [30].

2. Radioactivity: Two ml of serum were extracted for total lipid by the method of Folch et al (1957). The chloroform layer was transferred to a scintillation vial and the chloroform evaporated under a stream of nitrogen. Ten ml of scintillation fluid, prepared by dissolving 5.0 gm PPO [31] and 0.3 gm POPOP [32] in 1.0 litre scintillation grade toluene [33], was added. The samples were counted for

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[29] Model No. 6A-36715, Coleman Instrument Inc., Maywood, Illinois.

[30] Cholesterol reference from Fisher Scientific Company, Winnipeg, Manitoba.

[31] Diphenyloxazole, Amersham/Searle Corp., 2636S Clearbrook Drive, Arlington Heights, Illinois 60005.

[32] 1,4 - bis - 2 - (5 - phenyl azdyl) benzene, Packard Instruments Corporation Inc., 2200 Warrenville Road, Downers Grove, Illinois 60515.

[33] Fisher Scientific Company, Winnipeg, Manitoba.

20 minutes or 4000 counts in a liquid scintillation spectrometer [34] using the following settings: Data H.V. and Gate H.V. -9.0; dial A, level 3 -0.5 and level 5 -9.9; and dial B, levels 3 and 4 -0.5 and 2.3 respectively. The data attenuator was set at zero. Quenched tritium standards were used to determine the efficiency of counting by the spectrometer. Disintegrations per minute were adjusted for extraction losses, 15%,on the basis of recovery of a known amount of ^{14}C- cholesterol [22] analyzed in the same manner.

3. Triglycerides: The phospholipids in blood serum were adsorbed directly onto florisil and the phospholipid-free lipid was extracted with diethyl ether and isopropanol according to the method of Ryan and Rasho (1967), except that 0.2 ml serum were used for each determination. The saponification with ethanolic KOH and the colour reaction between chromatropic acid and glycerol were by the method of Van Handel and Zilversmit (1967), except that the sodium bisulphite replaced sodium arsenate as suggested by Jagannathan (1964). The optical density of the coloured solution was measured at 570 nm in a Coleman Junior Spectrophotometer [29] standardized by a reagent blank. A serum blank, unsaponified sample, was analyzed to correct the optical density reading for each serum sample. Milligrams of triglyceride were determined from a standard curve.

4. Lipid Phosphorus: The phospholipids in serum were analyzed by the method of Chen et al (1956) except ashing was carried out by heating in sulphuric acid at 250°C in a heating block (Model 120C) [35]

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[34] Model 725, Nuclear Chicago Corp., 333 East Howard Avenue, Des Plaines, Illinois.

[35] Hallikainen Instrument, Slaco Division, Richmond, California.

for one hour. The colour complex produced by the reaction of free phosphorus with ascorbic acid and ammonium molybdate in sulphuric acid, was measured at 820 nm in a Unicam SP600 Series 2 Spectrophotometer [36] standardized with a reagent blank. Micrograms of phosphorus were obtained by comparing the samples to a single phosphorus standard prepared from reagent grade Potassium Phosphate Monobasic.

5. Cholesteryl ester fatty acid patterns: Total lipid was extracted from 2 ml serum by the method of Folch et al (1957). Cholesteryl esters were separated on silicic acid microcolumns with 1% ethyl ether under nitrogen as described by Lis et al (1961). Saponification was carried out according to the procedure of Mann (1961) as modified by Tu et al (1967). After addition of 1% Thymol Blue indicator, the saponified mixture was acidified to a red end point with 50% HCL and the fatty acids were extracted in 10 ml petroleum ether. The petroleum ether was evaporated and the fatty acids redissolved in 5 ml methanolic NaOH and methylated with 3 ml BF_3- methanol as described by Metcalfe et al (1966). The fatty acid methyl esters were separated and quantified as previously outlined under section E) Diet and Ingredient Analysis.

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[36] Canlab Laboratory Equipment, Winnipeg, Manitoba.

RESULTS AND DISCUSSION

A. SUBJECTS

The subjects remained in good health throughout the dietary trials and appeared to adjust well to the experimental diets as no unusual digestive problems were reported. Although some weight fluctuation occurred, for most subjects, body weight was essentially unchanged over the six week metabolic trial. However, subject BD lost 3.5 kg and subject HM lost 2.2 kg. These losses may have had some bearing on the changes seen in serum lipid values. Nestel et al (1969) have reported a decrease in serum cholesterol in response to a decrease in body weight. It has also been found that for men who lost weight serum cholesterol remained at that lower level even when their weight had stabilized or when they were actually gaining (National Diet Heart Study Group, 1968). Galbraith et al (1964) reported that negative calorie balance had a greater influence upon serum lipids than dietary cholesterol.

One subject (SD) did not serve successfully for the duration of the study and was replaced by subject BH for the entire Diet Period II. Only data for subject BH are included in the analysis as it was believed that SD did not completely adhere to the experimental regimen.

B. TOTAL SERUM CHOLESTEROL

The level of cholesterol in the diet had a significant effect

($P < 0.05$) on serum cholesterol levels. The relationship between dietary cholesterol and serum cholesterol was linear ($P < 0.01$) and highly correlated ($r^2=0.90$). For each additional egg in the diet (300 mg cholesterol) serum cholesterol increased by approximately 12 mg/dl. The relationahip between the pure treatment effects (Appendix Table 3) and the number of whole eggs consumed is diagrammed in Figure 2 and is defined by the regression equation: serum cholesterol (mg/dl serum) = 147.7 + 11.9 (the number of whole eggs in the diet).

Weekly serum cholesterol values for each subject are presented in Table 9 and statistical analysis of the data is shown in Appendix Tables 3 and 4. The experimental design, an Incomplete Latin Square, did not permit the feeding of all diets to all subjects. As all subjects do not respond identically to a given level of dietary cholesterol statistical adjustments in the calculated treatment means were necessary (Cochran and Cox, 1957). The mean serum cholesterol levels, adjusted for intersubject variability, were 141, 165, 184, 172, and 220 mg/dl serum for subjects fed the 0-, 1-, 2-, 3- and 6-Egg diets, respectively.

The adjusted mean serum cholesterol values are compared with preducted values, obtained from the regression equation, in Table 10. There exists an apparent discrepancy between observed and predicted serum cholesterol values when subjects were fed the 2-Egg and 3-Egg diets. The predicted value for the 2-Egg diet was 172 mg/dl serum and the observed value was 184 mg/dl. The predicted

FIGURE 2.

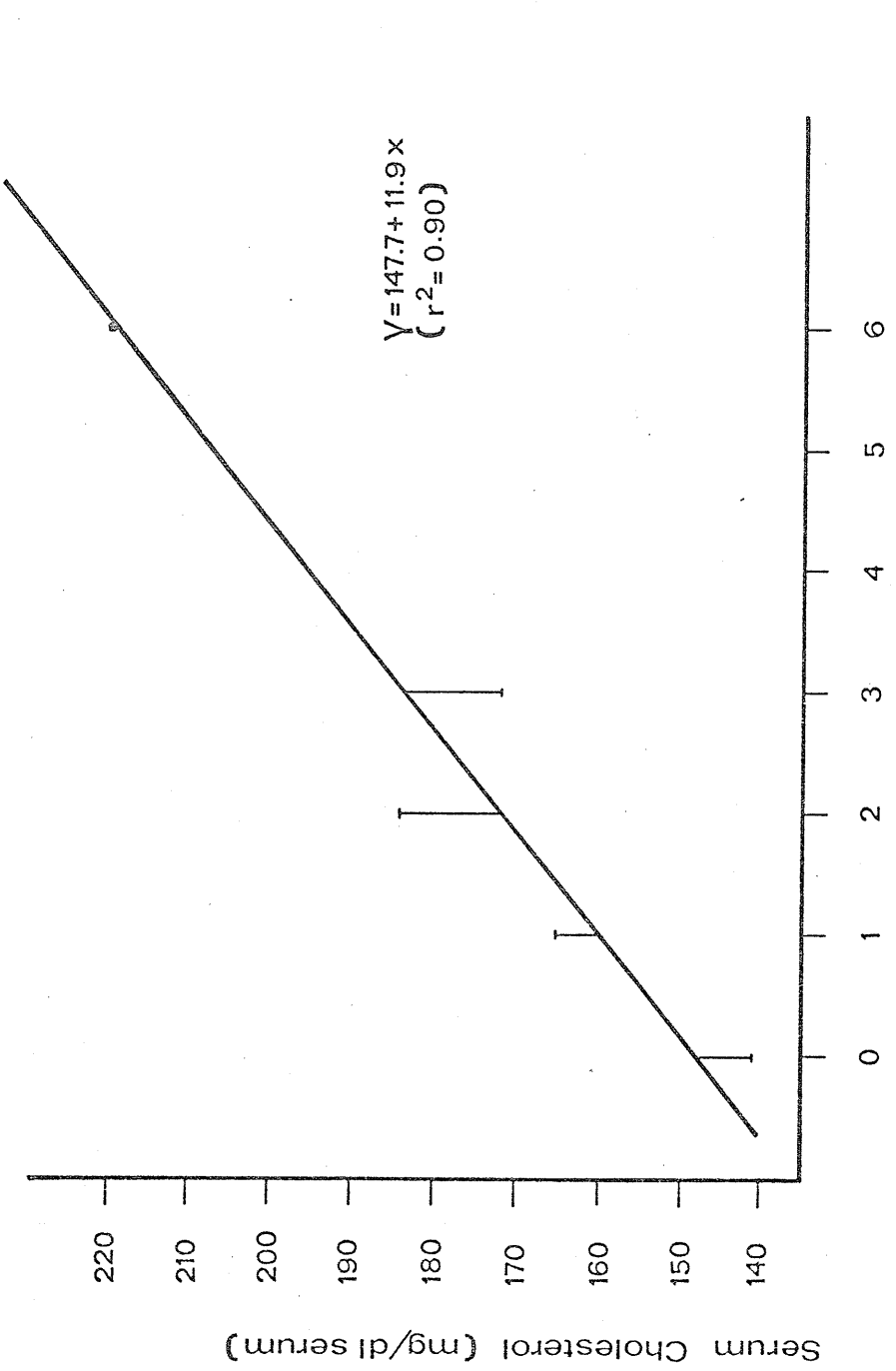
Effect Of Dietary Cholesterol On Serum Cholesterol
Y=147.7+11.9x
(r2= 0.90)
Serum Cholesterol (mg/dl serum)
220
210
200
190
180
170
160
150
140
0
1
2
3
4
5
6
Eggs/Day

TABLE 9

TOTAL SERUM CHOLESTEROL OF SUBJECTS IN RESPONSE TO DIETARY CHOLESTEROL[1]

SUBJECTS	DIETARY REGIMEN														
	0 EGG DIET			1 EGG DIET			2 EGG DIET			3 EGG DIET			6 EGG DIET		
	Week			Week			Week			Week			Week		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
BH[2]	I[3]	-	-	II 180	173	154									
JM				I 153	151	148							II 159	160	187
BD							I 203	199	190	II 179	178	176			
EC	II 168	173	171							I 192	188	188			
HM							II 163	159	151				I 173	178	178
LC	I 169	151	137				II 166	177	173						
BS				I 165	164	162				II 188	183	180			
DG				II 195	189	214	I 192	198	192						
RE										I 156	173	173	II 183	194	205
JB	II 177	177	175										I 220	213	210

1. Values are the mean of duplicate analyses expressed in mg/dl serum.
2. Missing values. Subject BH participated in Diet Period II only.
3. I and II indicate Diet Period I and II.

TABLE 10

OBSERVED AND PREDICTED ADJUSTED MEAN SERUM CHOLESTEROL FOR SUBJECTS FED DIFFERENT AMOUNTS OF EGG YOLK CHOLESTEROL[1]

Dietary Regimen	Observed Values mg/dl	Predicted Values mg/dl
0 - Egg Diet	141	148
1 - Egg Diet	165	160
2 - Egg Diet	184	172
3 - Egg Diet	172	183
6 - Egg Diet	220	219

1. For statistical adjustment of mean serum cholesterol values see Appendix Table 3.

value for the 3-Egg diet was 183 mg/dl serum and the observed value was 172 mg/dl. This disagreement can be explained to some extent, by the response of the only subject (BD) who was assigned to both of these diets. The serum cholesterol for this subject was 195 mg/dl ($\bar{X}$ of week 2 and week 3 results) while consuming the 2-Egg diet in Diet Period I and 177 mg/dl while consuming the 3-Egg diet in Diet Period II. The atypical response to an increased intake of dietary cholesterol is in part the result of weight loss experienced by this subject throughout the dietary trials.

Although it has been unequivocally demonstrated that serum cholesterol levels are influenced by the presence of cholesterol in the diet, controversy exists over the linear responsiveness of serum cholesterol to incremental increases of cholesterol in the diet. As early as 1960 Beveridge and coworkers reported a linear relationship between dietary cholesterol and serum cholesterol over a range of 0-634 mg cholesterol per day. These authors also reported that when synthetic cholesterol was added to a formula diet, which provided 30% of calories as fat, serum cholesterol did not increase further as dietary cholesterol was increased from 634 to 1300, 2500 and 4500 mg per day. Wells and Bronte-Stewart (1963) observed that incremental supplements of crystalline cholesterol from 25 to 500 mg per day were associated with increasing concentrations of cholesterol in the serum. These authors reported that at levels of dietary cholesterol greater than 500 mg per day, there was not a further increase in serum cholesterol concentration. A mathematical description of the relation-

ship between dietary and serum cholesterol was not reported in either study.

Later dietary trials demonstrated that cholesterol in the serum was a linear function of cholesterol in the diet at levels above 500 - 600 mg per day. Mattson and coworkers (1972) demonstrated that dietary cholesterol, fed as egg yolk, at levels of 0, 126, 212 and 317 mg/1000 kcal had a pronounced effect on serum cholesterol levels. These researchers found that the change in serum cholesterol was related to a change in dietary cholesterol by the equation: change in serum cholesterol (mg/dl serum) = 1.60 + 0.118 (dietary cholesterol mg/1000 kcal). Thus, for each additional 300 mg cholesterol in a 3000 kcal diet they predicted an increase in serum cholesterol of 12 mg/dl. In the present study it was observed that for each additional egg (300 mg cholesterol) in the 3000 kcal diets serum cholesterol was increased 11.9 mg/dl.

Hegsted and associates (1965) also reported that serum cholesterol was a linear function of the level of cholesterol in the daily diet. These investigators observed that cholesterol fed as a component of egg yolk and butterfat in a mixed diet was a significant variable in the determination of serum cholesterol. They concluded that the effect was an increase in plasma cholesterol of approximately 5 mg/dl for each additional 100 mg of dietary cholesterol. Thus, for each additional egg or 300 mg cholesterol in a diet an increase of 15 mg cholesterol per dl of serum would be expected.

Grande and coworkers (1965) reported that changing the cholesterol content of the diet produced a change in serum cholesterol in the same direction. They derived a prediction equation, by regression analysis, to express this relationship. In this prediction equation, serum cholesterol is a linear function of the square root of the amount of cholesterol in the daily diet. They have subsequently extended this relationship to predict the change in serum cholesterol which would result from a change in cholesterol intake: change in serum cholesterol (mg/dl serum) = $1.5\ (Z_2-Z_1)$[37]. From Figure 3, it can be seen that the prediction equation derived by Grande et al underestimates the effect of a change in dietary cholesterol on serum cholesterol at higher intakes and overestimates this effect at lower intakes with respect to the studies by Mattson et al (1972), Hegsted et al (1965) and the study reported here.

It has often been cautioned that regression equations are primarily descriptive of the information from which they are derived. The much lower response to high levels of cholesterol intake observed in the study by Grande and coworkers (1965) was influenced by a variable in the diet that sets that study apart from the other three presented. Synthetic cholesterol was fed to subjects rather than cholesterol as a natural component of foods such as egg yolk, butterfat or meat. The use of crystalline cholesterol in dietary trials undertaken to obtain quantitative information on the serum cholesterol response to different levels of dietary cholesterol has been questioned. Many early studies

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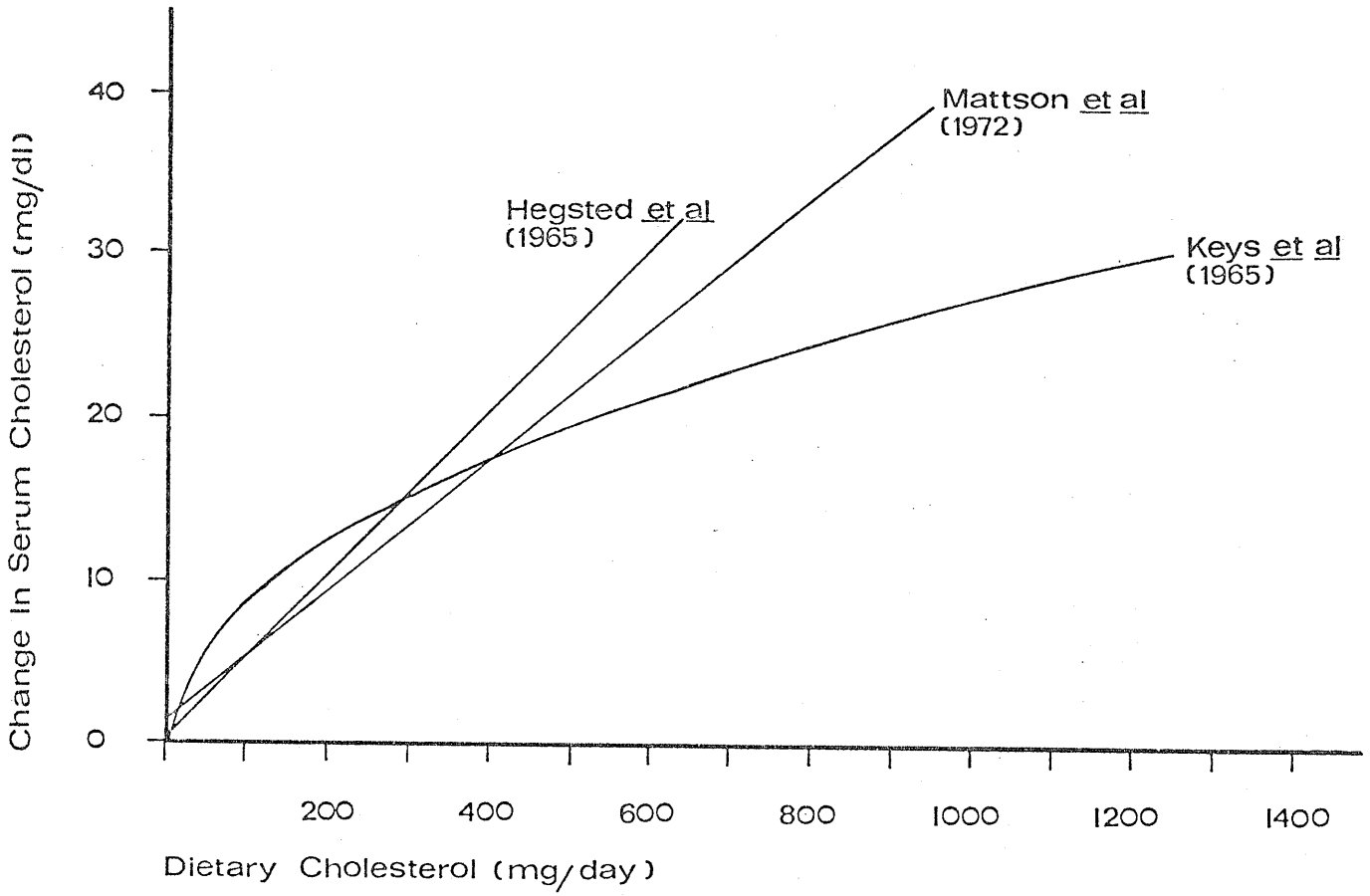
[37] Z_1 and Z_2 represent the square root of the cholesterol content in mg/1000 kcal of diet 1 and diet 2, respectively.

FIGURE 3.

Linear Responsiveness Of Serum Cholesterol To Dietary Cholesterol



were unable to demonstrate a significant effect on serum cholesterol by adding large quantities of synthetic cholesterol to diets (Keys et al, 1956; Turner et al, 1939; Ahrens et al, 1957). Wells and Bronte-Stewart (1963) clearly demonstrated that crystalline cholesterol has little effect on serum cholesterol unless fed with a suitable amount and type of fat. Connor and associates (1961) reported that egg yolk cholesterol was a more potent hypercholesterolemic dietary component than pure crystalline cholesterol dissolved in oil. Grande and coworkers (1965) reported that an egg diet containing 144 mg cholesterol/1000 kcal produced a somewhat higher cholesterol response than a diet containing 200 mg crystalline cholesterol/1000 kcal.

It has been repeatedly reported that the major changes in serum cholesterol, following a change of diet, occurs within two to three weeks (National Diet Heart Study Group, 1968; Hegsted et al 1965; Erickson et al, 1964; Grande et al, 1965). The present study appears to support this observation. For most subjects, serum cholesterol levels had reached a stable plateau by the end of the second week in each experimental period. The effect of weeks was not significant (Appendix Table 4) when serum cholesterol values determined at the end of week 2 and week 3 of each experimental period are included in the statistical analysis of the data. However, serum cholesterol did not appear to stabilize within this time for three subjects (JM, BH and DG) during Diet Period II as the largest change in cholesterol values occurred after the end of the second week (Table 11). During the third week in Diet Period II serum cholesterol increased 27 mg/dl for JM compared to an increase of only 12 mg/dl over the first two

TABLE 11

CHANGE IN SERUM CHOLESTEROL (mg/dl) IN RESPONSE TO DIFFERENT AMOUNTS OF DIETARY CHOLESTEROL

Subject	Diet Period I		Diet Period II	
	Week 1 + Week 2	Week 3	Week 1 + Week 2	Week 3
JM	-59	-3	+12	+27[1]
BD	-11	-9	-12	-2
EC	-7	0	-15	-2
HM	+19	0	-19	-8
LC	-32	-14	+40	-4
BS	-27	-3	+21	-3
DG	-2	-6	-3	+25[1]
RE	+2	0	+21	+11
JB	+29	-3	-33	-2
BH[2]			-16	-19[1]

1. Major change in serum cholesterol occurred after the end of the second week in the diet period.
2. Subject participated in Diet Period II only.

weeks. For subject BH serum cholesterol decreased 19 mg/dl in the third week compared to a decrease of 16 mg/dl over the first two weeks. And, for subject DG a sudden increase in serum cholesterol of 25 mg/dl was observed during the final week of Diet Period II. A slower rate of stabilization has also been reported by other laboratories. Mattson and coworkers (1972) have reported that up to four weeks are required before serum cholesterol concentrations were stabilized following a change of diet. Dam et al (1970) reported that serum cholesterol values were more elevated after six weeks than three weeks when subjects were fed increasing amounts of cholesterol as egg yolk. A recent report by Slater and associates (1976) has also shown that serum cholesterol does not stabilize within two to three weeks when there is a change in dietary cholesterol. This group of investigators fed free-living subjects one or two additional eggs per day with their regular diet for a period of eight weeks. In the first of the series of studies reported serum cholesterol values were significantly elevated at the end of three weeks for subjects who had consumed two additional eggs per day. But, following this peak, serum cholesterol values decreased until by the end of the eighth week they were no longer significantly elevated. From these findings, it appears that controlled metabolic studies to investigate the long term effect of a change in dietary cholesterol on serum cholesterol are in order.

The data from the present study supports the hypothesis that serum cholesterol is a linear function of the level of cholesterol in the daily diet. However, it must be noted that at levels of cholesterol intake greater than 1800 mg per day there were no subjects who had a

serum cholesterol which was elevated beyond that which is considered the normal physiological range for healthy young Canadian men, that is, 240 mg/dl (National Health and Welfare Canada, 1973).

C. SERUM LIPID PHOSPHORUS

The level of cholesterol in the daily diet did not have a significant effect on the level of serum lipid phosphorus (Appendix Table 6). The mean serum lipid phosphorus values, adjusted for inter-subject variability (Appendix Table 5), were 7.57, 7.88, 7.72, 8.10 and 9.63 mg/dl for subjects fed the 0-, 1-, 2-, 3- and 6-Egg diets, respectively. Corresponding serum phospholipid values (Table 12) were calculated by multiplying serum lipid phosphorus values by a factor of 25 (Tietz, 1970).

Weekly serum lipid phosphorus values for each subject are presented in Table 13. As with the serum cholesterol results, serum lipid phosphorus values determined at the end of week 2 and week 3 of each diet period were included in the statistical analysis of the data.

Although the serum phospholipids were not significantly altered by the level of dietary cholesterol, they generally parallelled changes which occurred in serum cholesterol. In 15 of 19 observations a decrease or an increase in serum cholesterol was accompanied by a corresponding decrease or increase in serum phospholipids (Table 14). This observation is consistent with results reported by a number of other laboratories (Connor et al, 1961a, 1961b; 1964; Erickson et al, 1064; Grande et al, 1965; Hegsted et al , 1965; Anderson et al, 1976). The magnitude of change in serum phospholipids with respect to the magnitude of change in serum cholesterol was variable. For some of the subjects the degree of change

TABLE 12

ADJUSTED MEAN SERUM LIPID PHOSPHORUS AND PHOSPHOLIPIDS FOR SUBJECTS FED 0, 1, 2, 3 OR 6 EGGS PER DAY[1,2]

Dietary Regimen	Serum Lipid Phosphorus mg/dl	Serum Phospholipid[3] mg/dl
0 Egg Diet	7.57	189
1 Egg Diet	7.88	197
2 Egg Diet	7.72	193
3 Egg Diet	8.10	203
6 Egg Diet	9.63	241

1. Values are means of duplicate analysis.
2. For statistical adjustments see Appendix Table 5.
3. Phospholipid = 25 X serum lipid phosphorus.

TABLE 13

SERUM LIPID PHOSPHORUS OF SUBJECTS IN RESPONSE TO DIETARY CHOLESTEROL[1]

Subjects	Dietary Regimen														
	0 Egg Diet			1 Egg Diet			2 Egg Diet			3 Egg Diet			6 Egg Diet		
	Week			Week			Week			Week			Week		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
BH[2]	I[3] -	7.50	7.50	II 7.80	7.88	7.80									
JM				I 7.92	7.71	7.74							II 8.51	8.76	9.47
BD							I 9.17	8.59	8.17	II 8.26	8.38	8.63			
EC	I 7.34	7.42	7.59							I 7.92	7.84	8.13			
HM							II 7.26	7.26	7.21				I 8.01	8.17	7.51
LC	I 8.47	8.09	7.63				II 8.55	7.51	7.96						
BS				I 8.42	8.30	7.92				II 8.09	8.05	8.13			
DG				II 8.40	8.76	9.13	I 8.67	8.97	8.34						
RE										I 8.09	8.97	8.67	II 9.05	9.13	10.09
JB	I 7.42	7.46	7.84										I 8.97	8.59	8.38

1. Values are means of duplicate analyses, mg/dl.
2. Missing values. Subject BH participated in Diet Period II only.
3. I and II refer to Diet Period I and II respectively.

TABLE 14

CHANGE IN SERUM TRIGLYCERIDES, PHOSPHOLIPIDS AND CHOLESTEROL IN RESPONSE TO THE NUMBER OF EGGS IN THE DIET

Subject and Diet	Triglycerides mg/dl	Phospholipids mg/dl	Cholesterol mg/dl
BH			
0 Egg Diet	-	-	-
1 Egg Diet	- 24	- 39	- 35
JM			
1 Egg Diet	- 15	- 70	- 64
6 Egg Diet	+ 61	+ 43	+ 39
BD			
2 Egg Diet	-108	- 66	- 20
3 Egg Diet	+ 25	+ 12	- 14
EC			
3 Egg Diet	- 28	- 20	- 7
0 Egg Diet	+ 6	- 13	- 17
HM			
6 Egg Diet	- 28	- 20	+ 19
2 Egg Diet	- 8	- 8	- 27
LC			
0 Egg Diet	- 49	- 77	- 46
2 Egg Diet	+ 1	+ 36	+ 8
BS			
1 Egg Diet	- 68	- 27	- 30
3 Egg Diet	+ 30	+ 5	+ 18
DG			
2 Egg Diet	- 3	- 15	- 8
1 Egg Diet	+ 37	+ 19	+ 22
RE			
3 Egg Diet	- 16	- 39	+ 2
6 Egg Diet	- 3	+ 35	+ 32
JB			
6 Egg Diet	- 15	- 19	+ 26
0 Egg Diet	+ 16	- 14	- 35

in both lipid fractions was comparable but for other subjects the magnitude of change was very different for the two lipid elements. This variability in the magnitude of response may explain why dietary cholesterol had no effect on serum phospholipid levels whereas serum cholesterol levels were correlated with dietary cholesterol.

D. SERUM TRIGLYCERIDES

The level of cholesterol in the diet did not have a significant effect on the level of serum triglycerides (Appendix Table 8). The adjusted mean serum triglyceride values (Appendix Table 7) were 67, 73, 94, 79 and 82 mg/dl for subjects fed the 0-, 1-, 2-, 3- and 6-Egg diets, respectively (Table 15).

Weekly serum triglyceride values for each subject are presented in Table 16. Serum triglyceride values determined at the end of weeks 2 and 3 of each diet period were included in the statistical analysis of the data.

The response of serum triglycerides to a change of diet more closely parallelled changes in serum phospholipids than changes in serum cholesterol (Table 14). For 16 of the 19 observations a decrease or an increase in serum phospholipids coincided with a corresponding decrease or increase in serum triglycerides whereas, the serum triglyceride response parallelled that of serum cholesterol in 12 of the 19 observations. The magnitude of response in serum triglycerides was variable with respect to the magnitude of response of either serum phospholipids or cholesterol.

E. CHOLESTERYL ESTER FATTY ACID PATTERNS

The proportion of arachidonic acid in the serum cholesteryl

TABLE 15

ADJUSTED MEAN SERUM TRIGLYCERIDES FOR SUBJECTS FED 0, 1, 2, 3 OR 6 EGGS PER DAY[1]

Dietary Regimen	Serum Triglyceride mg/dl
0 Egg Diet	67
1 Egg Diet	73
2 Egg Diet	94
3 Egg Diet	79
6 Egg Diet	82

1. For statistical adjustments see Appendix Table 7.

TABLE 16

SERUM TRIGLYCERIDES OF SUBJECTS IN RESPONSE TO DIFFERENT LEVELS OF DIETARY CHOLESTEROL[1]

Subjects	Dietary Regimen														
	0 Egg Diet Week			1 Egg Diet Week			2 Egg Diet Week			3 Egg Diet Week			6 Egg Diet Week		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
BH[2]	I[3] -	47	47	II 73	55	59									
JM				I 40	47	61							II 97	110	121
BD							I 52	31	34	II 48	46	59			
EC	II 42	43	46							I 39	36	40			
HM				II 52	52	50							I 66	68	59
LC	I 48	53	48				II 55	59	49						
BS				I 75	75	59				II 69	88	88			
DG				I 124	109	117	II 66	52	80						
RE										I 45	58	61	II 39	46	58
JB	II 80	75	80										I 58	57	64

1. Values are the mean of duplicate analyses, mg/dl.
2. Missing values. Subject BH participated in Diet Period II only.
3. I and II refer to Diet Period I and II.

esters increased slightly as egg consumption increased and it appears that this was primarily at the expense of cholesteryl linoleate. This change in cholesteryl esters cannot be attributed to a change in dietary fatty acids as there were essentially no differences in the fatty acid composition of the diets (Table 4). The proportion of polyunsaturated, monounsaturated and saturated fatty acids of the cholesteryl esters was unaffected by a change in the number of whole eggs in the diet. The fatty acid patterns of the serum cholesteryl ester fraction, determined at the beginning of the dietary trial and at the end of Diet Period I and Diet Period II are presented in Table 17 and Table 18.

The fatty acid profiles of the cholesteryl esters reported here are in agreement with relative values observed in humans in other laboratories (Goodman, 1965; Swell et al, 1960; Allard et al, 1973). In all species, the esters of polyunsaturated fatty acids predominate in plasma. However, the relative proportions of the different polyunsaturated fatty acids of cholesteryl esters differ substantially from species to species. In all species, other than the dog and mouse, cholesteryl arachidonate comprises less than 10% of plasma cholesteryl esters and cholesteryl linoleate is the major plasma fatty acid ester (Goodman, 1965).

A number of studies with humans have indicated that conditions associated with higher levels of plasma cholesterol are generally associated with decreases in the relative proportion of total polyunsaturated fatty acids in the cholesteryl esters, and specifically cholesteryl linoleate. These conditions include advancing age,

TABLE 17

CHOLESTERYL ESTER FATTY ACID PROFILE OF SUBJECTS FED 0, 1, 2, 3 OR 6 EGGS PER DAY[1]

Subject	Dietary Regimen	Percent of Total Fatty Acids							
		C14:0[2]	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4
BH	Initial	0.9	10.6	5.2	1.0	21.8	51.4	1.3	6.3
	0 Egg Diet[3]	-	-	-	-	-	-	-	-
	1 Egg Diet	tr[4]	9.9	2.6	0.9	19.7	59.5	0.6	5.7
JM	Initial	1.0	10.8	5.7	0.8	22.5	49.6	0.9	6.9
	1 Egg Diet	0.5	10.1	2.3	1.0	19.3	58.8	0.9	5.6
	6 Egg Diet	0.7	10.9	2.8	1.4	19.5	54.8	0.6	8.8
BD	Initial	0.9	11.0	4.9	1.1	23.6	48.0	0.8	6.5
	2 Egg Diet	tr	10.5	3.0	1.0	19.9	59.0	tr	5.2
	3 Egg Diet	tr	10.3	2.6	1.0	19.5	59.6	-	6.0
BS	Initial	0.5	12.4	3.8	1.4	27.1	50.3	-	3.6
	1 Egg Diet	0.5	13.7	2.8	2.3	27.2	43.6	0.7	6.6
	3 Egg Diet	tr	10.8	2.8	1.4	22.5	51.4	0.7	8.9
RE	Initial	0.8	11.7	3.4	1.0	22.4	54.9	-	4.8
	3 Egg Diet	tr	10.5	2.4	1.2	19.7	57.4	0.6	7.2
	6 Egg Diet	tr	11.1	2.6	1.3	19.4	55.2	-	9.6

continued on next page

TABLE 17 continued

Subject	Dietary Regimen	Percent of Total Fatty Acids							
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4
EC	Initial	0.7	11.5	3.4	1.1	24.5	49.8	0.7	7.1
	3 Egg Diet	tr	11.1	2.4	1.2	21.2	50.0	0.5	7.7
	0 Egg Diet	0.5	10.6	2.6	1.1	21.0	55.9	0.9	6.9
HM	Initial	0.6	12.5	3.0	1.0	22.2	50.8	0.7	8.5
	6 Egg Diet	tr	11.8	2.4	1.6	22.1	52.2	0.5	8.4
	2 Egg Diet	tr	10.6	2.2	1.2	21.2	56.8	0.7	6.3
DG	Initial	tr	10.5	3.2	0.8	24.3	52.9	0.6	6.8
	2 Egg Diet	tr	10.1	2.0	1.1	18.2	60.2	0.7	6.7
	0 Egg Diet	0.6	10.1	2.5	1.1	18.9	59.2	1.0	6.2
JB	Initial	0.7	10.1	3.0	1.2	21.5	57.0	0.5	5.2
	6 Egg Diet	tr	10.7	2.3	1.4	19.3	56.3	tr	8.1
	0 Egg Diet	tr	9.7	2.2	1.1	18.7	61.1	0.8	4.4
LC	Initial	1.3	11.2	5.0	1.4	23.3	48.9	1.1	5.7
	0 Egg Diet	0.6	9.7	2.3	1.0	18.9	61.7	0.9	3.3
	2 Egg Diet	0.5	10.0	2.6	1.3	20.1	58.1	0.9	5.7

1. Values are means of duplicate determinations of serum samples taken on the first day of the experimental trial (initial value) and on the last day of Diet Period I and II.
2. Carbon number: number of double bonds.
3. Subject participated only in Diet Period II.
4. Assigned to any value less than 0.5% of total fatty acids.

TABLE 18

PERCENTAGE POLYUNSATURATED, MONOUNSATURATED AND SATURATED FATTY ACIDS OF CHOLESTERYL ESTERS

Subject and Dietary Regimen			Polyunsaturated Fatty Acids
BH			
Initial	12.5	27.0	59.0
0 Egg Diet[1]	-	-	-
1 Egg Diet	10.8	22.3	65.8
JM			
Initial	12.6	28.2	57.4
1 Egg Diet	11.6	21.6	65.3
6 Egg Diet	13.0	22.3	64.2
BD			
Initial	13.0	28.5	55.3
2 Egg Diet	11.5	22.1	64.2
3 Egg Diet	11.3	22.1	65.6
BS			
Initial	14.3	30.9	53.9
1 Egg Diet	16.5	30.0	50.9
3 Egg Diet	12.2	25.3	61.0
RE			
Initial	13.5	25.8	59.7
3 Egg Diet	11.7	22.1	65.2
6 Egg Diet	12.4	22.0	64.8
LC			
Initial	13.9	28.3	55.7
0 Egg Diet	11.3	21.2	65.9
2 Egg Diet	11.8	22.7	64.7
EC			
Initial	13.3	27.9	57.6
3 Egg Diet	12.3	23.6	58.2
0 Egg Diet	12.2	24.6	63.7
HM			
Initial	14.1	25.2	60.0
6 Egg Diet	13.4	24.5	61.1
2 Egg Diet	11.8	23.4	63.8
DG			
Initial	11.3	27.5	60.3
2 Egg Diet	11.2	20.2	67.6
1 Egg Diet	11.2	21.4	66.8
JB			
Initial	12.0	24.5	62.7
6 Egg Diet	12.1	21.6	64.4
0 Egg Diet	10.8	20.9	65.5

1. Subject participated in Diet Period II only.

diabetes mellitus, atherosclerotic heart disease and idiopathic hyperlipemia (Goodman, 1965; Kingsbury et al, 1969). In most cases the differences have been small and approximately proportional to the degree of elevation of plasma cholesterol. Not all reports in the literature are in agreement with this observation. Laudat and associates (1966) and Allard and coworkers (1973) observed that atherosclerosis was not associated with a decreased proportion of either total polyunsaturates or linoleic acid in the cholesterol ester fraction.

According to Nestel and associates (1966) changes in the composition of cholesteryl esters are evident within twenty-four hours after a change in diet and the changes are apparent in all classes of lipoproteins. Both the type and amount of dietary fat have been shown to influence the composition of cholesteryl esters (Nestel, 1970). It has also been reported that in the rat, rabbit, guinea pig, monkey and gerbil, additional cholesterol in the diet resulted in a marked decrease in the proportion of polyunsaturated cholesteryl esters with increased plasma cholesterol ester concentration (Goodman, 1965). In contrast to this finding in animal experimentation, in the present study an increase in dietary cholesterol intake and the subsequent increase in serum cholesterol was not associated with a proportional decrease in the polyunsaturated fatty acids of the cholesteryl ester fraction. The increase or decrease in cholesteryl arachidonate in response to a corresponding increase or decrease in egg consumption, although small, less than 5%, was consistent.

PART II

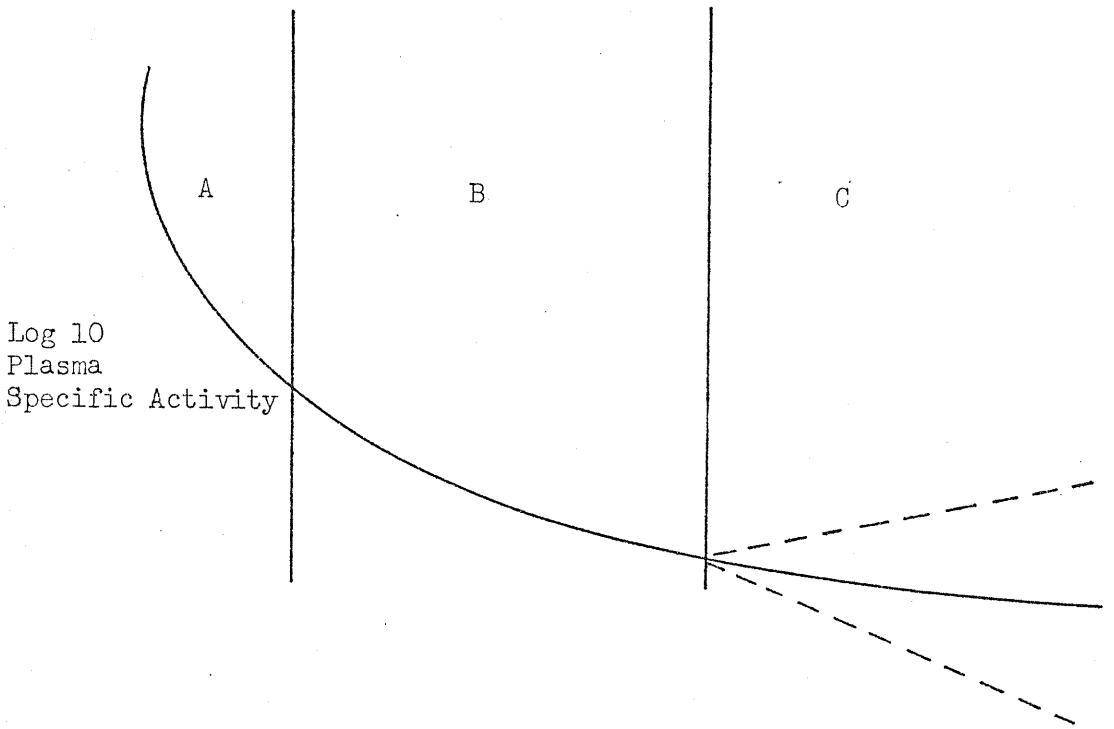
THE EFFECT OF DIETARY CHOLESTEROL ON THE RATE OF CHANGE OF SERUM CHOLESTEROL SPECIFIC ACTIVITY

REVIEW OF LITERATURE

The observation that the concentration of cholesterol in the serum can be altered by diet manipulation (Grande et al, 1965; Mattson et al, 1972) has created an interest in the effect of dietary cholesterol on tissue cholesterol turnover (Nestel, 1970). As it is believed that localized accretion of cholesterol in tissues is critical in the development of atherosclerosis, it is imperative that it be determined if a decrease in serum cholesterol is affected by increased deposition or a decreased rate or removal of cholesterol from soft tissue.

Turnover of cholesterol reflects the balance between input of cholesterol, from endogenous biosynthesis and from absorption of dietary cholesterol, and the loss of cholesterol which occurs in the feces in the form of bile acids and neutral sterols. Plasma, as one of the more accessible tissues, has been used extensively to study the rate of turnover of cholesterol. It has been consistently shown (Nestel et al, 1969; Noble et al, 1973; Quardford et al, 1973) that during the first four weeks after a single intravenous infusion of radioactive cholesterol the semilogarithmic plot of the plasma cholesterol specific activity versus time is curvilinear and after the fourth week the rate of disappearance of specific activity becomes essentially linear (Figure 4).

FIGURE 4: SCHEMATIC LOG PLASMA SPECIFIC ACTIVITY - TIME CURVE FOR SERUM CHOLESTEROL CONCENTRATION OF A SINGLE DOSE OF RADIOACTIVE CHOLESTEROL.



A: PERIOD OF RAPID DECAY - equilibration of plasma and other tissue pools of cholesterol

B: PERIOD OF ISOTOPIC EQUILIBRIUM

C: A change in the slope of the specific activity time curve can be effected by a change in dietary regimen

Grundy and Ahrens (1966)

Cholesterol exists in the body as a number of separate tissue pools that turnover at different rates (Chobanian et al, 1962a, 1962b). The curvilinear portion of the specific activity time curve, during the first four weeks after isotope administration, reflects the different rates of equilibration between the plasma pool and other tissue pools of cholesterol. Goodman and Noble (1968) described the plasma specific activity die-away curve in terms of two general body pools of cholesterol with different exchange rates. In this model, Pool A, estimated to contain one-third of the total body exchangeable cholesterol, comprises the liver, bile, plasma, erythrocytes and possibly some of the cholesterol in the intestine, lung, spleen and kidney. The flux of cholesterol among these tissues is rapid as the body moves to a new equilibrium. The equilibration of subpools within Pool A accounts for the rapid decline of plasma specific activity during the first four days after isotope administration.

Tissues which have a slower rate of cholesterol turnover were designated as Pool B. This pool includes the remainder of the cholesterol in viscera, that in skeletal muscle, skin and adipose tissue. The changing slope of the plasma cholesterol specific activity time curve during the first four weeks reflects the slower rate of equilibration between cholesterol in the plasma and that in the tissues which comprise Pool B.

Dietschy and Wilson (1970) and Goodman et al, (1973) have modified this model by the addition of a third pool, Pool C, made up of neural tissue, with negligible exchange rate. It appears that the three pool model fits the specific activity data for long term cholesterol turnover studies. However, data from the specific activity die-

away curve for studies of less than twelve weeks duration are best described by the two pool model (Goodman et al, 1973).

With the two pool model of cholesterol turnover it is assumed that a steady state equilibrium between tissues in Pool A and Pool B has been attained when the log-specific activity time curve becomes linear (Figure 4); approximately four weeks after intravenous administration of labelled cholesterol. The subsequent removal of cholesterol from the plasma represents the turnover of the slowly miscible pool of cholesterol. The rate of turnover is reflected by the slope of the log-linear portion of the plasma specific activity curve (Nestel, 1965; Grundy et al, 1966).

Grundy and Ahrens (1966) proposed that a change in dietary regimen would effect a change in the slope of the log-linear portion of the plasma specific activity curve (Figure 4). Entry into and exit from the total miscible body pool of cholesterol occurs almost exclusively through Pool A (Figure 5). In the steady state, the production rate of Pool A (endogenous synthesis plus absorption of dietary cholesterol) equals the rate of cholesterol excretion from Pool A. A change in the slope of the log-linear portion of the plasma specific activity curve can be brought about by changing the production rate of Pool A. After a change in dietary regimen, and attainment of a new steady state, an upward inflection or decreased slope of the curve would indicate an increase in the total body cholesterol pool or a decrease in the rate of endogenous biosynthesis or cholesterol absorption (Grundy et al, 1966). An increased slope or downward deflection of the curve would indicate a faster fractional turnover rate as a result of a decreased

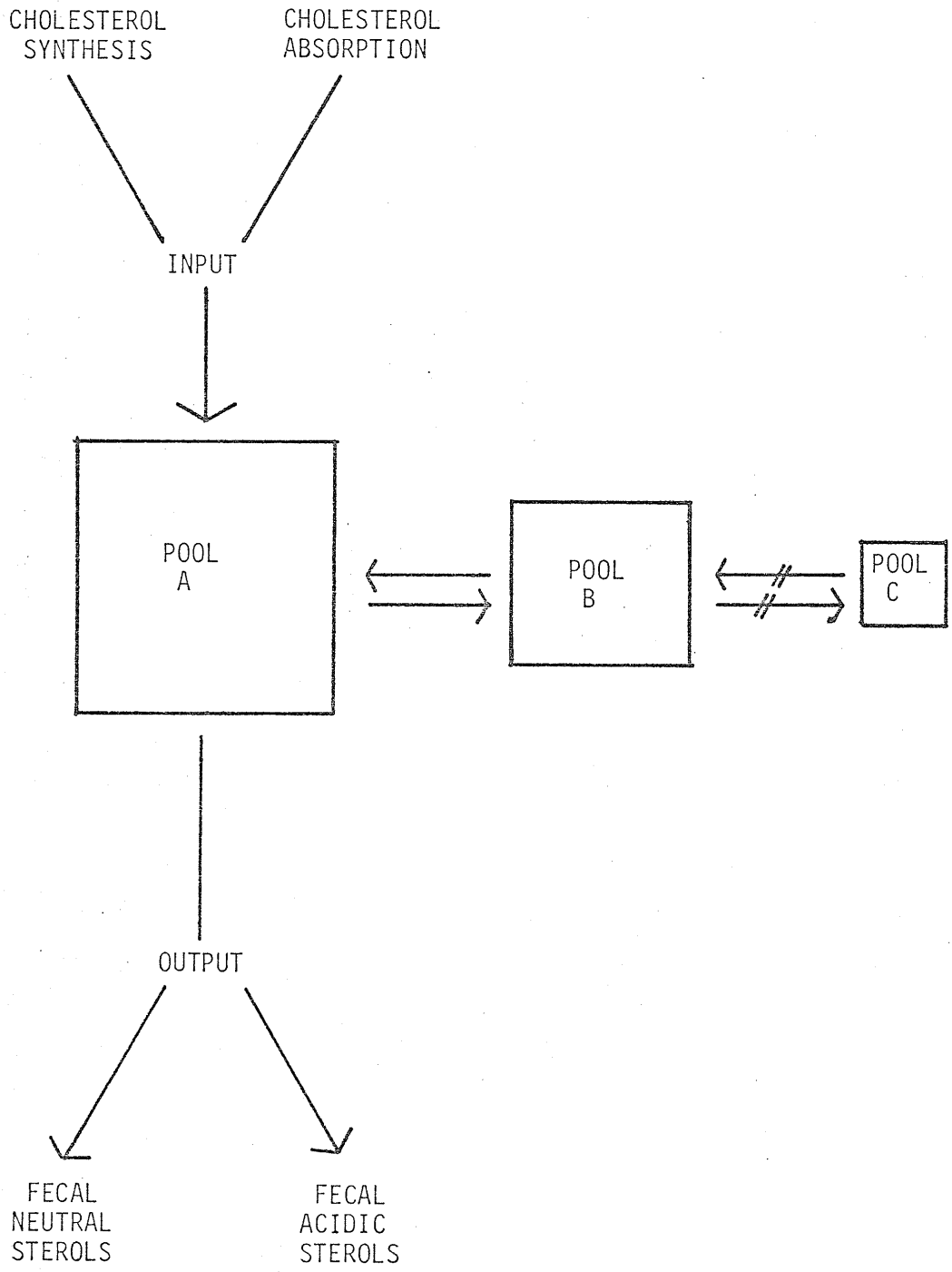


FIGURE 5: THREE POOL MODEL OF CHOLESTEROL TURNOVER.

Dietschy and Wilson (1970).

body pool of cholesterol or an increase in the rate of endogenous synthesis or cholesterol absorption. The fractional turnover rate of tissue cholesterol is directly proportional to the total body turnover rate of cholesterol (Nestel, 1965).

This technique has been used by Nestel (1965), Grundy and coworkers (1969) and Sodhi et al, (1973) to assess the effects of various drugs and dietary manipulations on the turnover rate of tissue cholesterol. In the study reported by Grundy and associates (1969) it was observed that an increase in the level of dietary cholesterol resulted in an increase in the plasma cholesterol concentration. A new steady state was achieved, following the change in cholesterol intake, and measurement of the plasma specific activity revealed an increased slope of the log-linear portion of the plasma specific activity curve. They concluded that more nonlabelled cholesterol entered the body pools per day when cholesterol intake increased. Hence, the production rate of Pool A had increased which, in the steady state, is equivalent to an increased metabolic turnover rate of cholesterol.

In the study reported here an attempt was made to assess the effects of dietary cholesterol on cholesterol turnover by a combination of analysis of plasma specific activity time curves and a sterol balance technique. [38]

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[38] The sterol balance portion of this study is the topic of a thesis to be submitted by Ms. Marie Fuller and will not be discussed further in the present thesis.

RESULTS AND DISCUSSION

PLASMA SPECIFIC ACTIVITY

The rate of change in plasma specific activity was monitored by measuring the radioactive ^{3}H-cholesterol (dpm) and expressing it in terms of total serum cholesterol. As isotope administration was carried out four weeks prior to the start of the experimental dietary periods it was assumed that equilibration between cholesterol in tissues in Pool A and Pool B was complete; the rate of change in the $\log_{10}$ of the plasma specific activity (Figure 4) was linear. An F test for the homogeneity of regression (Appendix Table 9) indicated that the rate of change in the log-serum specific activity did not differ significantly between Diet Period I and Diet Period II for any subject despite the change in dietary cholesterol intake and serum cholesterol levels. Values for serum radioactivity (dpm/ml serum) and specific activity (dpm/mg serum cholesterol) for each subject are presented in Tables 19 and 20, respectively.

In the steady state, a change in the log-plasma specific activity would reflect a change in cholesterol turnover rate (Nestel, 1965). It is assumed that the metabolic steady state is present when the following conditions are met: a constant plasma cholesterol concentration, unchanging fecal excretion of sterols and constant body weight (Grundy et al, 1969). In the present study, following the change in dietary regimen in Diet Period I and Diet Period II, the plasma cholesterol concentration was not constant for subjects prior to sampling for measurement of serum specific activity. When the size of the plasma pool

TABLE 19

MEAN SERUM RADIOACTIVITY (dpm/ml) OF SUBJECTS IN RESPONSE TO DIETARY CHOLESTEROL[1]

Subjects	DIETARY REGIMEN														
	0 EGG DIET			1 EGG DIET			2 EGG DIET			3 EGG DIET			6 EGG DIET		
	Week			Week			Week			Week			Week		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
BH[2]	I[3] -	-	-	II 714	620	551									
JM				I 689	572	460							II 413	364	327
BD							I 1233	1122	862	II 787	708	637			
EC	II 565	446	422							I 968	773	694			
HM							II 1141	1018	1558				I 1129	987	828
LC	I 952	637	441				II 485	385	309						
BS				I 1063	959	736				II 619	540	473			
DG				II 883	728	768	I 1726	1266	1071						
RE										I 1231	1223	1051	II 823	649	662
JB	II 1438	1239	1090										I 2451	2158	1846

1. Mean of duplicate analyses expressed in dpm/ml of serum.
2. Missing values. Subject BH participated in Diet Period II only.
3. I and II indicate Diet Period I and II, respectively.

TABLE 20

MEAN SERUM SPECIFIC ACTIVITY (dpm/mg) OF SUBJECTS IN RESPONSE TO DIETARY CHOLESTEROL[1]

Subjects	DIETARY REGIMEN																	
	0 EGG DIET			1 EGG DIET			2 EGG DIET			3 EGG DIET			6 EGG DIET					
	Week			Week			Week			Week			Week					
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3			
BH[2]	I[3]	-	-	-	II	397	359	358										
JM					I	451	379	311								II	260	228	175
BD									I	608	565	454	II	440	399	362		
EC	II	332	258	247									I	506	411	369		
HM									II	700	640	1032					I	652	555	465
LC	I	564	422	322					II	293	218	178						
BS					I	644	581	455					II	329	295	263		
DG					II	453	385	359	I	899	640	558						
RE													I	789	707	607	II	450	334	323
JB	II	813	700	623													I	1104	1013	879

1. All values are means of duplicate analyses expressed in dpm/mg serum cholesterol.
2. Missing values. Subject BH participated in Diet Period II only.
3. I and II indicate Diet Period I and II.

of cholesterol was changed a new steady state would only be achieved after four weeks due to the slow rate of equilibration between plasma cholesterol and cholesterol in tissues which contribute to Pool B. As stated previously, it is only in the steady state condition that the slope of the log-linear portion of the cholesterol specific activity curve reflects the fractional turnover rate of cholesterol. Hence, conclusions about the quantitative effect of dietary cholesterol on the rate of cholesterol turnover cannot be drawn from the results of this study as the metabolic steady state conditions were not present.

Nevertheless, monitoring of the specific activity of the plasma can be useful in isolating the possible mechanisms by which the size of the plasma cholesterol pool is altered by a change in dietary cholesterol. In the present study, cholesterol intake was found to affect the plasma pool of cholesterol in a linear fashion. There are several mechanisms whereby dietary cholesterol may effect this change. A hypercholesterolemic response may be brought about by (a) an increase in the amount of dietary cholesterol absorbed (Figure 6.1), (b) a failure to excrete this additional cholesterol or an overall reduction in the excretion of cholesterol from the plasma pool (Figure 6.2), (c) a net flux of cholesterol from other tissues to the plasma (Figure 6.3a and 6.3b) or (d) a combination of these mechanisms. Alternatively, a decrease in cholesterol intake resulted in a decrease in the size of the plasma pool of cholesterol and this could be caused by similar mechanisms operating in the opposite direction.

It is only possible to speculate on which of these mechanisms was operative. Analysis of the fecal steroids should help to resolve

FIGURE 6.1 INCREASED CHOLESTEROL ABSORPTION

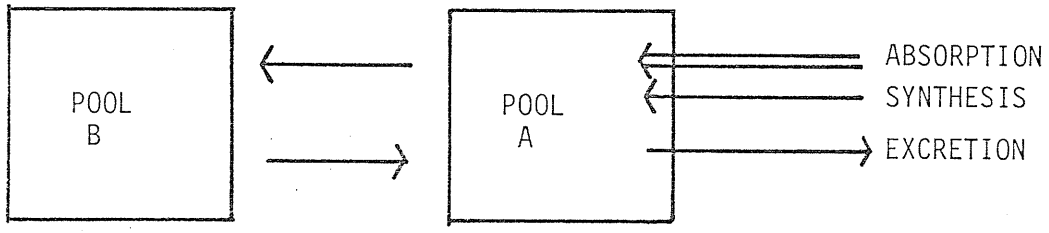


FIGURE 6.2 DECREASED CHOLESTEROL EXCRETION

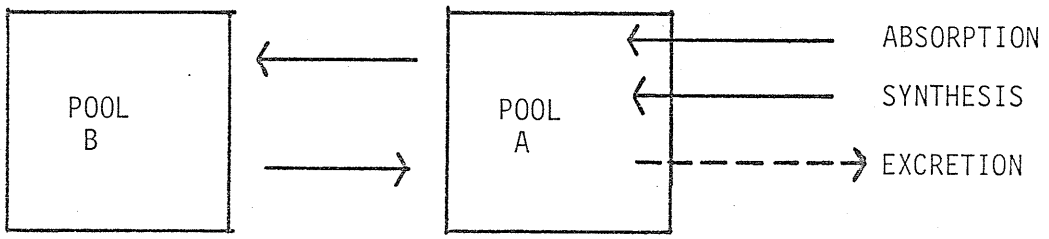


FIGURE 6.3a INCREASED TRANSFER TO PLASMA

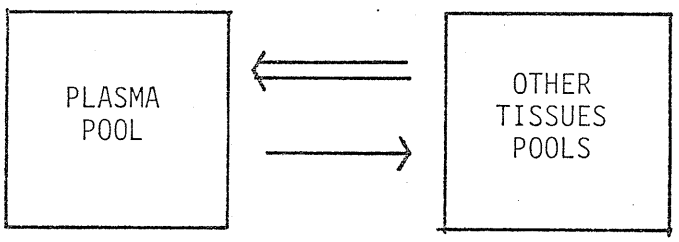


FIGURE 6.3b DECREASED TRANSFER FROM PLASMA

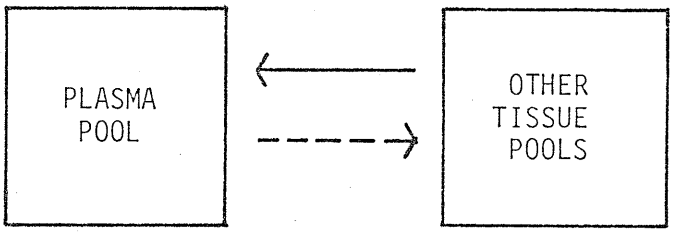


FIGURE 6: MECHANISMS WHEREBY DIETARY CHOLESTEROL CAN BRING ABOUT AN INCREASE IN THE SIZE OF THE PLASMA CHOLESTEROL POOL.

the mechanisms. Results of the quantitative and qualitative patterns of fecal neutral and acidic sterols, obtained from sterol balance data, will be reported elsewhere (Fuller, unpublished thesis). If the change in plasma cholesterol resulted from a change in the absorption of non-labelled dietary cholesterol then the rate of decline of plasma specific activity would be expected to change because the ratio of the radioactive label to total cholesterol in the plasma would be altered. The slope of the plasma specific activity - time curves did not change significantly in response to changes in dietary cholesterol intake despite changes in the size of the plasma cholesterol pool of subjects. However, following the change in the diet, there were small shifts in the slopes of the plasma specific activity - time plots which reflected the change that had occurred in the plasma cholesterol concentration for several of the subjects (Figure 7.1, 7.2, 7.3, 7.5, 7.6, 7.8) These changes were undetected by the statistical test used and cannot be considered significant but, nonetheless, are apparent from the semi-log plot of specific activity versus week of the study.

For three of four subjects (HM, EC, JB) a decrease in dietary cholesterol intake resulted in a marked decrease in serum cholesterol concentration. For these subjects the decrease in serum cholesterol was accompanied by a decrease in the slope of the plasma specific activity - time curve (Figure 7.1, 7.2, 7.3). The slower decline in plasma specific activity for EC and JB during the week following the diet change and the increase in plasma specific activity for HM suggest a decreased rate of dilution of the plasma labelled cholesterol either as a result of decreased absorption of nonlabelled dietary cholesterol, the entry

Figure 7.1 : EC

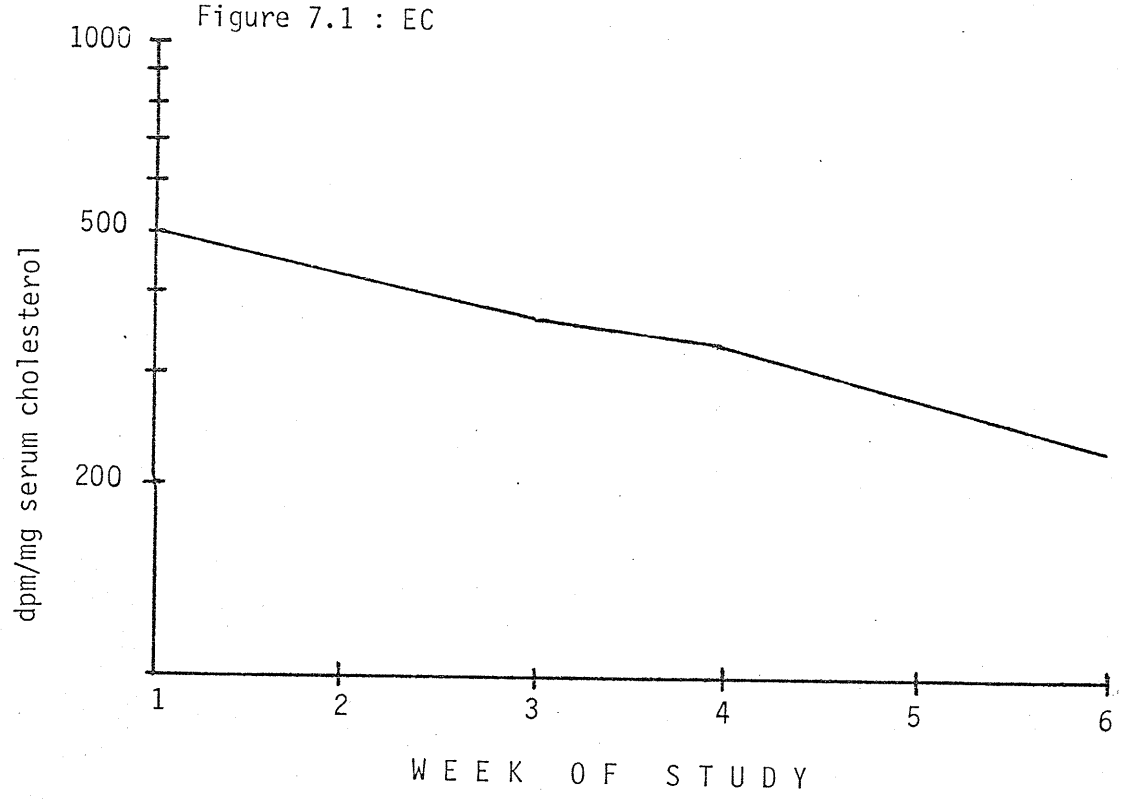


Figure 7.2 : JB

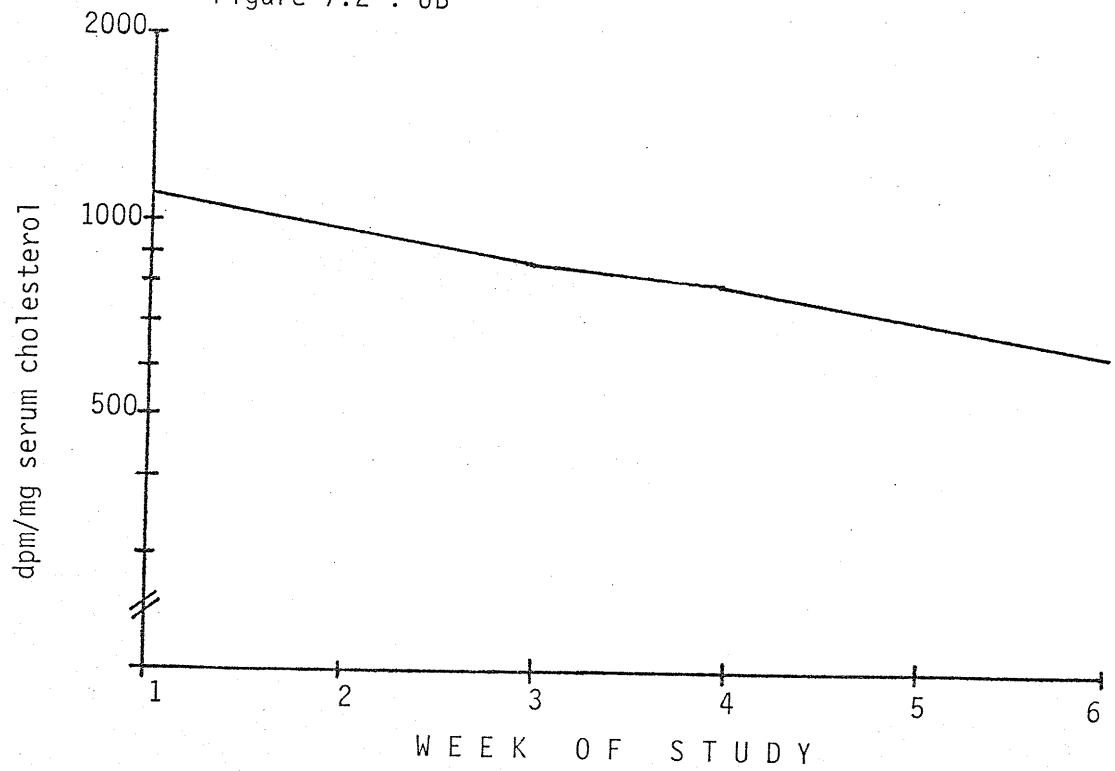


Figure 7.3 : HM

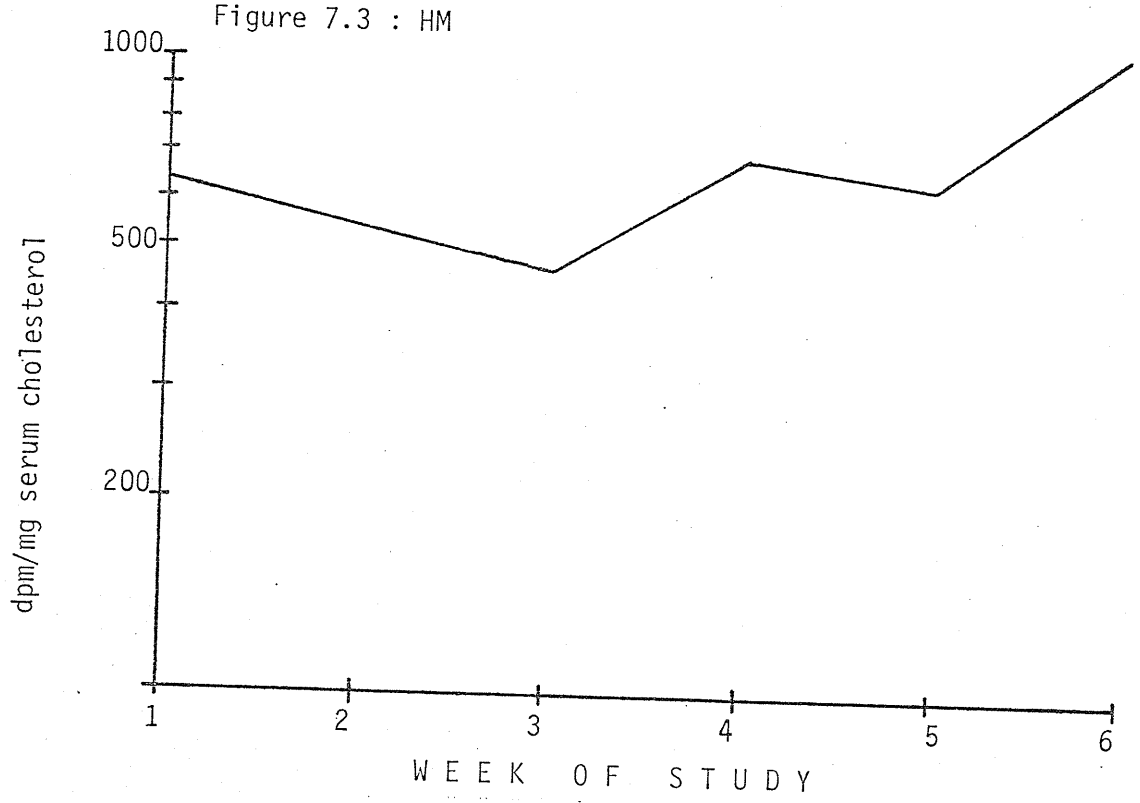
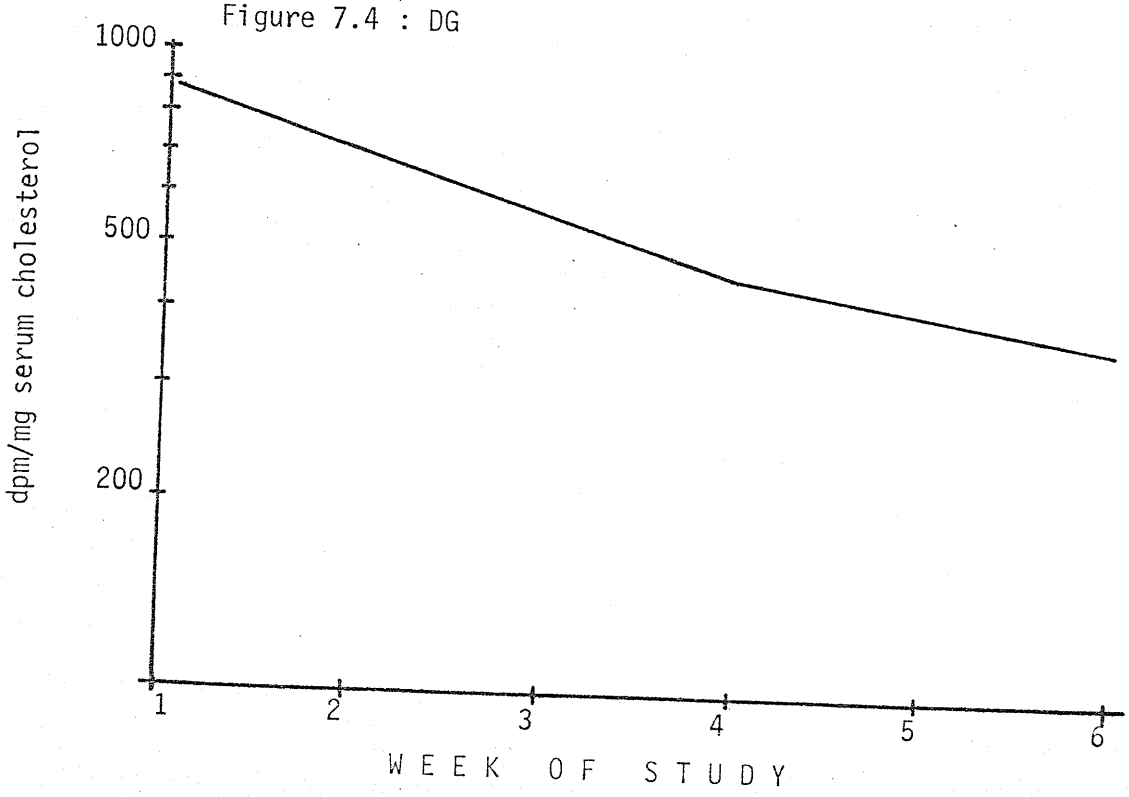


Figure 7.4 : DG



of higher specific activity cholesterol from the tissues into plasma or a combination of these mechanisms. According to Sodhi et al (1973) only the rapidly exchanging tissue pools of cholesterol have the same specific activity as the plasma pool of cholesterol. After labelling the plasma cholesterol, the specific activity of some of the tissue pools in rats, rabbits, baboons and man have been shown to be greater than that of plasma cholesterol. After labelling the plasma cholesterol pool and equilibration of plasma and tissue cholesterol, the specific activity of the pools of cholesterol in some tissues will remain higher than that of plasma. The magnitude of difference in their specific activities depends upon the relative size and the rate of equilibration between plasma and the tissue pool. The more rapid the equilibration and the smaller the tissue pool, the closer are the specific activity values of the plasma and tissue pool. Thus, the tissue pools expected to have higher specific activity than plasma cholesterol are the slowly exchanging pools of the tissues that comprise Pool B (Sodhi et al, 1973). Hence, an increase in the rate of exchange of cholesterol between tissues with a higher cholesterol specific activity than plasma and the plasma would result in a slower decline of plasma specific activity. Rather than an increase in the rate of exchange between plasma and other tissue pools, the change in the rate of decrease in the specific activity in the plasma may have resulted because of a net flux of cholesterol out of the tissues with a higher specific activity than plasma. In this instance, as the plasma cholesterol concentration decreased with a decrease in cholesterol intake, the additional cholesterol was not held in the plasma compartment. Preliminary observations of the fecal steroid

data suggest that the absolute amount of cholesterol absorbed decreased with a decrease in cholesterol intake. However, if the changes in serum cholesterol levels, in response to diets in the present study, are found to be partially due to redistribution of cholesterol between plasma and tissue pools, it will not be possible to determine from the data gathered the mechanism by which the new equilibrium between these pools was established.

For the fourth subject (DG) the slope of the specific activity - time plot decreased with a decrease in dietary cholesterol (Figure 7.4). However, it must be noted that a decrease in dietary cholesterol from 781 to 441 mg per day (ie. a change from the 2-Egg to the 1-Egg diet) did not result in a decrease in serum cholesterol for this subject. Thus, if decreased absorption of nonlabelled dietary cholesterol was the reason for decreased dilution of the plasma label then decreased excretion of cholesterol or a net flux of cholesterol from tissues to plasma must also have occurred in order for the subject's serum cholesterol concentration to remain constant.

The response of five subjects to an increase in dietary cholesterol was less uniform. For four subjects (RE, BS, JM, LC) an increase in dietary cholesterol intake caused a marked increase in serum cholesterol in the first week following the diet change. For two of these subjects (RE, BS) the increase in serum cholesterol was accompanied by a more rapid rate of decrease in plasma cholesterol specific activity during this week (Figure 7.5, 7.6). This more rapid decline in specific activity most likely resulted from increased dilution of the labelled plasma cholesterol as a result of increased absorption of nonlabelled

Figure 7.5: BS

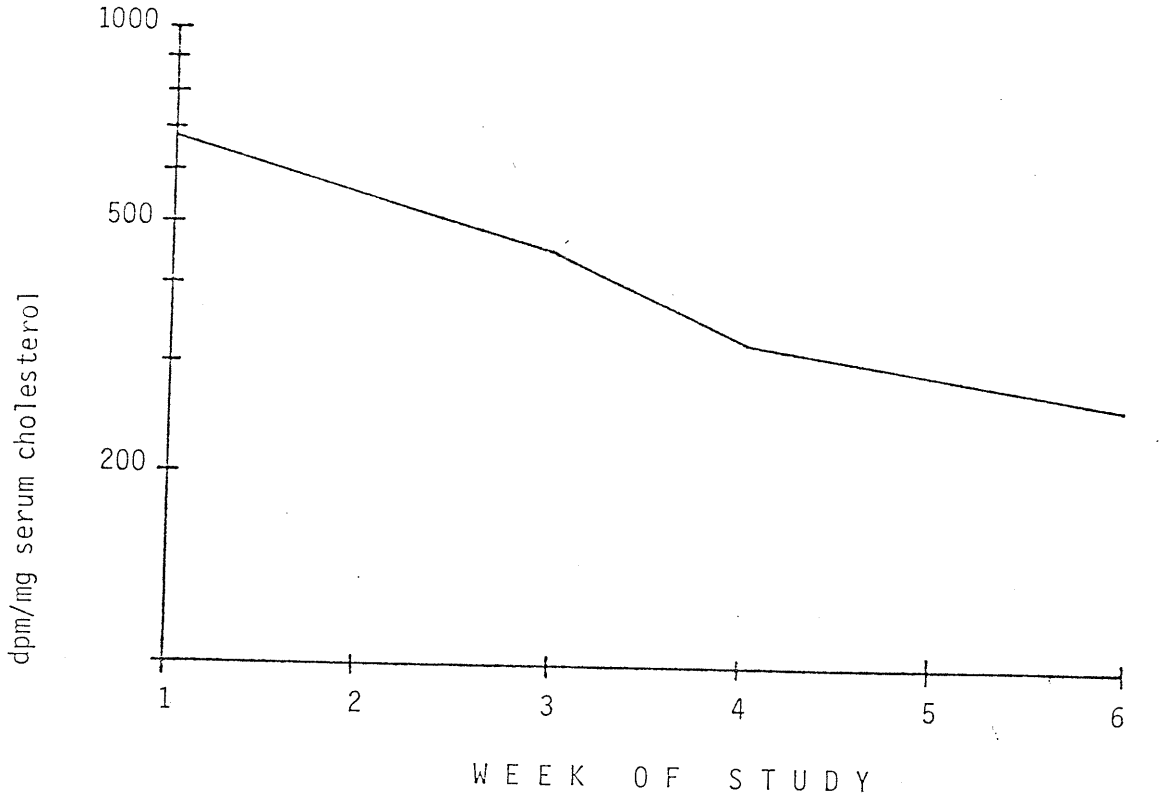
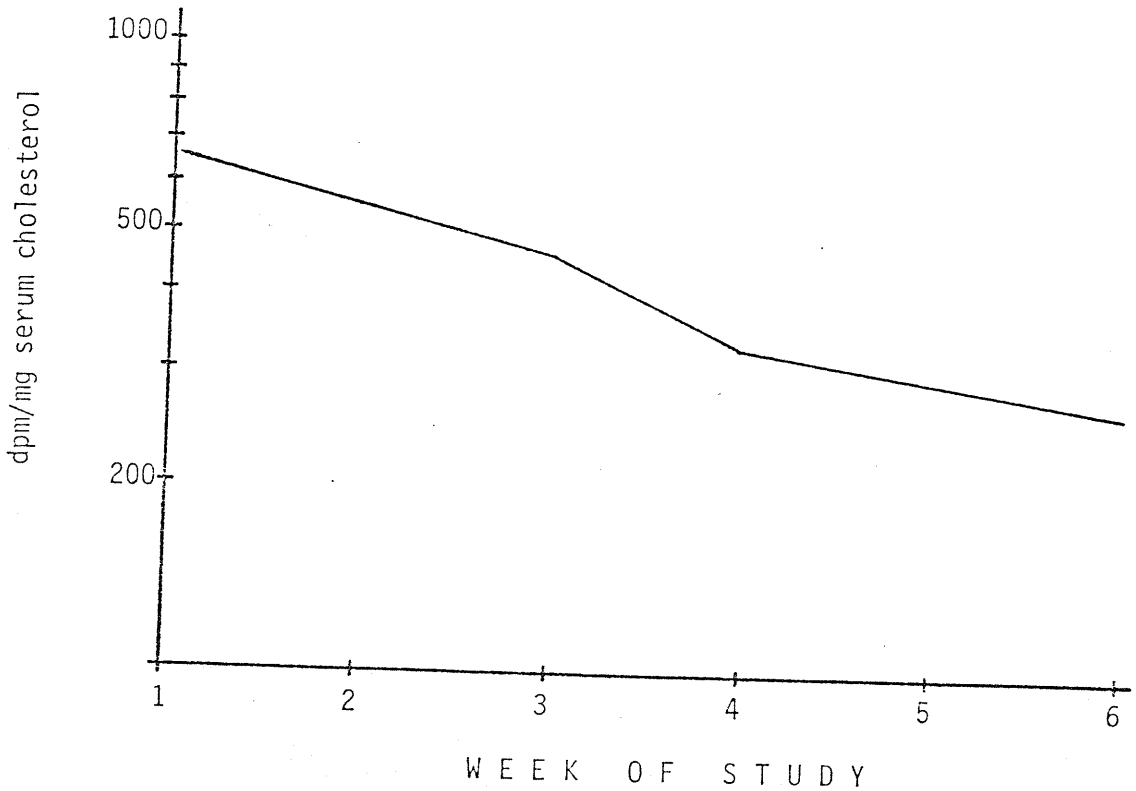


Figure 7.6: RE



dietary cholesterol, but may also reflect a decreased rate of exchange of cholesterol between tissues in Pool B and plasma.

The increase in serum cholesterol observed for subject JM did not result in any change in the rate of decrease of serum specific activity (Figure 7.8). On the other hand, the observed increase in serum cholesterol for subject LC was accompanied by a transient decrease in the rate of dilution of the plasma label in the week following the change in dietary regimen (Figure 7.7). If dietary cholesterol absorption increased with increased intake then one would expect either the exchange between plasma cholesterol and cholesterol of tissues in Pool B was increased or that there was a net flux of cholesterol from tissues with a higher specific activity than plasma cholesterol. This would have occurred only to the extent that it countered the effects of increased entry of unlabelled dietary cholesterol into the plasma for JM. In the case of subject LC there appears to have been an overcompensation in these mechanisms.

The remaining subject (BD) exhibited a decline in serum cholesterol and a slower rate of decrease in serum specific activity when dietary cholesterol was increased (Figure 7.9). The mechanism whereby this occurred is uncertain but it is possible that weight loss experienced by this subject had a greater effect on cholesterol metabolism than had an increase in dietary cholesterol.

Grundy and associates (1969) have observed that an increase in the level of dietary cholesterol resulted in an increase in the plasma cholesterol concentration as well as an increase in the rate of dilution of the plasma label and hence have concluded that more non-

Figure 7.7: JM

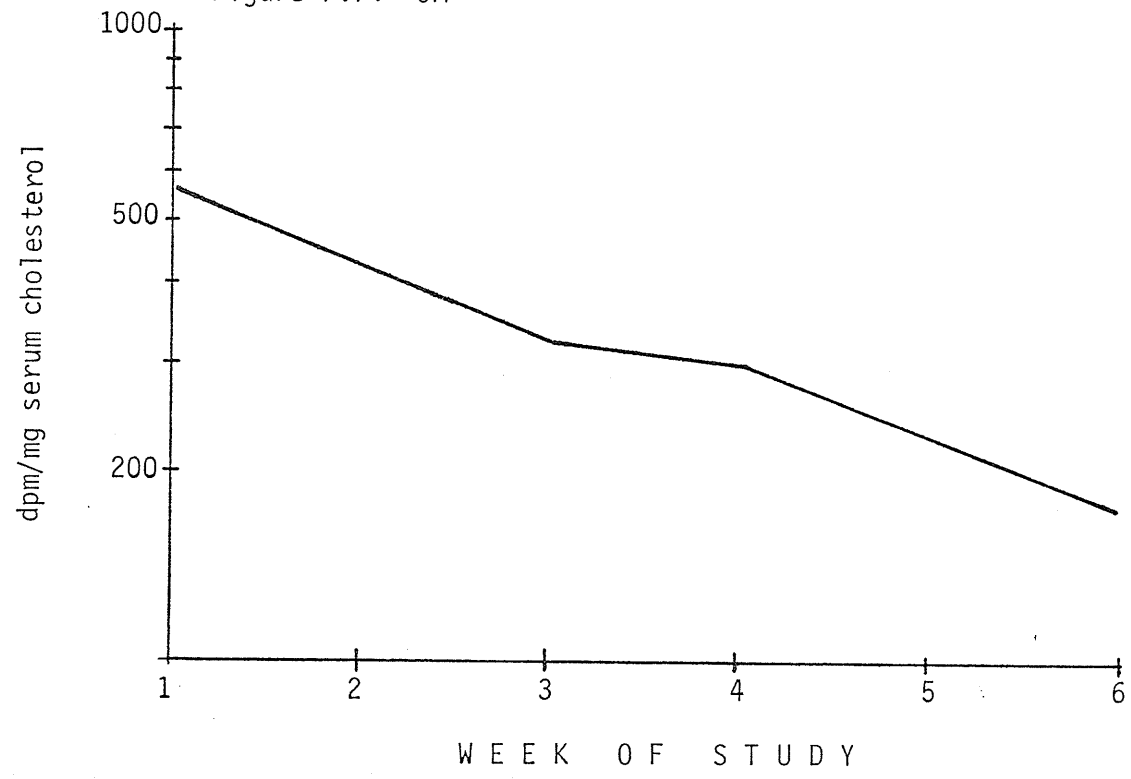


Figure 7.8: LC

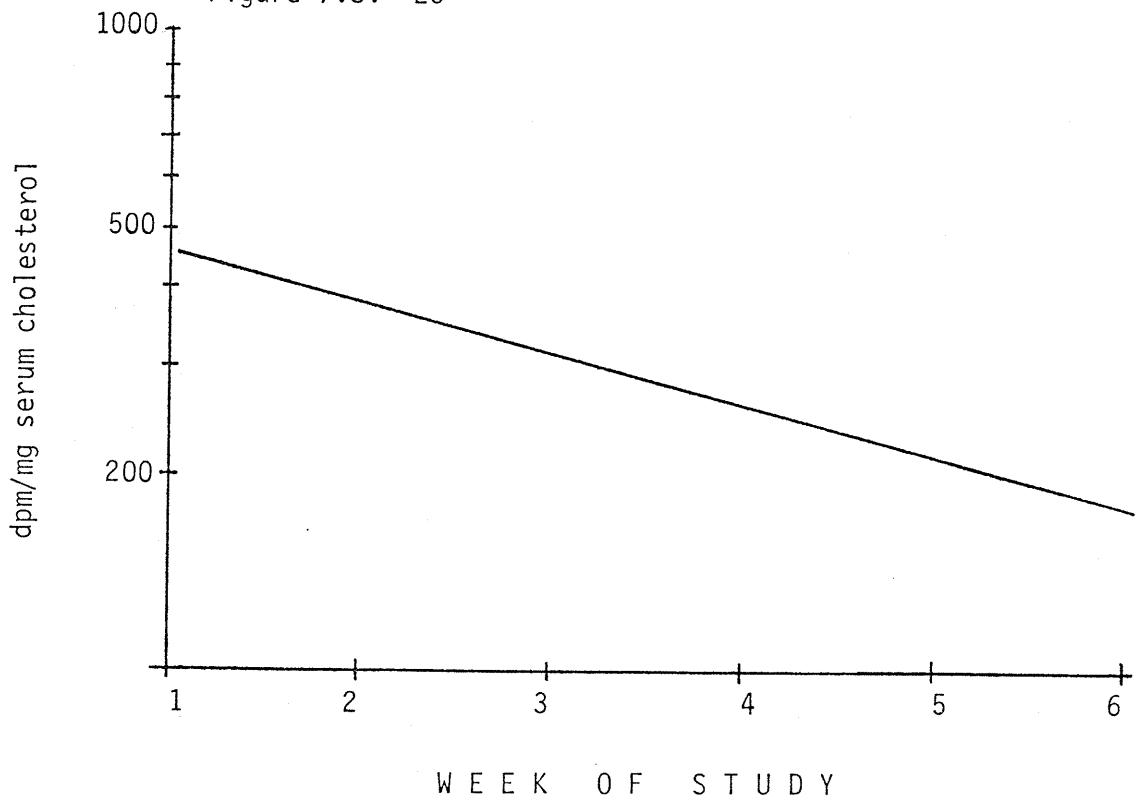
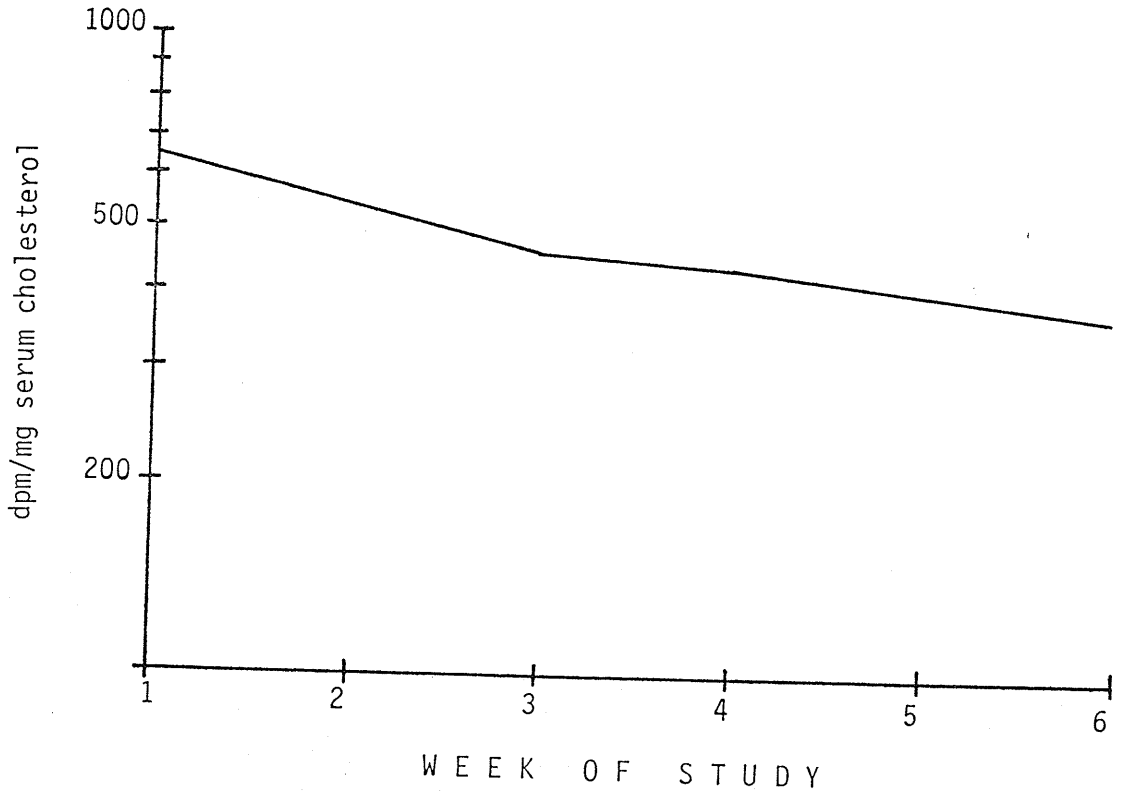


Figure 7.9: BD



labelled cholesterol entered the body pools per day when cholesterol intake was increased. This finding is consistent with reports in the literature which indicate that the percent cholesterol absorption is consistent over a broad range of intakes (Kudchodkar et al, 1973; Nestel et al, 1975; Ho et al, 1974). Wilson and Lindsey (1965) reported that a change in dietary cholesterol intake did not affect the rate of cholesterol turnover in the two subjects studied.

For subjects RE, BS, EC, JB and LC the transient change in the slope of the specific activity - time plot coincided with the week following the diet change. After that time the slope became the same as it was prior to the change in the diet which indicates that compensation by alteration of cholesterol synthesis, excretion, absorption or turnover occurred rapidly in some of the subjects. For subject JM no change in the slope of the specific activity - time curve, despite an increase in serum cholesterol concentration which was likely to be the result of increased cholesterol absorption would suggest that compensation occurred within less than one week. Metabolic compensation to a change in cholesterol intake, however, was incomplete as the changes in the serum cholesterol concentration were not transient over the three week period investigated.

SUMMARY AND CONCLUSIONS

The present study investigated the effects of dietary cholesterol, fed as egg yolk, on serum lipid patterns and the mechanisms by which dietary cholesterol effects a change in serum cholesterol in healthy free-living young men. Mixed diets, containing 40% of calories as fat, were designed to provide five levels of dietary cholesterol by the addition of 1, 2, 3 or 6 eggs to the basal diet which contained 113 mg cholesterol. The cholesterol content of the five experimental diets was determined to be 113, 441, 781, 1058 and 1819 mg cholesterol and from these data the average cholesterol content of the eggs was calculated to be 300 mg. The study, a 42-day metabolic trial, included two three-week diet periods. In Diet Period I two subjects were fed each of the experimental diets and for Diet Period II subjects were reassigned so that no subject consumed the same diet in the two diet periods.

Serum cholesterol was found to be a linear function of the level of cholesterol in the daily diet. The relationship between the level of serum cholesterol and the number of whole eggs consumed was defined by the regression equation: serum cholesterol (mg/dl) = 147.7 + 11.9 (number of eggs in the diet). Thus, for each additional egg in the diet (300 mg cholesterol) serum cholesterol was increased by approximately 12 mg/dl.

Although the level of serum phospholipids was not significantly altered by a change in dietary cholesterol intake the overall pattern of response was similar to that of serum cholesterol. When

the level of dietary cholesterol increased or decreased, serum phospholipids were correspondingly elevated or decreased. The magnitude of response in serum phospholipids was variable, however, compared to the magnitude of response in serum cholesterol. The level of serum triglycerides was not significantly affected by a change in dietary cholesterol intake and changes in serum triglycerides were less consistent than serum phospholipids with respect to the corresponding changes in serum cholesterol.

Serum cholesterol levels had stabilized for most subjects by the end of the second week after the diet change. However, as cholesterol levels had not completely stabilized for all subjects, studies of longer duration are indicated if the quantitative effects of dietary cholesterol on serum cholesterol are being investigated. This would make allowance for subject variability in the rate of adaptation to a change in dietary cholesterol intake and, therefore, the true effect of dietary cholesterol on serum cholesterol to be measured. Such a change in methodology may be of particular importance when large variations in dietary cholesterol intake are being investigated within one subject.

An increase in dietary cholesterol intake and in many cases an accompanying change in serum cholesterol was not accompanied by a change in the proportion of polyunsaturated, monounsaturated and saturated fatty acids in the plasma cholesteryl esters. There was a small, less than 5%, but consistent increase in cholesteryl arachidonate with an increase in egg consumption. This increase in arachidonate appeared to be at the expense of linoleate.

Approximately four weeks prior to the start of the study,

each subject was infused with 50 microcuries of tritium-labelled cholesterol and the decline in plasma specific activity was monitored throughout the dietary trials. There was no significant change in the slope of the specific activity-time curve for any subject when two different levels of dietary cholesterol were fed. However, small changes in the rate of decline of plasma specific activity were apparent for many of the subjects following the diet change. When dietary cholesterol intake was decreased serum cholesterol concentration decreased and the slope of the specific activity curve showed a small transient decrease. This result suggests that the decrease in serum cholesterol was consequent to a decrease in cholesterol absorption and hence a decreased rate of dilution of the plasma label. When dietary cholesterol intake was increased the effect on plasma specific activity was variable. For two subjects the increase in plasma cholesterol was accompanied by a small transient increase in the rate of decline of plasma specific activity, likely as a result of increased absorption of unlabelled dietary cholesterol. For two other subjects the increase in serum cholesterol, consequent to an increase in dietary cholesterol, coincided with either a small decrease in the rate of decline of the plasma specific activity or no change in the rate of decline. Analysis of fecal steroids will help to further resolve which mechanisms provided the additional source of serum cholesterol in these subjects.

The data obtained in the present study indicates that in healthy young men metabolic compensation for an increase or decrease in dietary cholesterol occurs rapidly, that is, within one to two weeks, but, that this compensation is incomplete as significant changes in

serum cholesterol concentration also occur.

The results of the present study indicate that dietary cholesterol has a measureable effect on serum lipids. However, it must be emphasized that in healthy young Canadian men the ingestion of more than 1800 mg cholesterol per day did not result in lipid values that exceeded the normal physiological range for serum cholesterol, phospholipids or triglycerides. These findings do not discredit the theory that a restriction in dietary cholesterol consumption would be beneficial in controlling serum lipids in persons with elevations beyond the level considered as high risk in connection with the incidence of atherosclerotic heart disease. The importance of a restricted cholesterol intake will become increasingly clear once it has been established what constitutes a desireable serum cholesterol level as opposed to a normal serum cholesterol concentration. A normal serum cholesterol is generally regarded as being less than 240 mg/dl for healthy young Canadian men, but some investigators (Connor et al, 1972; Wynder et al, 1972) continue to insist that a desireable serum cholesterol concentration would be less than 200 mg/dl.

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APPENDIX TABLE 1

COMPOSITION OF THE 6-EGG DIET

Menu I		Menu II	
Breakfast[1]	Amount		Amount
Orange Juice	6 ounces	Apple Juice	6 ounces
Scrambled Eggs	3 whole	Scrambled Eggs	3 whole
Toast	1 slice	Toast	1 slice
Jam or Jelly	14 gm	Jam or Jelly	14 gm
Butter[2]	18.5 gm	Butter[2]	18.5 gm
		Skim Milk	8 ounces
Lunch[1]			
Macaroni and Cheese[3]		Spaghetti in Tomato Sauce[3]	
Peas	50 gm	Coleslaw	
Bread	1 slice	Cabbage	50 gm
Strawberries, frozen	130 gm	Green Pepper	15 gm
Skim Milk	8 ounces	Corn Oil	10 gm
		Vinegar	5 gm
		Bread	1 slice
		Fruit Cocktail	150 gm
		Chocolate Eggnog[3]	
Dinner[1]			
Chili[3]		TVP Beef Stew[3]	
Rice	60 gm	Mashed Potato	40 gm
Tossed Salad		Celery	20 gm
Lettuce	50 gm	Bread	1 slice
Tomato	50 gm	Baked Custard[3]	
Egg, hard cooked	1 whole	Skim Milk	8 ounces
Corn Oil	10 gm		
Vinegar	5 gm		
Bread	1 slice		
Peaches, canned	130 gm		
Rum and Butter Eggnog[3]			
Snacks[1]			
7 Up or Fruit Juice	10 ounces	7 Up or Fruit Juice	10 ounces
Pineapple-Carrot Square[3]		Oatmeal Cookies[3]	2
		Orange	1 medium

1. Clear coffee and tea allowed ad lib.

2. Butter at breakfast to be used throughout the day.

3. For amounts see recipes, Fuller (unpublished thesis).

APPENDIX TABLE 2

AVERAGE NUTRIENT COMPOSITION OF THE EXPERIMENTAL DIETS [1,2]

NUTRIENT		0-Egg	1-Egg	2-Egg	3-Egg	6-Egg	RECOMMENDATION
Calories	(kcal)	2976	2997	3007	3007	3031	3000
Protein	(gm)	90	90	95	93	106	56
Fat	(gm)	133	133	132	133	133	--
Carbohydrate	(gm)	314	369	365	367	360	--
Calcium	(mg)	1176	1148	1280	1159	1437	800
Phosphorus	(mg)	1307	1360	1539	1524	1950	800
Iron	(mg)	13.3	14.6	15.3	16.7	19.5	10.0
Vitamin A	(ug RE)	1063	1240	1425	1595	2255	1000
Thiamine	(mg)	1.7	1.7	1.8	1.8	2.0	1.5
Riboflavin	(mg)	3.3	3.3	3.4	3.3	3.6	1.8
Niacin	(NE)	34	34	35	35	37	20
Vitamin C	(mg)	230	246	229	246	246	30

1. Calculated values using USDA Handbook No. 8 Composition of Foods (Watt and Merrill, 1963).
2. Average of values for Menu I and Menu II.
3. 1975 Revised Canadian Dietary Standard, males 19 - 35 years.

APPENDIX TABLE 3

CALCULATION OF PURE TREATMENT EFFECTS AND ADJUSTED MEAN SERUM CHOLESTEROL VALUES

Treatment	Total[1]	Tb[2]	Q[3]	Pure Treatment Effect[4]	Adjusted Mean Serum Cholesterol[5] (mg/dl)
0 - Egg Diet	1298	2774	-178	-35.60	141
1 - Egg Diet	1356	2770	- 58	-11.60	165
2 - Egg Diet	1439	2840	+ 38	+ 7.60	184
3 - Egg Diet	1439	2898	- 20	- 4.00	172
6 - Egg Diet	1525	2832	+218	+43.60	220

1. Sum of serum cholesterol values (mg/dl) from weeks 2 and 3 for all subjects on the given treatment.
2. Sum total of all blocks containing the given treatment.
3. k(Total) - Tb, where k = 2 observations/block.
4. $tj = \frac{Q}{2(rEf)}$, where $rEf = \frac{rk - r + \lambda}{k} = 2.5$. With the design used in the present study r = 4 replicates, $\lambda = 1$, ie., the number of times each treatment appears with each other treatment, and k = 2 observations/block.
5. Grand mean (176.4 mg/dl) plus the pure treatment effect for the given treatment.

APPENDIX TABLE 4

ANALYSIS OF VARIANCE: SERUM TOTAL CHOLESTEROL

Source of Variance	df	SS	MS	F-Value	p[1]
Treatments (adjusted)	4	4220.80	1055.20	15.02	<0.05
Regression	1	3024.07	3024.07	43.03	<0.01
Lack of Fit	3	1196.73	398.91	5.68	n.s.
Blocks (unadjusted)	9	7430.02			
Replicate 1 vs Replicate 2	1	135.02	135.02	1.92	n.s.
Replicate 3 vs Replicate 4	1	432.45	432.45	6.15	n.s.
Error A[2]	3	210.80	70.27		
Weeks	1	5.62	5.62	0.09	n.s.
Weeks X Treatments	4	193.98	48.38	0.77	n.s.
Error B					
Weeks X Blocks Weeks X Replicates Weeks X Error	15	938.50	62.57		
Total	39	13567.77			

1. P= probability of chance occurrence.
2. Lose 1 df in error for replacement of missing values.

APPENDIX TABLE 5

CALCULATION OF PURE TREATMENT EFFECTS AND ADJUSTED MEAN SERUM LIPID PHOSPHORUS VALUES

Treatment	Total[1]	Tb[2]	Q[3]	Pure Treatment Effect[4]	Adjusted Mean Serum Lipid Phosphorus[5] (md/dl)
0 - Egg Diet	61.03	125.12	-3.06	-0.61	7.57
1 - Egg Diet	65.24	131.96	-1.48	-0.30	7.88
2 - Egg Diet	64.01	131.96	-2.29	-0.46	7.72
3 - Egg Diet	66.80	134.01	-0.41	-0.08	8.10
6 - Egg Diet	70.10	132.96	+7.24	+1.45	9.63

1. Sum of serum lipid phosphorus values (mg/dl) from weeks 2 and 3 for all subjects fed the given treatment.
2. Sum total of all blocks containing the given treatment.
3. k(Total) - Tb, where k = 2 observations/block.
4. $tj = \frac{Q}{2(rEf)}$, where $rEf = \frac{rk - r + \lambda}{k} = 2.5$. With the design used in the present study r = 4 replicates, $\lambda = 1$, ie., the number of times each treatment appears with each other treatment, and k = 2 observations/block.
5. Grand mean (8.18 mg/dl) plus the pure treatment effect for the given treatment.

APPENDIX TABLE 6

ANALYSIS OF VARIANCE: SERUM LIPID PHOSPHORUS

Source of Variance	df	SS	MS	F-Value	p[1]
Treatment (adjusted)	4	3.47	0.87	5.44	n.s.
Blocks (unadjusted)	9	10.44	-	-	-
Replicate 1 vs Replicate 2	1	0.12	0.12	0.75	n.s.
Replicate 3 vs Replicate 4	1	0.01	0.01	0.06	n.s.
Error A[2]	3	0.48	0.16	-	-
Weeks	1	0.01	0.01	0.08	n.s.
Weeks X Treatments	4	0.03	0.01	0.08	n.s.
Error B					
Weeks X Blocks Weeks X Replicates Weeks X Error	15	1.76	0.02	-	-
Total	39	16.32	-	-	-

1. P = probability of chance occurrence.
2. Lose 1 df in Error A for replacement of missing values.

APPENDIX TABLE 7

CALCULATION OF PURE TREATMENT EFFECTS AND ADJUSTED MEAN SERUM TRIGLYCERIDE VALUES

Treatment	Total[1]	Tb[2]		Pure Treatment Effect[4]	Adjusted Mean Serum Triglyceride[5] (mg/dl)
0 - Egg Diet	439.4	856.7	+ 22.1	+ 4.4	67
1 - Egg Diet	580.6	1213.9	+ 52.7	+10.5	73
2 - Egg Diet	408.4	967.9	-151.1	+30.2	94
3 - Egg Diet	476.7	869.4	+ 84.0	+16.8	79
6 - Egg Diet	581.6	1065.5	+ 97.7	+19.5	82

1. Sum of serum triglyceride values (mg/dl) from weeks 2 and 3 for all subjects on the given treatment.
2. Sum total of all blocks containing the given treatment.
3. k(Total) - Tb, where k = 2 observations/block.
4. $tj = \frac{Q}{2\,(rEf)}$, where $rEf = \frac{rk - r + \lambda}{k} = 2.5$. With the design used in the present study r = 4 replicates, $\lambda = 1$, ie., the number of times each treatment appears with each other treatment and k = 2 observations/block.
5. Grand mean (62.2 mg/dl) plus the pure treatment effect for the given treatment.

APPENDIX TABLE 8

ANALYSIS OF VARIANCE: SERUM TRIGLYCERIDES

Source of Variance	df	SS	MS	F-Value	p[1]
Treatments (adjusted)	4	2134.91	533.73	0.63	n.s.
Blocks (unadjusted)	9	10508.17	-	-	-
Replicate 1 vs Replicate 2	1	1520.77	1520.77	1.79	n.s.
Replicate 3 vs Replicate 4	1	1320.31	1320.31	1.55	n.s.
Error A[2]	3	2550.24	850.08	-	-
Weeks	1	1.48	1.48	0.02	n.s.
Weeks X Treatments	4	71.88	17.97	0.29	n.s.
Error B Weeks X Blocks Weeks X Replicates Weeks X Error	15	945.77	63.05	-	-
Total	39	19053.87	-	-	-

1. P - probability of chance occurrence.
2. Lose 1 df in Error A for replacement of missing values.

APPENDIX TABLE 9

ANALYSIS OF COVARIANCE FOR HOMOGENEITY OF REGRESSION: DISAPPEARANCE RATE OF LOG_{10} SERUM SPECIFIC ACTIVITY FOR EACH SUBJECT IN DIET PERIOD I vs DIET PERIOD II

Subject	df	xx	xy	yy	df	SS	MS	F
RE								
line 1	2	2	-0.1138	0.0065	1	0.0000		
line 2	2	2	-0.1438	0.0125	1	0.0022		
individual regressions pooled					2	0.0022	0.0011	
common regression	4	4	-0.2576	0.0190	3	0.0024		
homogeneity of regression					1	0.0022	0.0022	0.18 n.s.
LC								
line 1	2	2	-0.2429	0.0295	1	0.0000		
line 2	2	2	-0.2151	0.0234	1	0.0003		
individual regressions pooled					2	0.0003	0.0002	
common regression	4	4	-0.4580	0.0529	3	0.0005		
homogeneity of regression					1	0.0002	0.0002	1.00 n.s.
BD								
line 1	2	2	-0.1268	0.0087	1	0.0007		
line 2	2	2	-0.0843	0.0036	1	0.0000		
individual regressions pooled					2	0.0007	0.0004	
common regression	4	4	-0.2111	0.0123	3	0.0012		
homogeneity of regression					1	0.0005	0.0005	1.25 n.s.

Subject	df	xx	xy	yy	df	SS	MS	F
JB								
line 1	2	2	-0.0990	0.0050	1	0.0001		
line 2	2	2	-0.1154	0.0067	1	0.0000		
individual regressions pooled					2	0.0001	0.0001	
common regression	4	4	-0.2144	0.0117	3	0.0002		
homogeneity of regression					1	0.0001	0.0001	1.00 n.s.
BS								
line 1	2	2	-0.1514	0.0121	1	0.0006		
line 2	2	2	-0.0980	0.0048	1	0.0000		
individual regressions pooled					2	0.0006	0.0003	
common regression	4	4	-0.2494	0.0169	3	0.0013		
homogeneity of regression					1	0.0007	0.0007	2.33 n.s.
EC								
line 1	2	2	-0.1364	0.0096	1	0.0003		
line 2	2	2	-0.0192	0.0096	1	0.0094		
individual regressions pooled					2	0.0097	0.0049	
common regression	4	4	-0.1556	0.0192	3	0.0131		
homogeneity of regression					1	0.0034	0.0034	0.69 n.s.

Subject	df	xx	xy	yy	df	SS	MS	F
DG								
line 1	2	2	-0.1364	0.0228	1	0.0013		
line 2	2	2	-0.1009	0.0054	1	0.0003		
					2	0.0016	0.0008	
individual regressions pooled								
common regression	4	4	-0.3081	0.0282	3	0.0045		
homogeneity of regression					1	0.0029	0.0029	3.63 n.s.
HM								
line 1	2	2	-0.1470	0.0108	1	0.0000		
line 2	2	2	0.1683	0.0242	1	0.0100		
					2	0.0100	0.0050	
individual regressions pooled								
common regression	4	4	0.0213	0.0350	3	0.0349		
homogeneity of regression					1	0.0249	0.0249	4.98 n.s.
JM								
line 1	2	2	-0.16135	0.01303	1	0.00002		
line 2	2	2	-0.17119	0.01518	1	0.00053		
					2	0.00055	0.00028	
individual regressions pooled								
common regression	4	4	-0.33254	0.02821	3	0.00056		
homogeneity of regression					1	0.00001	0.00001	0.04 n.s.