IOWA STATE UNIVERSITY
Digital Repository

1988

Effects of diets and genetics on cholesterol metabolism in mice

Son-iu Kuan

Iowa State University

INFORMATION TO USERS

Effects of diets and genetics on cholesterol metabolism in mice

Kuan, Son-iu, Ph.D.

Iowa State University, 1988

Effects of diets and genetics on cholesterol metabolism

in mice

by

Son-iu Kuan

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Food and Nutrition
Major: Nutrition

Approved:

Members of the Committee:

In Charge of Major Work

For the Major Department

For the Graduate College

Iowa State University
Ames, Iowa

1988

TABLE OF CONTENTS

v

LIST OF TABLES

LIST OF FIGURES

DEDICATION

TO MY PARENTS AND MY HUSBAND

INTRODUCTION

Cholesterol is required for normal cell function. It is the precursor of steroid hormones and bile acids and is a major component of membranes and plasma lipoproteins. However, it is also a major constituent of atherosclerotic plaques and most gallstones.

A major disease related to cholesterol is coronary heart disease (CHD), which is the leading cause of death in the United States (Levy, 1981). Most CHD is caused by atherosclerosis, a slowly progressive disease of the large arteries that begins early in life but rarely produces symptoms until middle age. Often the disease goes undetected until the time of the first heart attack, and this first heart attack is often fatal. So, the major progress against this disease should focus on finding preventive measures. A number of risk factors have been identified. Cigarette smoking, high blood pressure, and high blood cholesterol levels are the most clearly established of these factors. Risk is greater in men, increases with age, and has a strong genetic component. Obesity, diabetes mellitus, physical inactivity and behavior pattern are also risk factors (Consensus Conference, 1985). Although some factors such as sex, age and genetics cannot be changed, others may be modified. Epidemiology and human

and animal experimentation suggest that blood cholesterol
and thus CHD incidence can be lowered by dietary
modifications.

Epidemiology

The mortality of CHD in this country increased during
the first half of this century, stabilized in 1964, declined
after 1968, and is still declining (Levy, 1981). Coinciding
with such change was a steady rise in the consumption of
animal fats both from dairy products and meats before the
1950s and a decrease in the consumption of animal fats and
an increase in the consumption of polyunsaturated fat from
vegetable sources afterwards (Brown and Karmally, 1985).
Blood cholesterol also changed during that interval. Data
from the National Health and Nutrition Examination Surveys
(NHANES) (National Center for Health Statistics, 1987)
showed that age-adjusted mean serum cholesterol levels
decreased by 6 to 8 mg/dl, or 3 to 4 %, between the 1960 to
1962 and the 1976 to 1980 surveys. The parallel changes in
CHD incidence, diet trend and blood cholesterol levels
indicated that there might be a causal relationship among
them. Although the discovery of new drugs, advancement of
surgical techniques and improvement of medical services can
explain part of the decline in CHD mortality, the importance

of identification and modification of risk factors such as
cigarette smoking cessation, hypertension control, diet
change and reduction in blood cholesterol level cannot be
ignored (Levy, 1981). An apparent decline in CHD mortality
happened only in those countries with an aggressive approach
to risk factor change. In fact, unlike the United States,
Australia and Canada, where changes have taken place, some
countries including the Soviet Union, Ireland, Scotland,
Germany, Denmark and some Eastern Europe nations have seen
increasing death rates from CHD (Levy, 1984). The
experience of North Karelia, the most easterly county of
Finland, is another example. North Karelia had the highest
death rate from CHD in the world in 1971. A community
program was then set up and everyone was advised to stop
smoking, eat less fat and more vegetables, avoid obesity,
and have their blood pressure checked. By 1979 coronary
mortality had fallen by 24% in men and 51% in women, a
significantly greater fall than the general decline in
coronary deaths in Finland during the same period (Truswell,
1985).

Several studies have been conducted to search for the
association between cardiovascular mortality and food intake
among countries. A strong correlation was found between
cardiovascular mortality and percentage calories from fat

(Lopez-S et al., 1966), percentage calories from saturated fat (Lopez-S et al., 1966; Keys, 1970) and egg consumption (Armstrong et al., 1975). Similar findings were reported comparing vegetarians and nonvegetarians (Sacks et al., 1975). Comparison of individuals within populations, however, failed to find a strong association between diet and cardiovascular mortality (McGill et al., 1981).

Thirty years of follow-up from the Framingham study (Anderson et al., 1987) has provided a strong proof that cardiovascular mortality is positively related to blood cholesterol level. Under age 50 years, cardiovascular death increased 9% for each 10 mg/dl increase in blood cholesterol concentration although no such relationship was found after age 50 years as the data were confounded by people whose cholesterol levels were falling due to certain diseases predisposing to death. Comparisons of various countries throughout the world also revealed a direct correlation between blood cholesterol levels and cardiovascular mortality. No population has been reported to have a high rate of coronary heart disease and low blood cholesterol levels (Consensus Conference, 1985). Stamler et al. (1986) concluded that the relationship between serum cholesterol concentration and CHD mortality was not a threshold one, but rather was a continuously graded one with increased risk confined to the two highest quintiles.

Results of epidemiology studies on the relationship between dietary fats and blood cholesterol levels were not quite consistent. A positive correlation was identified between average dietary cholesterol or saturated fat intake and average plasma cholesterol concentrations when comparisons were made among population groups (McGill et al., 1981). Studies on Japanese populations also supported this conclusion. In the Japanese fishing villages of Kyushu and Tanushimaru, people ate rice supplemented by fish as their major food. The diet contained 10% of calories (Kcal) from fat and 3% from saturated fat. Their blood cholesterol averaged less than 170 mg/dl (Kimura, 1967). The urban Japanese in Hiroshima and Nagasaki ate 15% of calories from fat and 7% from saturated fat, and their blood cholesterol averaged 181 mg/dl (Kagan et al., 1974). The Japanese who have migrated to Honolulu and San Francisco increased their fat consumption; 23-26% of Kcal in their diet came from fat, and their average blood cholesterol concentration was 228 mg/dl (Worth et al., 1975). The incidence of nonfatal myocardial infarction and CHD death among Japanese men had tripled in the population moving to California. Such conclusions, however, could not be drawn when comparisons were made among individuals within the population (Morris et al., 1963; Hodgson et al., 1976). McGill (1979) and Keys

(1970) interpreted this discrepancy as a natural consequence of the large within-subject variation in serum cholesterol levels and measurement errors, and the lack of association did not negate a real causal relationship between dietary fat and serum cholesterol concentration or CHD mortality. Glueck (1979a and b) also pointed out that the difference in nutrient intake within a population group was relatively small, recent diet habits did not necessarily represent lifetime patterns, and it was misleading to relate recent dietary practice to the risk of CHD since the development of atherosclerosis took decades to occur.

Human Experimentation

Controlled metabolic studies

Controlled human studies have clearly demonstrated that diets in which polyunsaturated fats have been substituted for saturated fats lowered serum total and low density lipoprotein (LDL) cholesterol levels in healthy subjects (Shepherd et al., 1978 and 1980; Kraemer et al., 1982; Blaton et al., 1984; Reiser et al., 1985) as well as in hypercholesterolemic patients (Blaton et al., 1984), but the effects on high density lipoprotein (HDL) cholesterol were inconsistent. Some reported a decrease (Shepherd et al., 1978 and 1980), some reported no change (Kraemer et al.,

1982) and some reported an increase (Blaton et al., 1984).
Such discrepancies might be due to differences in fat
sources, ratio of polyunsaturated to saturated fat (P/S),
experimental subjects and experimental design. Several
mechanisms have been proposed to explain the
hypocholesterolemic effect of polyunsaturated fats. These
include increasing fecal excretion of bile acids and neutral
sterols (Wood et al., 1966), shift of cholesterol from
plasma to tissues due to greater volume of polyunsaturated
fatty acids in the lipoprotein particles (Glueck, 1979a) and
altering the production (Vega et al., 1982) or clearance
(Shepherd et al., 1980) of LDL from the plasma. It is
possible that the hypocholesterolemic action of
polyunsaturated fat diets is effected by multiple mechanisms
the expression of which may vary from person to person
(Shepherd et al., 1980).

The discoveries that Greenland Eskimos had a low
incidence of cardiovascular diseases and that might be
related to their high intake of marine lipids (Dyerberg and
Bang, 1982) have stimulated many studies on fish oil. Fish
oil was reported to be anti-thrombotic (Herold and Kinsella,
1986; Knapp et al., 1986; Norum and Drevon, 1986). Its
effects on cholesterol metabolism, however, were not very
large. Nestel (1986) reported a cholesterol-lowering effect

of fish oil (40 g of MaxEPA per day) in plasma very low
density lipoprotein (VLDL), LDL and HDL. He also found
lower apoprotein (apo) A-2 and apo B levels with fish oil
intake. Sanders et al. (1985) and Sullivan et al. (1986)
used a lower dose of fish oil (15 g of MaxEPA per day) and
found different results. LDL-cholesterol and apo B were
higher with fish oil supplementation in both normal and
hypertriglyceridemic patients. So, the cholesterol-lowering
effect of fish oil occurred only at very high doses. Herold
and Kinsella (1986) warned that the health professionals
should consider the potentially deleterious effects of fish
oil when making recommendations on fish oil supplementation.
These could result from excessive oxidation, vitamin E
deficiency, vitamin A and D excesses, or increased intake of
contaminants such as PCBs and DDT.

Dietary effects of monounsaturated fats on cholesterol
metabolism have long been a neglected area. People used to
think of monounsaturated fats as being neutral in
cholesterol metabolism. The fact that low fat (Grundy,
1986) or high polyunsaturated fat diets frequently reduced
plasma cholesterol as well as HDL-cholesterol encouraged the
search for a diet which did not reduce the protective
lipoprotein HDL while reducing plasma cholesterol.
Epidemiologic study indicated a low incidence of coronary

heart disease and low plasma cholesterol levels in the Mediterranean countries, which might be due in part to the greater use of olive oil, a high monounsaturated fat oil (Keys, 1970). Mattson and Grundy (1983) and Grundy (1986) reported that monounsaturated fats lowered plasma cholesterol and LDL-cholesterol, but had no effect on HDL-cholesterol. The cholesterol-lowering effect was described as at least as effective as a diet low in fat and high in carbohydrate. More research in this area needs to be done to verify the findings.

The effects of dietary cholesterol on plasma cholesterol levels are somewhat controversial. Mattson et al. (1972) found a linear relationship between dietary cholesterol and elevation of serum cholesterol using egg yolk to modify the amount of dietary cholesterol. The elevation in serum cholesterol was about 12 mg/dl for each 100 mg of cholesterol added per 1000 Kcal over the range of 0-317 mg of cholesterol per 1000 Kcal per day. Ginsberg et al. (1981), however, found no effect of dietary cholesterol on concentrations of plasma cholesterol and LDL-cholesterol, and rate of production or clearance of apo B in VLDL and LDL in their subjects. The conflicting results brought about the recognition of responders and nonresponders to dietary cholesterol and raised the question about the role of

genetics in the regulation of cholesterol metabolism. This variability did not appear to be due to individual differences in the absorption of dietary cholesterol as the mass of cholesterol absorbed was linearly related to the amount of dietary cholesterol in both normocholesterolemic (Simons et al., 1978) and hypercholesterolemic patients (Connor and Lin, 1974). It is possible that other mechanisms such as changes in endogenous cholesterol synthesis, excretion of cholesterol or bile acids or lipoprotein metabolism are working differently in the responders and nonresponders.

Intervention trials

A number of intervention trials have been carried out to test the diet-heart hypothesis. The Chicago Coronary Prevention Trial (Farinaro et al., 1977), the Oslo study (Hjermann et al., 1981) and the Multiple Risk Factor Intervention Trial (MRFIT) (Dolecek et al., 1986) all demonstrated that blood cholesterol can be lowered by decreasing saturated fat and cholesterol and increasing polyunsaturated fats in the diet.

The beneficial effects of lowering blood cholesterol were further supported by the results of the Lipid Research Clinics Coronary Primary Prevention Trial (CPPT) (The Lipid Research Clinics Coronary Primary Prevention Trial Results I

and II, 1984a and b). In this study, cholesterol-lowering was achieved by a combination of diet and cholestyramine in the treatment group and diet alone in the control group. They found that a 1% reduction in plasma cholesterol was accompanied by a 2% reduction in the incidence of coronary heart disease morbidity and mortality in men with high blood cholesterol levels. Kronmal (1985) criticized the way this conclusion was reached. First, the criterion to test the difference between the placebo and the treatment groups was originally set at 0.01 level, but the results were reported using a one-sided 0.05 level. Second, the use of percent change in plasma cholesterol could lead to "spurious" correlation. In other words, the relationship between the risk of a CHD end point and the percent cholesterol change might not be caused by the absolute change in plasma cholesterol, but rather to the relationship between the baseline cholesterol level and the CHD event rate. Rahimtoola (1985) questioned the efficacy of reduction of hypercholesterolemia since the difference in CHD incidence between the two groups was small. Anyway, CPPT provided important results concerning the effects of diet plus cholestyramine on the lowering of plasma cholesterol and LDL-cholesterol levels and its effects on CHD mortality and morbidity in hyperlipidemic men.

Animal Models

Both the controlled metabolic studies and clinical intervention trials have suggested that diet can affect blood cholesterol and high blood cholesterol has a causal relationship with atherosclerosis. However, because of ethical reasons and limitations, human studies alone are not sufficient to investigate the mechanisms of the relationships. A number of animal models have been developed to provide a means for mechanistic study. There are wide variations in cholesterol metabolism among species. No one animal model is a perfect model for the human. But animal models do provide valuable information of what may be happening in humans.

Squirrel monkey

Although marked differences in plasma cholesterol concentrations were not apparent among squirrel monkeys fed cholesterol-free diets, marked differences occurred when they were fed a high cholesterol diet (1 mg/Kcal) (Clarkson et al., 1971). Hyperresponders developed considerable hypercholesterolemia with total serum cholesterol levels in the range of 700 to 1,000 mg/dl, while hyporesponders were around 200 to 300 mg/dl, and still others were in between. Moreover, the hyporesponders fed high cholesterol diets for

long periods of time had minimal arterial lesions while hyperresponders fed the same diet had markedly exacerbated atherosclerosis. By different combinations of matings of hyperresponders and hyporesponders, Clarkson et al. (1971) showed that more than half of the variability in plasma cholesterol values of the progeny when challenged with a high cholesterol diet was attributable to genetic factors. Lofland et al. (1972) demonstrated that hyporesponders fed a cholesterol-containing diet had a greater and more rapid increase in fecal excretion of bile salts than did hyperresponders. They speculated that the mechanism of control of the level of plasma cholesterol was related to the rate of conversion of cholesterol to bile acids. Jones et al. (1975) further studied the mechanism by feeding hyper- and hyporesponders safflower oil or butter as 40% of calories. Hyperresponders had higher plasma cholesterol than hyporesponders, and butter induced a greater response than did safflower oil. Also, hyperresponders absorbed a higher mean percentage of ingested cholesterol and were more sensitive to a given amount of absorbed cholesterol than were hyporesponders.

Rhesus monkey

Rhesus monkeys showed marked individual differences in
serum cholesterol levels not only when fed an atherogenic
diet (high saturated fat and high cholesterol) but also when
fed a basal diet. Unlike squirrel monkeys, hyperresponders
of rhesus monkeys excreted more bile acids in the feces than
did hyporesponders when fed an atherogenic diet, and both
groups had similar excretion of neutral sterols (Eggen,
1976). So, differences in the excretion of bile acids could
not explain the higher plasma cholesterol level in
hyperresponders. Furthermore, endogenous cholesterol
synthesis could not explain the differences either, as
hyperresponders had a greater degree of feedback inhibition
of cholesterol biosynthesis (Bhattacharyya and Eggen, 1981).
The major difference was intestinal absorption;
hyperresponders absorbed more cholesterol than did
hyporesponders (Bhattacharyya and Eggen, 1980 and 1981).
The investigators concluded that such differences might
reflect differences in rates of one or more of the
biochemical reactions involved in the absorption process,
differences in surface area of the intestinal lumen or
differences in motility of the intestine. Baker et al.
(1983) studied the lipoprotein profiles in rhesus monkeys.
They reported higher plasma apo B and E and lower A-1

concentrations in hyperresponders than in hyporesponders fed a high fat (38% Kcal) and high cholesterol (0.4 mg/Kcal) diet.

Baboon

The baboon also showed variability in serum cholesterol when fed an atherogenic diet as did squirrel and rhesus monkeys, and its serum cholesterol and HDL-cholesterol responses to diet were heritable (Flow et al., 1981). Sire effects were observed for serum cholesterol concentration, cholesterol turnover rate, cholesterol production rate and several cholesterol pool parameters derived from a two-pool model (Flow and Mott, 1982). Flow and Mott (1984) provided evidence that the size of the rapidly miscible pool of body cholesterol and the movement of cholesterol in and out of that pool were influenced to a large degree by the same genes that regulate the plasma concentration of HDL. The differences among sire progeny groups in cholesterol metabolism were likely due to genetically mediated differences in the hepatic receptors or enzymes which regulated HDL-cholesterol metabolism.

Pigeon

Two strains of Show Racer Pigeons (SR) were identified and selectively bred as hypo (SR-39) and hyper (SR-37)

responders to diet-induced hypercholesterolemia and atherosclerosis (Wagner et al., 1973). The defect in the SR-37 strain was not overproduction of cholesterol since SR-39 pigeons displayed much higher hepatic hydroxymethylglutaryl coenzyme A reductase activity (HMGR) (a rate-limiting enzyme in cholesterol synthesis) than did the SR-37 strain (Hulcher et al., 1976). A turnover study by Wagner and Clarkson (1974) indicated that SR-39 pigeons had a larger rate constant for excretion of cholesterol from pool A, whereas SR-37 pigeons had a much lower rate constant. Hulcher and Margolis (1982) further studied cholesterol metabolism in these two strains of pigeons by assaying the activity of cholesterol 7a-hydroxylase (COH), a key regulatory enzyme in bile acid synthesis. They found that the enzyme activity was consistently lower in SR-37 than in SR-39 pigeons no matter whether they were fed a cholesterol-free diet or a cholesterol-containing diet. The data implied that the basis for inefficient regulation of serum cholesterol in the SR-37 strain resided in inefficient up-regulation of the activity or synthesis of cholesterol 7a-hydroxylase.

St. Clair et al. (1986) studied the relationship between β-VLDL and atherosclerosis in White Carneau (WC) (atherosclerosis-susceptible) and Show Racer (SR)

(atherosclerosis-resistant) pigeons. β-VLDL is a cholesteryl ester-rich beta-migrating VLDL present in animals fed cholesterol and is suspected as the primary atherogenic lipoprotein in cholesterol-fed animals. The two strains of pigeon had similar plasma cholesterol levels but different susceptibility to atherosclerosis. No relationship between β-VLDL concentration or the ability of β-VLDL to stimulate macrophage cholesterol accumulation in vitro and the extent of atherosclerosis was found. The authors concluded that differences in susceptibility to atherosclerosis among pigeons probably were mediated at the level of the arterial wall, perhaps by genetic differences that influence the way an individual animal's arterial cells (endothelial, smooth muscle, macrophage) interact with specific plasma lipoproteins.

Quail

Hypercholesterolemia was induced in a strain of Japanese quail by feeding 1% of cholesterol or 1% of cholesterol plus 0.5% of cholic acid in the diet. Radcliffe and Liebsch (1985) found that the severity of atherosclerosis and arterial cholesterol concentration were positively correlated with serum cholesterol concentration.

Miniature swine

In a 2 by 2 factorial experiment, Walsh-Hentges et al.
(1985a and b) studied the effects of protein (soy protein /
egg white) and fat (beef tallow / soybean oil) on plasma
lipid and lipoprotein metabolism in miniature swine. They
found that proteins and fats affected cholesterol metabolism
by different mechanisms. The dietary fat source affected
plasma lipid concentration and tissue cholesterol
accumulation while the dietary protein source affected the
catabolic rate of LDL. Compared to beef tallow, soybean oil
consumption resulted in decreased plasma cholesterol
concentration, increased LDL-cholesterol concentration,
decreased HDL-cholesterol concentration, and greater
accumulation of cholesterol in aorta, heart, large and small
intestines, liver, adipose tissue and skeletal muscle. Egg
white consumption, on the other hand, decreased fractional
catabolic rate of LDL-cholesterol compared to soy protein.
LDL disappearance was higher when vegetable products were
fed, i.e., soy protein or soybean oil, which suggested that
receptor-dependent degradation might be sensitive to changes
in the degree of unsaturation of dietary lipid and to
changes in protein source.

Clow et al. (1987) studied the effects of dietary fats
(SFA diet: 40 en% beef fat, P/S=0.17; PUFA: 8 en% beef

fat, 32 en% safflower oil, P/S=4.16) on cholesterol and bile acid kinetics in miniature swine. Cannulae were implanted to allow sampling of the portal vein, aorta and jejunal contents. Diets did not affect the half-life of serum cholesterol, but the fractional turnover rate from pool 2 to pool 1 was greater for the SFA group than the PUFA group. The PUFA-fed pigs had a larger cholesterol pool and greater flow rate between pools compared to SFA-fed pigs. Jejunal cholesterol kinetics were similar to those for serum from SFA-fed pigs, but not PUFA-fed pigs in which the flow rates between pools in jejunal pools were lower than those in serum pools. Bile acid specific activity reached a maximum within 15 hours and showed a plateau for about two weeks before beginning to decrease. The authors concluded that pools of cholesterol available in the liver for resecretion to the serum, excretion into the gut and formation of bile acids were not in rapid equilibrium.

Rabbit

Beynen et al. studied the responses of serum cholesterol to diet in random-bred (1986a) and inbred (1986b) rabbits. Among random-bred rabbits, individual variations in cholesterolemic responses to dietary fat and cholesterol were large, and the association between responses to dietary cholesterol and responses to dietary

coconut oil (high saturated fat) was weak. Hyper- and hyporesponders to dietary cholesterol and fat were also found in inbred rabbits. The hyperresponders to dietary cholesterol were also hyperresponders to dietary coconut fat and casein. The effects of monounsaturated fats, polyunsaturated fats and fish oil were also studied in rabbits. Compared to butter (high saturated fat), both corn oil (high polyunsaturated fat) and olive oil (high monounsaturated fat) caused a decrease in plasma cholesterol and an increase in HDL-cholesterol, and the effect of corn oil was higher than that of olive oil (Masi et al., 1986). Beynen et al. (1987) also found an intermediate effect of olive oil between corn oil and coconut fat in both plasma total and HDL-cholesterol. Ten percent menhaden oil (a fish oil) or menhaden oil plus 1% cholesterol added to the diet of rabbits caused an increase in plasma cholesterol, a decrease of HMGR activity in both liver and intestine, an increase of acyl-CoA:cholesterol acyltransferase (ACAT) activity in liver and no change in intestine, and an increase in n-3 polyunsaturated fatty acids and a decrease in cholesterol in liver microsomes (Field et al., 1987). It was concluded that dietary manipulation caused changes in membrane fatty acid saturation in liver and intestine which changed HMGR and ACAT activities.

A special strain of rabbits called the Watanabe heritable hyperlipidemic (WHHL) rabbit has been the focus of many studies as an animal model to investigate the genetic defect of familial hypercholesterolemia (FH) in the human. Like FH patients, the WHHL rabbit has hypercholesterolemia despite the ingestion of a cholesterol-free diet, has elevation in LDL-cholesterol and develops atherosclerosis. It is now known that the WHHL rabbit has a single gene defect in the LDL receptor which results in inefficient removal of LDL from the blood and leads to hypercholesterolemia (Goldstein et al., 1983). Tissue culture studies using human skin fibroblasts showed that the same gene is defective in FH patients.

Rat

The rat is rather resistant to hypercholesterolemia and atherosclerosis. In order to produce hypercholesterolemia in the rat, people often use antithyroid drugs, female sex hormone (estradiol), bile acid (cholic acid) in addition to dietary cholesterol, or induction of essential fatty acid deficiency plus cholesterol supplementation. However, these treatments can cause some side effects such as hypothyroidism, sex hormone imbalance, etc. So, not much research has been done using hypercholesterolemic rats. Huang et al. (1986) induced hypercholesterolemia in rats and

investigated the effects of dietary fats. They found that
polyunsaturated fat was more effective than saturated fat in
lowering both plasma and liver cholesterol, and the
reduction of saturated and monounsaturated fatty acids in
plasma and liver cholesteryl esters was correlated with the
reduction of plasma and liver cholesterol. Sugano et al.
(1986) compared the hypocholesterolemic effects of evening
primrose oil (linoleic plus γ-linolenic acids), safflower
oil (linoleic acid) and olive oil (low linoleic acid) in
hypercholesterolemic rats induced by feeding cholic acid and
cholesterol. They found that the order of cholesterol
lowering was: evening primrose oil > safflower oil > olive
oil.

More research was done using normocholesterolemic rats.
The LDL to HDL ratio was lower when a low-fat diet (10 wt%)
was fed than when a high-fat diet (25 wt%) was fed, and the
ratio was lower when corn oil was the fat in the diet than
when peanut oil was the fat source (Worthington and Miller,
1986). Replacing sucrose by sunflower oil (Haug et al.,
1985) or increasing the polyunsaturated to saturated fatty
acid ratio (P/S ratio) (Lim et al., 1985) in the diet
lowered plasma cholesterol as well as HDL-cholesterol.
Cholesterol-lowering was accompanied by an increase in fecal
cholesterol excretion and ACAT activity. Cholesterol

synthesis was reported to be higher in rats fed corn oil
than those fed beef tallow or low fat diets (Dupont, 1966).
Studies on the lipoprotein profiles in normal and
streptozotocin-induced diabetic rats (Hennig and Dupont,
1983) suggested that excess intake of a diet high in
saturated fat might induce a metabolic pattern similar to a
diabetic state. The metabolic changes might be due in part
to enzyme changes resulting from membrane lipid environment
changes induced by diet. An increase in dietary linoleic
acid was found to cause an increase in linoleic acid and a
decrease in cholesterol content in hepatic plasma membranes
and a suppression of glucagon-stimulated adenylate cyclase
activity (Marson and Clandinin, 1985).

Mouse

Members of an inbred strain of mice of the same sex are
more than 99.44% homozygous for the same genome or
combination of gene pairs (Russell, 1969). As mice present
a variety of inbred strains, they are useful in genetic
experimentation. Mice exhibited marked strain differences
in plasma cholesterol levels (Weibust, 1973; Yamamoto et
al., 1963). Genetic factors governing the plasma
cholesterol concentration in mice were polygenic and
additive (Yamamoto et al., 1963). The mode of inheritance
was neither dominant nor recessive, but was intermediate
(Bruell, 1963).

Inbred strains of mice also differed in their responses to diet in terms of blood cholesterol and in susceptibility to atherosclerosis. Roberts and Thompson (1975) screened 13 strains of male inbred mice by feeding them either a regular laboratory diet or an atherogenic diet (a high-fat, high-cholesterol diet containing 30 wt% cocoa butter, 5 wt% cholesterol and 30 wt% protein in the form of casein). Mice showed marked differences in plasma cholesterol concentrations and extent of atherosclerotic lesions. The C57BR/cdJ strain had the highest response (highest plasma cholesterol level and largest lesions with many foam cells) while the CBA/J strain had the smallest response. After crossing the most susceptible strain, C57BR/cdJ, with the least susceptible strain, CBA/J, these investigators concluded that atherosclerosis susceptibility and plasma cholesterol level showed polygenic inheritance (Roberts and Thompson, 1977).

Paigen et al. (1985) fed ten strains of inbred mice an atherogenic diet containing 1.25 wt% cholesterol, 0.5 wt% cholic acid and 15 wt% fat. They confirmed Roberts and Thompson's work showing that inbred strains of mice differed in plasma cholesterol response to diet and in susceptibility to lesion formation. Aubert et al. (1987) also demonstrated genetic variations of blood cholesterol in inbred mice in

response to a hypercholesterolemic diet. However, there were inconsistencies among the reports. Aubert et al. (1987) did not find the CBA mouse a resistant strain to diet-induced hypercholesterolemia as did Roberts and Thompson (1975 and 1977), and they reported that DBA/2 mice had the lowest increase of serum cholesterol level when fed a hypercholesterolemic diet whereas Paigen et al. (1985) found that the same strain had the highest increase. Walker (1984) fed male CBA/J and C57BR/cdJ mice purified diets containing 10 wt% fat from different sources (sunflower oil, lard, olive-sunflower oil 77:23, or hydrogenated soybean-sunflower oil 73:27). The CBA/J strain was more resistant while C57BR/cdJ mice were more susceptible to diet-induced plasma cholesterol changes, further confirming Roberts and Thompson's work.

A similar conclusion was drawn by Morrisett et al. (1982). In addition, they found that on an atherogenic diet, CBA/J mice had a higher apo E/total lipoprotein ratio, and lower levels of VLDL and LDL than C57BR/cdJ mice. They then concluded that genetic resistance to diet-induced aortic atherosclerosis in mice was correlated with capacity to prevent large increases in serum cholesterol, to suppress abnormal α- and pre-β migrating lipoproteins, and to maintain an elevated serum apo E/total lipoprotein ratio.

Breckenridge et al. (1985) also investigated the lipoprotein profiles in these two strains of mice and got similar findings. Furthermore, they showed that the phosphatidylcholine/free cholesterol ratio of the VLDL, intermediate density lipoprotein (IDL) and LDL in C57BR/cdJ mice fed an atherogenic diet was noticeably lower than that of CBA/J mice; both strains fed the atherogenic diet had a marked decrease in the HDL fraction, but a greater reduction was seen in the C57BR/cdJ strain. Based on these findings, the investigators concluded that the development of atherosclerosis in C57BR/cdJ mice resulted from a greatly increased accumulation of cholesteryl ester-rich VLDL and IDL and a depletion of HDL, all of which were characterized by decreased phosphatidylcholine/free cholesterol ratio when compared to CBA/J mice.

Regulation of Cholesterol Balance

Cholesterol is present in all human tissues as free cholesterol and cholesteryl esters. Free cholesterol concentrations range from 0.9 mg/g of skeletal muscle to 3.9 mg/g of adrenal gland. In adrenal gland, the ester form is approximately 83% of the total, and, in serum, the ester form is about 70%. Adipose tissue is a major cholesterol storage organ. It contains 0.6 - 1.6 mg of cholesterol per gram wet weight (Oh, 1982).

No enzymes in the body are capable of degrading the
sterol nucleus of the cholesterol molecule. Since it would
be detrimental to accumulate cholesterol within the body, it
is important that the rate at which the body acquires
cholesterol from all sources must be essentially balanced by
the rate at which the body can excrete the sterol molecule.
Cholesterol enters the body pool from two sources. It may
be absorbed from the diet (300-500 mg/day in man) or
synthesized within the various organs (700-900 mg/day in
man). There are also two mechanisms for removal of
cholesterol from the body. Either cholesterol itself is
excreted into the gastrointestinal tract (approximately 600
mg/day), or is lost through the sloughing of skin
(approximately 85 mg/day), or the cholesterol is first
converted to bile acids (approximately 400 mg/day) or
steroid hormones (approximately 50 mg/day) which, in turn,
are excreted from the body in bile or urine. During the
active growth phase, some cholesterol (approximately 1.5 g
cholesterol/Kg body weight gained) is used to build new body
tissue (Dietschy, 1984). To maintain this critically
important balance of cholesterol across the body and to
ensure adequate supplies of cholesterol to all major organs,
elaborate regulatory and transport mechanisms have evolved.

Cholesterol synthesis

The overall rate of cholesterol synthesis is determined
by the activity of hydroxymethylglutaryl coenzyme A
reductase (HMGR) which catalyzes the reductive deacylation
of hydroxymethylglutaryl coenzyme A (HMG-CoA) to mevalonate
by two molecules of NADPH. HMGR has been detected in many
species and probably is present in any life form capable of
synthesizing isoprenoids. In mammals, HMGR activity has
been detected in many tissues and is associated almost
exclusively with the microsomal membrane fraction except
adrenal gland in which a major fraction is associated with
mitochondria (Gill et al., 1985).

Cholesterol synthesis and HMGR activity vary with the
physiological states of the organism. For instance,
cholesterol feeding and fasting decrease while
cholestyramine feeding increase the enzyme activity and
cholesterol synthesis in the liver. Also, HMGR activity and
cholesterol synthesis showed similar patterns of diurnal
variations and developmental changes (Gill et al., 1985).
In vitro studies with human skin fibroblasts and leukocytes
showed that HMGR and cholesterol synthesis were depressed by
the addition of serum and derepressed by the removal of
serum (Avigan et al., 1970; Brown et al., 1973; Fogelman et
al., 1975). This serum factor was found to be LDL. A

receptor-mediated pathway was postulated by Brown and Goldstein (1986) to explain cholesterol homeostasis.

Four mechanisms have been proposed to describe the regulation of HMGR activity. These include interconversion of active and inactive forms by dephosphorylation-phosphorylation, modulation by membrane fluidity, changes in enzyme synthesis rate or changes in enzyme degradation rate. It is possible that different types of cells exhibit different mechanisms or that several mechanisms operate in the same cell. Tissues such as liver and steroid-secreting organs, which may have sudden demands for large amounts of cholesterol, may use the phosphorylation-dephosphorylation mechanism to maintain a reservoir of inactive HMGR that can be quickly activated. On the other hand, tissues that have lower and more constant demands for sterol, such as cultured fibroblasts, may not need such a mechanism for short term control and use enzyme synthesis and degradation mechanisms instead (Brown et al., 1979).

Cellular uptake of cholesterol

Brown and Goldstein (1986) used human skin fibroblasts as a cultured cell model to investigate the regulation of cholesterol homeostasis. They discovered a receptor-mediated pathway for cholesterol uptake called the LDL pathway. The LDL pathway consists of an ordered sequence of

events. First, LDL is bound to a high-affinity receptor on
the cell surface. Through the clustering of LDL receptors
in coated pits, LDL receptors are internalized by
endocytosis. The receptors dissociate from LDL after
internalization and recycle back to the surface. LDL is
delivered to lysosomes where the cholesteryl esters are
hydrolyzed resulting in the release of free cholesterol.
The cholesterol (or an oxygenated derivative that is formed
within the cell) is responsible for at least three metabolic
controls: 1) it acts at several levels to reduce the
activity of HMGR, including suppression of transcription of
the HMGR gene and acceleration of the degradation of the
enzyme protein, thus decreasing the rate of cellular
cholesterolgenesis; 2) it activates ACAT, thus increasing
the rate of cellular esterification; and 3) it suppresses
synthesis of LDL receptors by lowering the concentration of
LDL receptor messenger RNA (mRNA). By receptor regulation,
the cells adjust to provide sufficient cholesterol for
metabolic needs without causing cholesterol
overaccumulation. The LDL receptor recognizes apo E when it
is present together with apo B-100 or apo B-48, or apo B-100
alone. So, it binds endogenous lipoproteins (IDL and LDL)
as well as exogenous lipoproteins (chylomicron remnants).

A different receptor, mediating the cellular uptake of the cholesterol-containing lipoproteins, called chylomicron remnant receptor has been described by Brown and Goldstein (1983). This receptor exists predominantly on hepatocytes. It recognizes apo E when it is present on a chylomicron remnant, but not when it is associated with apo B-100. This receptor mediates the uptake of lipoproteins that carry exogenous cholesterol, i.e., chylomicron remnants.

With constant infusion techniques and LDL preparations labeled with markers, Spady et al. (1983) quantitated the rates of LDL uptake and the proportion using the receptor-dependent process by different organs in the hamster. Approximately 75% of LDL turnover from the plasma compartment is accounted for by uptake into the liver. The small intestine accounts for 7% and the remaining organs account for only trivial amounts. However, the highest rate of uptake on a weight basis was seen in the adrenal gland. In the liver, ovary, and kidney, the vast majority of LDL uptake is by the receptor-mediated process, while in the spleen and intestine, receptor-independent LDL transport is relatively more important. As approximately 75% of circulating LDL is cleared by the liver and more than 90% of this clearance is mediated through the LDL receptor mechanism, the plasma LDL-cholesterol level will be very

sensitive to the number of LDL receptors present on the
sinusoidal membrane of the liver cell (Dietschy, 1984).

Catabolism and excretion

Cholesterol cannot be broken down by oxidation to
carbon dioxide and water, because mammalian tissues do not
have enzymes capable of catabolizing the steroid nucleus.
Cholesterol is mainly excreted into feces either as neutral
sterols or bile acids.

The neutral sterols represent a mixture of endogenous
cholesterol and its derivatives and unabsorbed dietary
sterols of animal and vegetable origin. They are delivered
to the intestine from the liver in the bile, through the
sloughing of intestinal mucosal cells or from the diet.
Some cholesterol in the intestinal tract is acted on by
intestinal bacterial enzymes and converted to other neutral
sterols before excretion in the feces. In humans the main
neutral sterol products in the stool are coprostanol and
cholestanone. Cholestanol is another metabolic product of
cholesterol that is formed in the liver and delivered into
the intestine in the bile.

Bile acids are hydroxyl derivatives of cholanic acids,
a 24-carboxyl compound of the parent hydrocarbons, 5α- and
5β-cholane. Primary bile acids are synthesized de novo from
cholesterol in the liver, including cholic acid and

chenodeoxycholic acid whereas secondary bile acids are formed from primary bile acids by intestinal microorganisms through deconjugation, dehydroxylation and reduction. The main secondary bile acids in humans are deoxycholic acid and lithocholic acid. Bile acids formed in the liver are conjugated at C24 with either glycine or taurine before being secreted into the bile. In most vertebrates, bile is continuously produced by the liver and stored in the gall bladder. When food enters the duodenum, cholecystokinin-pancreozymin is released; this facilitates the contraction and emptying of the gallbladder, and the bile, containing bile salts, enters the lumen of the small intestine via the common bile duct. Most of the bile acids excreted into the gastrointestinal tract are reabsorbed rapidly in the ileum by active transport or passive ionic and nonionic diffusion. Under normal conditions, about 95% of the bile acids are absorbed, and the rest are excreted via the feces. This process of recycling is called the enterohepatic circulation.

Bile acids are formed from cholesterol by hydroxylation, reduction and side chain cleavage. The first and the rate-limiting step is the insertion of a hydroxyl group at position C7, which is catalyzed by cholesterol 7α-hydroxylase (COH). COH is a microsomal enzyme requiring

cytochrome P450, NADPH, O_2 and succinate as a respiratory substrate. Its activity and thus bile acid biosynthesis are regulated by the amount of bile acids that are reabsorbed through enterohepatic circulation. In addition to being a catabolite and excretory product of cholesterol, bile acids also play some active roles such as facilitating the absorption of fat and fat-soluble vitamins by dispersing and solubilizing dietary fats and interacting with pancreatic lipase-colipase (Goswami and Dupont, 1982).

Cholesterol kinetics

Whole body cholesterol metabolism has been customarily studied by following the decline of plasma cholesterol specific activity over time after a single injection of tracer. Each change of slope of the curve is interpreted as indicating a different kinetic pool of cholesterol. In other words, the pools represent tissues having different rates of equilibrium of cholesterol with serum cholesterol. By such a curve-peeling technique, Goodman et al. (1973) described three kinetic pools of cholesterol in humans -- a rapidly miscible pool, a slowly miscible pool and a very slowly miscible pool. Studies of tissue cholesterol metabolism (Dupont, 1982) suggested that the rapidly miscible pool contained cholesterol in the liver, plasma, red blood cells and intestine; the slowly miscible pool

contained muscle and adipose cholesterol; and the very slowly miscible pool contained cholesterol of brain and nervous tissue.

Dietary Recommendations

Epidemiology and human and animal experimentations all suggest that diet can affect blood cholesterol, and blood cholesterol level has a causal relationship to atherosclerosis. There is no evidence that a moderate-fat, low-cholesterol diet is harmful. Considering the high prevalence of heart problems in the United States, a consensus development committee of the National Heart, Lung and Blood Institute and the National Institutes of Health recommended to all Americans a shift from the current average American diet to one that is lower in total fat, saturated fat and cholesterol -- reduction of calories from fat to 30%, calories from saturated fat to 10% or less, and dietary cholesterol to no more than 250 to 300 mg daily, and an increase of calories from polyunsaturated fat, but to no more than 10% of total calories (Consensus Conference, 1985). This diet is consistent with the earlier recommendations of the American Heart Association (AHA Committee Report, 1978).

Reaven (1986) challenged the recommendations of the consensus conference (1985) and questioned whether all Americans can benefit from the moderate-fat, high-carbohydrate diet. He suspected that such modifications would actually increase the risk of developing heart disease in some individuals such as carbohydrate-intolerant people.

There are still some uncertainties about the dietary recommendations. Since individual variation in response to diet is great, should recommendations be different for responders and nonresponders? Polyunsaturated fats decrease HDL-cholesterol as well as LDL and may cause some health problems such as gallstones; there is some suspicion of cancer also. How much polyunsaturated fat is safe but sufficient to protect people from heart disease? The present dietary recommendations are the best based on available information. However, more research is needed to resolve the uncertainties and give even better recommendations to the public and to individuals.

Purpose of the Study

This study was designed to investigate the effects of genetics and diets on cholesterol metabolism in mice. Mice are small and economic laboratory animals. Furthermore, the availability of different inbred strains facilitates genetic

experimentation. The first study tested whether mice responded to dietary pectin or fat manipulation and whether there were sex, light and genetic differences in the response using hepatic hydroxymethylglutaryl coenzyme A reductase activity as an index. The second and the third studies investigated the effects of dietary fat modifications on cholesterol metabolism in C57BR/cdJ and CBA/J mice. The C57BR/cdJ strain was reported as a hyperresponder to diet-induced hypercholesterolemia and susceptible to atherosclerosis, while the CBA/J strain was considered a hyporesponder and resistant to atherosclerosis. The dietary manipulations included a diet high in total fat and cholesterol and low in P/S ratio like the average American diet in 1974, and one moderate in total fat and cholesterol but high in P/S ratio close to the US Dietary Goals.

Explanation of Thesis Format

This thesis is composed of three papers. The first paper reports hepatic hydroxymethylglutaryl coenzyme A reductase activity in inbred strains of mice. The second and third papers report the effects of dietary fat on static metabolic measurements and whole body cholesterol kinetics, respectively, in mice genetically susceptible and resistant to atherosclerosis.

Dr. Jacqueline Dupont supervised all three studies. For the first paper, the Ph.D. candidate, Son-iu Kuan, with the assistance of David A. Warner, fed C57BL/6J mice, made diets, killed mice, prepared liver microsomes and performed all hydroxymethylglutaryl coenzyme A reductase assays including those of microsomes shipped from the Paris collaborators. Son-iu Kuan also did the whole set of experiments on CBA/J mice and all the statistical analyses. For the second paper, Son-iu Kuan designed and performed the entire study. For the third paper, Son-iu Kuan did the laboratory work, and Dr. Richard Seagrave and Leah Patterson helped with data analysis and mathematical modeling. Additional methodological testing with the help of Dr. Henry Stahr is reported in the general discussion.

PART 1. HEPATIC HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE

ACTIVITY IN INBRED STRAINS OF MICE

Hepatic hydroxymethylglutaryl coenzyme A reductase activity in inbred strains of mice

Son-iu Kuan[1], Jacqueline Dupont[1], Roberte Aubert[2],

David A. Warner[1], Marie-Claire Camus[2], and Jeanine Herzog[2]

Department of Food and Nutrition, Iowa State University,[1]

Ames, IA 50011, USA

Unite de Recherches sur la Nutrition et l'Alimentation,[2]

U.1 INSERM, Hopital Bichat, 170 Blvd. Ney,

75018 Paris, France

ABSTRACT

Hydroxymethylglutaryl coenzyme A reductase (HMGR)
activity is a major factor in regulation of cholesterol
homeostasis. The enzyme activity is known to vary with age,
sex, diurnal cycle and dietary properties in rats. Mice are
available in numerous genetic strains and could be a useful
inexpensive animal model for studying diet and genetic
interactions in regulation of cholesterol metabolism. Obese
and nonobese C57BL/6J, CBA/J and obese and nonobese DW db^{Pas}
mice were subjected to variations in light cycle, feeding
schedule and pectin and fat composition of their diets.
They were then killed by decapitation and hepatic microsomal
HMGR analyzed. The mice responded to light cycle, feeding
pattern and sex difference. They exhibited marked
differences due to age, genotype, strain and diet
variations. We conclude that they will, indeed, offer an
excellent animal model for the study of regulation of
cholesterol metabolism.

INTRODUCTION

Hydroxymethylglutaryl coenzyme A reductase (HMGR, EC.1.1.34) activity is well known to vary with age (Shefer et al., 1972), sex (Carlson et al., 1978) and diurnal cycle (Shefer et al., 1972; Carlson et al., 1978). Many factors in the diet including plant fiber (Eastwood and Kay, 1979) and type of fat (Faas et al., 1978) also act to influence HMGR activity. These results have been observed with rats as well as with some other species. Little has been reported regarding HMGR activity in the mouse. Energy metabolism is also an effector of regulation of cholesterol metabolism (Dupont, 1987), but studies of cholesterol metabolism in relation to energy usage have not been included in characterization of differences between genetically different animals. The following studies have been conducted to evaluate the value of use of genetically diverse strains of mice as models for studying cholesterol metabolism.

METHODS

Animals

Genetically obese (C57BL/6J) mice and nonobese littermates were bred in the ISU Food and Nutrition Department colonies. They were maintained at 24±3°C with twelve hour cycles of light from 0700 to 1900 and darkness from 1900 to 0700 h. During breeding, gestation and lactation, the mice were fed a commercial stock diet (Purina Rat Chow, Ralston Purina Co., St. Louis, MO) and had unlimited access to the food and to tap water. Genotype of the ob/ob mice was determined as described by Fanelli and Kaplan (1978). At weaning the mice were segregated by sex and genotype and caged in groups until used for this experiment.

At about 8-9 wk of age C57BL/6J mice were put into a room with normal (light 0600-1800 h) or a reversed light cycle (light 1800-0600 h) and placed on a purified control or high pectin diet at about 10 wk of age. After 15-18 d of consumption of the purified diets they were killed by decapitation at 1000-1100 h, livers were excised and microsomes were isolated for HMGR assay.

Six-wk-old male CBA/J mice were purchased from the Jackson Laboratory (Maine). They were kept in a reversed

light room (light 1700-0500 h) and fed a diet (US74)

corresponding to the average consumption in the U.S.A. in

1974 or a modified fat diet formulated to contain 10% of

linoleate and 10% of saturated fat. After 4 wk of feeding,

some mice were killed at their mid-dark cycle (1000-1100 h).

Blood was collected, livers were excised and liver

microsomes were prepared. Serum and liver microsomes were

frozen at -80°C for later analysis. The remaining mice were

switched to the other diet, i.e., those fed US74 diet were

changed to modified diet and vice versa (crossover). After

another 4 wk, mice were killed and serum and liver

microsomes were prepared.

Genetically obese DW db^{Pas} (Aubert et al., 1985) mice

were grown at the U.1 INSERM, Hopital Bichat, Paris. They

were maintained at 22+1°C with a regulated light cycle

corresponding to normal light and dark. They were caged in

groups segregated by sex and genotype. They had unlimited

access to food and water. At age 12 or 24 mo they were

subjected to a schedule of removal of food at 1800 h and

replacement at 0730 h for 2 d. On the second day they were

killed by decapitation at 1100-1200 h. This schedule is

analogous to the habit of food consumption when rodents

enter the dark cycle, that is, approximately 4 h into the

period of normal food consumption.

Experimental Diets

The C57BL/6J and CBA/J mice were caged singly and fed a purified diet based upon that devised by Fanelli and Kaplan (1978) with variation in fiber intake by manipulation of pectin or variation of fat composition. For evaluation of the effect of dietary fiber the diets were isocaloric (Table 1) with replacement of fat by pectin for the control and high pectin diets. The diets used to compare fat composition were isonutrient (Kuan and Dupont, 1987). The diets were pelleted and provided fresh at 3-d intervals. Food intake was determined for the comparison of high and low pectin diets in the C57BL/6J mice.

Serum Cholesterol Determination

Very low density and low density lipoproteins were precipitated by HDL precipitating reagent purchased from Sigma (St. Louis, MO). The serum total and HDL-cholesterol were determined using the enzymatic reagent kit from Sigma.

Hydroxymethylglutaryl CoA Reductase Assay

Microsomes were isolated from excised livers which were held on ice (4°C) throughout the procedure. The liver was minced, homogenized in a Potter-Elvehjem tissue grinder with teflon pestle with 4 volumes of potassium phosphate (KPi)

TABLE 1. Composition of control and high pectin diets

ingredient	control		high pectin	
	g	Kcal	g	Kcal
casein	20	80	20	80
DL-methionine	0.3		0.3	
minerals	5.0		5.0	
vitamins	1.0		1.0	
choline chloride	0.2		0.2	
beef tallow	16.5	148.5	26.5	238.5
corn oil	4.5	40.5	4.5	40.5
sucrose	24	96	13	52
corn starch	20	80	11	44
dextrin	5	20	2.5	10
cellulose	3.5		3.5	
pectin	0		12.5	
total	100	465	100	465

buffer and centrifuged at 20,000 x g for 30 min. The
supernatant was transferred to a tube for centrifugation at
100,000 x g in a 50 Ti rotor for 60 min. The pellet was
resuspended using a Kontes tube and hand-operated pestle in
the same buffer and stored at -80°C. Samples prepared in
Paris were transferred to Iowa stored in dry ice. HMGR
activity was determined in the samples from both
laboratories with a random distribution of treatments. The
methods of Nordstrom et al. (1977) were used. The Lowry
method as modified by Peterson (1977) was used to determine
microsomal protein.

RESULTS

Body and liver weights of the mice used in the feeding
experiments and their food intakes are shown in Table 2.
HMGR activities of C57BL/6J mouse liver microsomes are shown
in Table 3. The comparison of environment (light vs. dark)
was made only in female mice fed the control diet. There
was a significantly greater HMGR activity at 5 h into the
dark cycle for the mice when the activity was expressed as
nmoles mevalonate formed per milligram of microsomal
protein. The obese mice had significantly less HMGR
activity than did their lean littermates, and their response
to the environmental change was less marked which resulted
in a significant interaction of time vs. genotype. The
genotype effect was entirely caused by difference in liver
size as indicated by disappearance of the difference when
the values were calculated for the total liver. The enzyme
activity differences were analogous when expressed per
milligram of protein and per gram of body weight.

Males were compared with females only in the dark cycle
and with the control diet. There was no difference caused
by sex in the lean mice, but the obese males had
significantly greater HMGR activity than obese females. The
diet effect was studied only in males in the dark cycle.
There was a significantly elevated HMGR activity with the

TABLE 2. Body and liver weights of C57BL/6J, CBA/J, and DW
db^{Pas} mice (n=3-8 per group)

strain	diet	body weight	liver weight	food intake
		g	g	g/day
C57BL/6J, male				
lean	control	27.3^a	1.14^a	1.77^b
lean	pectin	25.2	1.22	2.11
obese	control	49.0	3.30	2.63
obese	pectin	43.3	1.91	3.08
SEM		2.39	0.20	0.15
CBA/J, male				
4 wk	US74	42.9^c	2.37^d	
4 wk	modified	35.8	1.76	
8 wk	US74	44.8	2.18	
8 wk	modified	43.4	1.91	
SEM		1.02	0.09	
DW db^{Pas}, 1 yr, male				
lean		36.6^e	1.67^e	
obese		76.8	2.27	
SEM				

^{a}Significant effects of diet (P<0.01), genotype (P<0.01) and diet x genotype interaction (P<0.01).

^{b}Significant effects of genotype (P<0.01) and diet (P<0.05).

^{c}Significant effects of age (P<0.01) and diet (P<0.05).

^{d}Significant effect of diet (P<0.05).

^{e}Significant effect of genotype (P<0.05).

TABLE 3. Hydroxymethylglutaryl CoA reductase activity in obese and nonobese C57BL/6J mice (n=6 per group)

sex	time	genotype	diet	HMGR per mg protein	HMGR per liver
				nmoles mevalonate formed	
F	light	lean	control	0.80[a]	9.4[b]
F	light	obese	control	0.44	11.9
F	dark	lean	control	2.43	25.9
F	dark	obese	control	0.76	20.3
SEM				0.21	4.3
M	dark	lean	control	2.89	41.9
M	dark	obese	control	1.44	41.4
M	dark	lean	pectin	5.00	99.7
M	dark	obese	pectin	3.65	90.9
SEM				0.46	15.6

[a]Significant effects of sex (P<0.05), time (P<0.01), genotype (P<0.01), diet (P<0.01) and time by genotype interaction (P<0.05).

[b]Significant effects of sex (P<0.01), time (P<0.01) and diet (P<0.01).

high pectin diet as compared to the control diet in both lean and obese mice. The effect of genotype was related to liver size and the genotype difference was lost when the activity of the whole liver was calculated.

Serum cholesterol concentrations and liver microsomal HMGR activity of male CBA/J mice are shown in Table 4. Diet did not affect either total or HDL cholesterol concentration. The enzyme activity was extremely responsive to dietary fat. The diet higher in total and saturated fat (US74) inhibited HMGR activity by 86% compared to the diet modified to contain 30% total fat with 10% saturated and 10% linoleate. The effect was partially due to differences in liver size in the younger group and the older mice had lower activity than did the younger group.

In the DW db^{Pas} mice genotype difference was studied only in males. There was a genotype difference in HMGR activity (Table 5) expressed per milligram of microsomal protein (P<0.05). The difference was enhanced when the enzyme activity was expressed as activity per liver in the 2-yr-old mice and diminished, but was not eliminated per liver in the 1-yr-old mice. These results are contrary to those observed in C57BL/6J mice. The two strains were not observed in the same experimental conditions. The comparison between the male C57BL/6J mice fed control diet

TABLE 4. Serum cholesterol and HMGR activity in liver
microsomes of CBA/J male mice (n=3-8 per group)

period	diet	HMGR		serum cholesterol	
		per mg protein	per liver	total	HDL
		nmoles mevalonate formed		mg/dl	
4 wk	US74	0.33[a]	9.06[a]	181	104
4 wk	modified	2.37	40.2	154	100
8 wk	US74	0.16	3.55	163	101
8 wk	modified	1.33	24.9	146	96
SEM		0.19	3.28	5.40	2.70

[a]Significant effects of age (P<0.01) and diet (P<0.01).

in the dark cycle and the younger male db^{Pas} mice is most analogous. The genotypic results for HMGR activity are opposite in this comparison, C57BL/6J being greater for the lean and db^{Pas} greater for the obese.

The differences in enzyme activity in the 2-yr-old db^{Pas} mice were not associated with differences in lipid composition of the livers (Table 6). No statistical differences in cholesterol, triglyceride or phospholipid concentration in the liver were observed.

TABLE 5. Hydroxymethylglutaryl CoA reductase activity in liver microsomes of male DW db^{Pas} mice (n=3-6 per group)

| genotype | HMGR[a] | | | |
| | 1-yr-old mice | | 2-yr-old mice | |
	per mg protein	per liver	per mg protein	per liver
	n moles mevalonate formed			
lean	2.73	31.5	1.68	16.4
obese	0.25	5.2	3.50	42.0
SEM	0.33	3.6	0.29	3.8

[a]Significant effects of genotype (P<0.01).

TABLE 6. Lipid composition of liver of 2-yr-old male DW db^{Pas} mice

lipid	lean (n=5)	obese (n=3)	SEM
	mg/g liver		
cholesterol	2.01	2.18	0.20
triglycerides	19.15	18.23	2.48
phospholipids	22.59	19.00	1.38

DISCUSSION

Mice responded to the factors which influence HMGR activity in rats in the same ways. Sex differences exist as reported for rats (Carlson et al., 1978), and there is a clear diurnal variation as reported for rats (Carlson et al., 1978; Shefer et al., 1972). As in rats (Mueller et al., 1983) increasing dietary pectin increased HMGR activity. Diets high in linoleate resulted in higher HMGR activity than did diets with high saturated fat as reported for rats (Faas et al., 1978). The results are analogous to reported effects in rats of such diets on cholesterol synthesis from acetate (Dupont, 1965). A decline in cholesterol synthesis with maturation also has been reported in rats (Story et al., 1976). Few observations have been made of old rats, but there may be a decrease in cholesterol synthesis after age 18 mo (Dupont et al., 1978). Obese db^{Pas} mice are also hypercholesterolemic (Aubert et al., 1985). The CBA/J mice have been reported to be resistant to development of atherosclerotic lesions when fed a high-fat, high-cholesterol diet (Paigen et al., 1985). Their ability to modify cholesterol synthesis with even a small change in dietary fat may be indicative of a general ability to adapt cholesterol metabolism to diet to maintain homeostasis.

These several strains of mice offer models for the study of the interaction between genetics and dietary factors in regulation of lipid metabolism. They may also provide a suitable model to identify the metabolic stresses which result in development of atherosclerosis.

BIBLIOGRAPHY

Aubert, R., J. Herzog, M. C. Camus, J. L. Guenet, and D. Lemonnier. 1985. Description of a new model of genetic obesity: The db Pas mouse. J. Nutr. 115:327-333.

Carlson, S. E., A. D. Mitchell, and S. Goldfarb. 1978. Sex-related differences in diurnal acitvities and development of hepatic microsomal HMG-CoA reductase and cholesterol 7a-hydroxylase. Biochim. Biophys. Acta 531:115-124.

Dupont, J. 1965. Relationship between utilization of fat and synthesis of cholesterol and total lipid in young female rats. J. Am. Oil. Chem. Soc. 42:903-907.

Dupont, J. 1987. Fat effects on fatty acid and cholesterol metabolism in animal experiments. In J. Beare-Rogers, ed. Fat requirements for development and health. Am. Oil Chemists' Society, Champaign. (In press)

Dupont, J., A. A. Spindler, and M. M. Mathias. 1978. Dietary fat saturation and cholesterol and fatty acid synthesis in aging rats. Age 1:93-99.

Eastwood, M. A., and R. M. Kay. 1979. An hypothesis for the action of dietary fiber along the gastrointestinal tract. Am. J. Clin. Nutr. 32:364-367.

Faas, F. H., W. J. Carter, and J. O. Wynn. 1978. Fatty acyl-CoA inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase activity. Biochim. Biophys. Acta 531(2):158-166.

Fanelli, M. T., and M. L. Kaplan. 1978. Effects of high fat and high carbohydrate diets on the body composition and oxygen consumption of ob/ob mice. J. Nutr. 108:1491-1500.

Kuan, S. I., and J. Dupont. 1987. Dietary fat effects on cholesterol metabolism in CBA/J and C57BR/cdJ mice. J. Nutr. (Submitted)

Mueller, M. A., M. P. Cleary, and D. Kritchevsky. 1983. Influence of dietary fiber on lipid metabolism in meal-fed rats. J. Nutr. 113:2229-2238.

Nordstrom, J. L., V. W. Rodwell, and J. U. Mitschelen. 1977. Interconversion of active and inactive forms of rat liver hydroxymethylglutaryl-CoA reductase. J. Biol. Chem. 252:8924-8934.

Paigen, B., A. Morrow, C. Brandon, D. Mitchell, and P. Holmes. 1985. Variation in susceptibility to atherosclerosis among inbred strains of mice. Atherosclerosis 57:65-73.

Peterson, G. L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83:346-356.

Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. HMG-CoA reductase of intestinal mucosa and liver of the rat. J. Lipid Res. 13:402-412.

Story, J. A., S. A. Tepper, and D. Kritchevsky. 1976. Age-related changes in the lipid metabolism of Fisher 344 rats. Lipids 11:623-627.

PART 2. DIETARY FAT EFFECTS ON CHOLESTEROL METABOLISM IN

CBA/J AND C57BR/CDJ MICE

Dietary fat effects on cholesterol metabolism in CBA/J and C57BR/cdJ mice

Son-iu Kuan and Jacqueline Dupont

Department of Food and Nutrition, Iowa State University,
Ames, IA 50011, USA

ABSTRACT

The aim of this study was to determine how small differences in dietary fats affect cholesterol metabolism in mice hypo- and hyperresponsive to diet-induced hypercholesterolemia. Six-wk-old male hyperresponders (C57BR/cdJ mice) and hyporesponders (CBA/J mice) were fed either a US74 diet (40 en% as fat, 347 mg cholesterol / 1000 Kcal, P/S=0.24) or a modified fat diet (30 en% as fat, 46 mg cholesterol / 1000 Kcal, P/S=0.91). After 8 wk of feeding, neither strain developed hypercholesterolemia. CBA/J mice had higher serum total, HDL, and esterified cholesterol concentrations than did C57BR/cdJ mice. CBA/J mice showed marked differences in responses to dietary fat compared with C57BR/cdJ mice. CBA/J mice responded to the US74 diet by decreasing the hepatic synthesis of cholesterol as a result of suppression of hydroxymethylglutaryl coenzyme A reductase (HMGR) activity. C57BR/cdJ mice, on the other hand, responded to the US74 diet compared to the modified fat diet by increasing the fecal excretion of cholesterol. Compared to the modified fat diet, the US74 diet caused an increase in serum total to HDL cholesterol ratio, liver microsomal total and free cholesterol, and hepatic microsomal cholesterol 7a-hydroxylase activity in the CBA/J strain, and caused an increase in fecal cholesterol and a decrease in

fecal ergosterol and β-sitosterol, and hepatic microsomal HMGR activity in both strains. We conclude that the metabolic responses to small differences in dietary fat are different in CBA/J and C57BR/cdJ mice and the genetic propensity to hypercholesterolemia of C57BR/cdJ mice is not expressed when they are young.

INTRODUCTION

There is a wide variation among individuals in response to dietary fat -- some individuals are especially susceptible (hyperresponders) to diet (high cholesterol, high saturated fat)-induced hypercholesterolemia while some are particularly resistant (hyporesponders) (Clarkson et al., 1985). Although this phenomenon has been known for some time, the underlying mechanisms are still unknown, probably because of the limitations of human experimentation. Moreover, the Dietary Goals for the United States (1977) suggest reducing average dietary fat to 30% of total energy, reducing cholesterol to 300 mg per day, and increasing the P/S ratio (polyunsaturated to saturated fatty acid) to 1.0. How this change will affect metabolism and how the metabolism differs between the hyper- and hyporesponders is not known. So, an appropriate animal model can be used to determine specific biochemical events.

Roberts and Thompson (1975) screened 13 strains of inbred mice and found that the C57BR/cdJ strain was very susceptible to and the CBA/J strain was resistant to dietary cholesterol and saturated fat-induced hypercholesterolemia and atherosclerosis. Walker (1984) confirmed their results. Mulvihill and Walker (1984) further characterized C57BR/cdJ mice and found that the serum cholesterol concentration was

inversely related to the dietary P/S ratio. These phenomena are very similar to those which occur in humans (Clarkson et al., 1985). The C57BR/cdJ and CBA/J strains of mice, therefore, may be good animal models to study cholesterol metabolism as related to genetic differences in response to dietary fat manipulation. In this study, the effects of dietary fat on cholesterol metabolism in C57BR/cdJ and CBA/J mice were investigated. The diets compared were a diet high in total fat and cholesterol and low in P/S ratio like the average American diet in 1974, and one moderate in total fat and cholesterol and high in P/S ratio as suggested by the US Dietary Goals.

MATERIALS AND METHODS

Experimental Design

C57BR/cdJ (susceptible to diet-induced atherosclerosis) and CBA/J (resistant) were purchased from the Jackson Laboratory (Maine) and bred in the Animal Care Facility of the Food and Nutrition Department, Iowa State University. The breeders and the post-weaning offspring were fed Purina Breeder Chow. At 6 wk of age, male mice were caged singly in a room with a reversed light cycle (light: 1700-0500 h; dark: 0500-1700 h) and fed either a US74 or a modified fat diet (Table 7). There were 8 mice per treatment. The two experimental diets used were isonutrient and were based upon that devised by Fanelli and Kaplan (1978) with variations in fat composition. The dietary fat composition was calculated using the USDA Handbook No. 8-4 (Consumer and Food Economics Institute, 1979) and was similar in energy yielding nutrients to that of a human study done in our department (Batres-Cerezo et al., 1987; Garcia et al., 1987). The US74 diet had a fat content and composition similar to that of the average American diet in 1974 in accord with the HANES I survey (Abraham and Carroll, 1979) (40% of energy from fat, 600 mg cholesterol / 2000 Kcal, and a P/S ratio of 0.3), while the modified fat diet was designed to meet the Dietary

Goals for the United States (1977) (30% of energy from fat, less than 300 mg cholesterol / 2000 Kcal, and a P/S ratio of 1.0). The feeding lasted for 8 wk. Feces excreted during the last three days of the dietary treatments were collected and frozen for later analysis of neutral sterols and bile acids.

Diet Analyses

The experimental diets, US74 and modified fat diets, were analyzed for fatty acid and neutral sterol compositions. An aliquot of the diet was ground and lipids were extracted with chloroform/methanol (2:1, v/v). The extract was saponified in 10% ethanolic KOH at 60°C for 1 hour. Neutral sterols were extracted with petroleum ether, silylated with Sil-prep (Alltech-Applied Science) and analyzed by using a Beckman gas chromatograph equipped with a flame ionization detector and an 8 ft x 1/8 in OD, 2 mm ID glass column packed with 5% OV210, 80-100 mesh. Instrument settings were: column temperature 220°C, detector temperature 250°C, inlet temperature 260°C, and detector line temperature 270°C, and nitrogen flow 20 ml/min. Identification of trimethylsilyl (TMS) derivatives of neutral sterols was by comparison of their retention times with those of silylated standards. Quantitation was done by comparison with an internal standard, 5α-cholestane.

TABLE 7. Compositions of US74 and modified fat diets

ingredient	US74		modified	
	g	Kcal	g	Kcal
high nitrogen casein[a]	18.0	72.0	18.0	72.0
DL-methionine[a]	0.3		0.3	
AIN76 mineral mixture[b]	5.0		5.0	
AIN76 vitamin mixture[b]	1.0		1.0	
choline chloride[a]	0.2		0.2	
beef tallow[c]	16.2	145.8	7.2	64.8
corn oil[d]	3.3	29.7	7.3	65.7
sucrose	22.0	88.0	27.0	108.0
corn starch	20.0	80.0	25.0	100.0
dextrin[b]	4.25	17.0	5.5	22.0
celufil[a]	3.48		3.48	
cholesterol[e]	0.132		0.012	
L-ascorbic acid[b]	0.010		0.010	
total	93.872	432.5	100.002	432.5

[a]Purchased from United States Biochemical Co., Cleveland, OH.

[b]Purchased from ICN Biochemicals, Cleveland, OH.

[c]Purchased from the Meat Lab., ISU, Ames, IA.

[d]Mazola corn oil, Englewood Cliffs, NJ.

[e]Purchased from Sigma Chemical Co., St. Louis, MO.

Table 7 (continued)

ingredient	US74	modified
fat (%Kcal)	40.6	30.2
S:M:P[f]	1.0:0.9:0.31	1.0:1.05:1.0
cholesterol (mg/1000 Kcal)	347	46

[f]Saturated: monounsaturated: polyunsaturated fatty acids.

The ethanolic fraction was acidified to pH<5 with 6 N
HCl and fatty acids were extracted with petroleum ether.
The fatty acids were methylated by heating with
borontrifluoride/methanol (Alltech-Applied Science) for 2
min in a boiling water bath. After the sample cooled,
methylated fatty acids were extracted with hexane and
separated by using a Beckman gas chromatograph equipped with
a flame ionization detector and a 6 ft x 1/8 in stainless-
steel column packed with Altex Chrom WAW 10% CS10, 100-120
mesh. Instrument settings were: column temperature 180°C,
detector temperature 250°C, inlet temperature 220°C,
detector line temperature 250°C, and nitrogen flow 20
ml/min. Identification of fatty acid methyl esters was by
comparison of their retention times with those of known
methylated standards.

Tissue Analyses

At the end of the feeding period, mice were
anesthetized with ether and killed by decapitation at
1000-1100 h. Blood was collected and allowed to clot at
room temperature. Serum was prepared by centrifuging the
blood at 2,000 x g for 10 min (Beckman Model J-6B
centrifuge) and stored at -80°C until analysis. Livers were
excised and microsomes were prepared (Angelin et al., 1982).
Liver microsomes were stored at -80°C until analysis.

Serum

Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) were precipitated with sodium phosphotungstate and magnesium chloride (Seigler and Wu, 1981) using a kit from Sigma Chemical Co. (St. Louis). The enzymatic method (Allain et al., 1974) was used to determine the total, free, and HDL-cholesterol in serum.

Liver

The activities of hydroxymethylglutaryl coenzyme A reductase (HMGR) and cholesterol 7a-hydroxylase (COH) in liver microsomes were determined using methods described by Nordstrom et al. (1977) and Dodd et al. (1982), respectively. The Lowry method (Lowry et al., 1951) as modified by Peterson (1977) was used to determine the protein content in the microsomes. The activity of HMGR was expressed as nmoles of mevalonate formed in 30 min per mg protein, per whole liver, and per 50 g body weight, while that of COH was expressed as percentage conversion of cholesterol into 7a-hydroxycholesterol in 40 min per 0.7 mg protein.

Liver microsomal lipid was extracted with chloroform/methanol (2:1, v/v) and washed with 0.05 M NaCl and 0.36 M $CaCl_2$/methanol (1:1, v/v). The enzymatic method (Allain et al., 1974) as modified by Carlson and Goldfarb

(1977) was used to determine the total and free cholesterol in liver microsomes.

Feces

Dried feces were ground in a mortar. An aliquot was extracted twice with 0.5 N HCl in absolute ethanol by incubation at 37°C for 1 hr (Oh and Dupont, 1975). The fecal extract was then saponified in 10% KOH in ethanol at 60°C for 1 hr. Neutral sterols were extracted with petroleum ether, silylated with Sil-prep and analyzed by gas chromatography (GC). The GC conditions were the same as those for analyzing dietary neutral sterols. The alcoholic fraction was acidified to pH 0-2 with 6 N HCl, and bile acids were extracted with ethyl ether. Free fatty acids were removed from bile acids by thin layer chromatography (TLC) using toluene/ethanol/methanol/water/ammonium hydroxide (50:20:14:3:1, v/v/v/v/v) as the developing solvent. The area of total bile acids was scraped off the plate, the bile acids were eluted with methanol, and quantitated by using a 3α-hydroxysteroid dehydrogenase assay (Weber et al., 1972). Data were expressed as μg neutral sterols or bile acids excreted per 50 g body weight per day.

Statistical Analyses

The mean and standard deviation of each parameter measured were calculated for each treatment group. The SAS (Statistical Analysis System) computer program for analysis of variance was used to test the diet effect, the strain effect, and the diet by strain interaction. Comparisons between groups were done using student t-test. Pearson correlation coefficients between parameters were calculated.

RESULTS

The results of diet analyses are shown in Tables 8 and
9. There were some discrepancies between the calculated and
the analyzed fatty acid compositions. The US74 diet had a
saturated: monounsaturated: polyunsaturated fatty acid ratio
of 1.0:0.55:0.24 as opposed to the calculated 1.0:0.90:0.31,
and the modified fat diet had a ratio of 1.0:0.87:0.91 as
opposed to 1.0:1.05:1.0. The discrepancies were due to the
slightly different fatty acid compositions of corn oil and
beef tallow we used compared with the values in USDA
Handbook No. 8-4 (Consumer and Food Economics Institute,
1979). Another reason was that diet ingredients other than
corn oil and beef tallow might contain a little fat which
was not counted by calculation. The analyzed cholesterol
values, however, were the same as the calculated values.
The US74 diet had 347 mg cholesterol per 1000 Kcal, while
the modified fat diet had 46 mg cholesterol per 1000 Kcal.
Corn oil, although containing a negligible amount of
cholesterol, if any, was high in the phytosterols,
ergosterol and β-sitosterol. This was reflected in the
higher phytosterol content in the modified diet than the
US74 diet.

The body and liver weights and food intakes of CBA/J
and C57BR/cdJ mice are summarized in Table 10. CBA/J mice

TABLE 8. Fatty acid compositions of diets and fat sources as determined by gas-liquid chromatography

| fatty acid | diet | | fat source | |
	US74	modified	corn oil	beef tallow
	weight percent of total fatty acids			
14:0[a]	2.7	1.6	0.1	3.3
15:0	0.7	0.4	trace	0.8
16:0	23.5	18.7	11.0	29.8
18:0	28.9	15.3	1.2	12.7
18:1	30.6	31.4	26.9	52.8
18:2	13.0	31.9	58.9	0.2
18:3	0.6	0.7	1.9	0.4

[a]Number of carbons:number of double bonds.

were heavier, had bigger livers, and ate more than did C57BR/cdJ mice. The body weight differences were proportional to differences in food intake. The liver weights, however, were not directly proportional to body weight or food intake. Even as a ratio to body weight, CBA/J mice had bigger livers than did C57BR/cdJ mice. The liver weight was influenced by the diet. Mice fed modified diet had smaller livers than did those fed US74 diet. The effect on CBA/J mice was especially obvious.

Contrary to previous reports, C57BR/cdJ mice had less serum total cholesterol than did CBA/J mice (Table 11). C57BR/cdJ mice also had less serum high density lipoprotein (HDL) cholesterol and esterified cholesterol (EC) than did CBA/J mice. The diets used in this experiment did not induce hypercholesterolemia as did those used by Roberts and Thompson (1975 and 1977). Mice fed the modified diet had a lower serum total to HDL-cholesterol ratio than those fed US74 diet.

The activities of HMGR (Table 12) and COH (Table 13) were determined in liver microsomes. C57BR/cdJ mice had higher cholesterol degradative but lower cholesterol synthesis activities than CBA/J mice, as reflected by the greater COH activity and lesser HMGR activity. The diet effect on these two enzymes was also obvious, especially

TABLE 9.   Neutral sterol compositions of diets and fat
           sources as determined by gas-liquid chromatography

| neutral sterol | diet | | fat source | |
	US74	modified	corn oil	beef tallow
			μg/g	
cholesterol+ dihydrocholesterol	1600	200	27	736
ergosterol	52	123	1970	-
β-sitosterol	270	480	5550	155

TABLE 10. Body and liver weights, and food intakes of 2
strains of mice fed diets with different fat
compositions (n=8 per group)

strain	diet	body[1,2] weight	liver[3] weight	food[2] intake
		g	g	g/day
CBA	US74	45.8 ± 2.2^a	2.72 ± 0.53^a	3.50 ± 0.34^a
CBA	modified	43.8 ± 3.2^a	1.86 ± 0.31^b	3.32 ± 0.06^a
C57BR	US74	39.1 ± 4.2^b	1.62 ± 0.24^b	2.69 ± 0.14^b
C57BR	modified	37.6 ± 3.8^b	1.35 ± 0.17^c	2.43 ± 0.19^b

[1]Values are means±SD. Values within a column not
sharing a common superscript are different (P<0.05).

[2]Significant strain effect (P<0.01).

[3]Significant strain effect (P<0.01), diet effect
(P<0.01), and strain x diet interaction (P<0.05).

HMGR. US74 diet suppressed HMGR activity but increased COH activity as an adaptation to the cholesterol content of the diet. CBA/J mice showed a greater response to diet than did C57BR/cdJ mice in terms of HMGR activity. US74 diet suppressed HMGR activity by 80% in CBA/J mice but only 40% in C57BR/cdJ mice.

The total and free cholesterol concentrations in liver microsomes were affected by diet, as shown in Table 13. US74 diet induced an accumulation of total and free cholesterol in liver microsomes. Correlation analysis using pooled data showed a negative correlation between HMGR activity and total cholesterol in liver microsomes ($r=-0.63$, $P<0.01$), but, when data within a treatment were used, no significant correlations were found. No significant correlations between COH activity and cholesterol concentration in liver microsomes or serum were found.

There were significant strain effect, diet effect, and strain x diet interaction in fecal excretion of cholesterol (Table 14). The US74 diet caused higher fecal cholesterol excretion than did the modified fat diet. C57BR/cdJ mice showed an especially great response. Those fed US74 diet excreted almost five times as much cholesterol as those fed the modified diet. The diet effect was also significant in fecal excretion of ergosterol, β-sitosterol and bile acids.

TABLE 11. Serum cholesterol of 2 strains of mice fed diets with different fat compositions (n=8 per group)

strain	diet	serum cholesterol			ratio	
		total[1,2]	free	HDL[2]	EC^2/free	$total^3$/HDL
		mg/dl				
CBA	US74	157 ± 17^a	26.3 ± 7.7^a	$132\pm10^{a,b}$	5.2 ± 0.9^a	1.20 ± 0.14^a
CBA	modified	152 ± 13^a	25.1 ± 3.2^a	143 ± 15^a	5.1 ± 0.7^a	1.07 ± 0.06^b
C57BR	US74	$140\pm19^{a,b}$	28.6 ± 5.0^a	$118\pm18^{b,c}$	4.1 ± 0.7^b	1.19 ± 0.06^a
C57BR	modified	135 ± 14^b	27.0 ± 4.0^a	117 ± 10^c	4.2 ± 0.4^b	1.15 ± 0.05^a

[1]Values are means±SD. Values within a column not sharing a common superscript are different (P<0.05).

[2]Significant strain effect (P<0.01).

[3]Significant diet effect (P<0.05).

TABLE 12. Liver microsomal hydroxymethylglutaryl CoA
reductase activity in 2 strains of mice fed diets
with different fat compositions

strain	diet	HMGR[1,2]		
		per protein	per liver	per 50g BW
		nmoles mevalonate formed/30 min		
CBA	US74	0.38 ± 0.08^a	12.0 ± 2.7^a	13.1 ± 2.7^a
CBA	modified	3.24 ± 1.04^b	57.7 ± 23.3^b	65.6 ± 24.0^b
C57BR	US74	0.19 ± 0.03^c	3.2 ± 0.5^c	4.1 ± 0.7^c
C57BR	modified	0.35 ± 0.13^a	5.2 ± 2.1^d	6.9 ± 2.6^d

[1]Values are means$\pm$SD. Values within a column not
sharing a common superscript are different (P<0.01).

[2]Significant strain effect (P<0.01), diet effect
(P<0.01), and strain x diet interaction (P<0.01).

TABLE 13. Liver microsomal total and free cholesterol and cholesterol 7a-hydroxylase activity (COH) in 2 strains of mice fed diets with different fat compositions

strain	diet	total[1,2]	free[3]	COH[4]
		μg/g liver		% conversion/ 0.7 mg protein/40 min
CBA	US74	303±26[a]	249±37[a]	1.55±0.24[a]
CBA	modified	188±20[b]	171±16[b]	1.13±0.22[b]
C57BR	US74	285±51[a,c]	234±50[a,c]	1.74±0.36[a]
C57BR	modified	244±48[c]	189±36[b,c]	1.50±0.37[a]

[1]Values are means±SD. Values within a column not sharing a common superscript are different ($P<0.01$).

[2]Significant diet effect ($P<0.01$) and strain x diet interaction ($P<0.05$).

[3]Significant diet effect ($P<0.01$).

[4]Significant strain effect ($P<0.05$) and diet effect ($P<0.01$).

Mice fed the modified diet excreted more ergosterol and β-sitosterol than those fed the US74 diet. This was due to the high phytosterol concentration in the modified fat diet. Bile acid excretion, on the other hand, was greater in mice fed the US74 diet than in those fed the modified fat diet.

TABLE 14. Fecal excretion of neutral sterols and bile acids in 2 strains of mice fed diets with different fat compositions

strain	diet	chol[1,2]	ergo[3]	sito[4]	BA[5]
			μg/50g body weight/day		
CBA	US74	646± 89[a]	150±24[a]	514± 57[a]	54±15[a]
CBA	modified	536± 76[b]	352±52[b]	1070±128[b]	40±15[a]
C57BR	US74	2580±631[c]	140±31[a]	543± 82[a]	92±36[b]
C57BR	modified	519± 93[b]	409±43[c]	1080±127[b]	26± 6[c]

[1]Values are means±SD. Values within a column not sharing a common superscript are different (P<0.05).

[2]Cholesterol. Significant strain effect (P<0.01), diet effect (P<0.01), and strain x diet interaction (P<0.01).

[3]Ergosterol. Significant diet effect (P<0.01) and strain x diet interaction (P<0.01).

[4]β-sitosterol. Significant diet effect (P<0.01).

[5]Bile acids. The values were calculated by using the molecular weight of cholic acid. Significant diet effect (P<0.05).

DISCUSSION

Diets of different fat contents can be made isocaloric by modifying the content of fiber. It is known that fiber can affect cholesterol metabolism (Anderson, 1985; Salvioli et al., 1985; Mueller et al., 1983). Adolf (1947) reported that rats fed a diet diluted with cellulose or kaolin consumed enough food to maintain energy intake. Dupont (1965) demonstrated that rats were able to adjust their energy intake when fat supplied 0 to 40% of the total calories. The experimental diets used in this study were made isonutrient rather than isocaloric to eliminate the effect of fiber.

Roberts and Thompson (1975 and 1977) reported that the C57BR/cdJ mouse was susceptible to and the CBA/J mouse was resistant to an atherogenic diet (30 wt% cocoa butter, 5 wt% cholesterol, and 30 wt% protein in the form of casein) -induced hypercholesterolemia and atheromatous lesions. Morrisett et al. (1982) reported similar results. In contrast to the extremely high fat, high saturated fat and high cholesterol diet used in those two studies, the present study used moderate fat diets similar to a human diet. We found that the C57BR/cdJ mouse had lower serum cholesterol than the CBA/J mouse when they were fed either a US74 diet or a modified fat diet, and there was no significant diet

effect on serum cholesterol. So, mice hyperresponsive to an extremely high fat diet may not be hyperresponsive to a moderate fat diet in terms of serum cholesterol level. Furthermore, cocoa butter is not present in the American diet in large quantities and is very different from beef tallow and corn oil in fatty acid compositions. It is likely that cocoa butter exerts a different effect from beef tallow and corn oil. The reported diet also is deficient in essential fatty acids.

However, Walker (1984) demonstrated that the C57BR/cdJ mouse was more responsive to a moderate-fat diet (10% fat as lard, olive-sunflower oil, or hydrogenated soy bean-sunflower oil)-induced elevation of plasma cholesterol than was the CBA/J mouse. The different results obtained by Walker and in our study may be due to differences in mouse age and treatment period. In the present study 6-wk-old mice were used compared with 16- to 18-wk-old mice in Walker's study, and the treatment period lasted for 8 wk instead of 11 wk. Studies with rats hyper- and hyporesponsive to diet-induced hypercholesterolemia (Takeuchi et al., 1976) showed that the genetic defects in hyperresponders were masked when they were young but were revealed in the aging process. The combined results of Walker and this study may indicate that the aging process

aggravates the expression of genetic defects in cholesterol metabolism of C57BR/cdJ mice.

Consistent with the report of Breckenridge et al. (1985), most of the plasma total cholesterol in both strains of mice used in this study was in the HDL fraction. The modified fat diet caused a significantly lower total to HDL cholesterol ratio than did the US74 diet. This result is consistent with Mulvihill and Walker's finding (1984) that the ratio of serum total to HDL cholesterol decreased with increasing P/S ratio in the diet. Significant strain effects in the distribution of serum cholesterol between free and esterified cholesterol were found. CBA/J mice had more cholesterol in the esterified form than C57BR/cdJ mice, resulting in a higher serum esterified to free cholesterol ratio.

In this experiment, C57BR/cdJ mice responded to dietary cholesterol and fat effects on serum cholesterol equally as well as CBA/J mice did although the mechanisms were different. CBA/J mice responded to US74 diet by decreasing hepatic cholesterol synthesis as a result of suppresssion of HMGR activity. C57BR/cdJ mice, on the other hand, responded to US74 diet by increasing fecal cholesterol excretion. Because both strains had efficient mechanisms to respond to the experimental diets used, hypercholesterolemia was not seen in either strain of mouse.

However, under all conditions, C57BR/cdJ mice had a lesser hepatic HMGR activity than did the CBA/J mice. Dietschy (1984) compared hepatic cholesterol synthesis in several species and concluded that species with high rates of hepatic cholesterol synthesis, e.g., the rat, can more readily adapt to large changes in the flux of cholesterol into and out of the body than can species with much lower rates of hepatic cholesterol synthesis, e.g., the hamster and man. Moreover, Nestel and Poyser (1976) reported two groups of human subjects differing in metabolic responses to increased dietary cholesterol intake. Increased fecal excretion of cholesterol was the predominant change in one group while reduction in cholesterol synthesis was the predominant change in the other group. However, a rise in the plasma cholesterol level was prevented only when cholesterol synthesis was suppressed. The metabolic differences between CBA/J and C57BR/cdJ mice were consistent with the characteristics of the hyper and hyporesponders in humans and other species.

The US74 diet caused an accumulation of both free and total cholesterol in liver microsomes compared to the modified fat diet. It also decreased the activity of HMGR and increased the activity of COH in the liver microsomes. Since both HMGR and COH are membrane-bound enzymes, it is

possible that their activities are modulated by the cholesterol present in the microsomal membranes either through end product inhibition or precursor activation, respectively. Correlation analysis using pooled data showed a negative correlation between HMGR activity and cholesterol concentration in the liver microsomes, but, when data within a treatment were used, no correlation was found. This suggests that cholesterol concentration may be one factor in the gross control of HMGR activity, but fine tuning of the enzyme activity is effected by other factors. No correlation was found between COH activity and liver microsomal cholesterol concentration. Balasubramaniam et al. (1973) demonstrated that there were compartments of cholesterol in the rat liver microsomes accessible to COH. It is likely that compartmentalization for metabolic channeling obscured a possible correlation between COH activity and COH-accessible cholesterol concentration in the liver microsomes.

In summary, this study has demonstrated marked differences in the metabolic response to small changes in dietary cholesterol and fat in CBA/J and C57BR/cdJ mice, and the genetic defects in cholesterol metabolism of C57BR/cdJ mice are not expressed when they are young. This phenomenon is very similar to what occurs in humans. CBA/J and

C57BR/cdJ mice may serve as good animal models to study the genetic predisposition to diet-induced hypercholesterolemia. Future research shall investigate the changes in cholesterol metabolism during the aging process.

BIBLIOGRAPHY

Abraham, S., and M. D. Carroll. 1979. Fats, cholesterol, and sodium intake in the diet of persons 1-74 years: United States. Advance Data, Vital and Health Statistics, No. 54. U. S. Department of Health and Human Services, Washington, D. C.

Adolf, E. F. 1947. Urges to eat and drink in rats. Am. J. Physiol. 151:110-125.

Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

Anderson, J. W. 1985. Health implications of wheat fiber. Am. J. Clin. Nutr. 41:1103-1112.

Angelin, B., L. Backman, K. Einarsson, L. Eriksson, and S. Ewerth. 1982. Hepatic cholesterol metabolism in obesity: Activity of microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase. J. Lipid Res. 23:770-773.

Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat-liver microsomes. Eur. J. Biochem. 34:77-83.

Batres-Cerezo, R., J. Dupont, P. A. Garcia, and C. C. Kies. 1987. Plasma from humans eating diets with different fatty acid composition alters prostaglandin synthesis by human endothelial cells growing in culture. J. Am. Coll. Nutr. (Submitted)

Breckenridge, W. C., A. Roberts, and A. Kuksis. 1985. Lipoprotein levels in genetically selected mice with increased susceptibility to atherosclerosis. Arteriosclerosis 5:256-264.

Carlson, S. E., and S. Goldfarb. 1977. A sensitive enzymatic method for determination of free and esterified tissue cholesterol. Clinica Chim. Acta 79:575-582.

Clarkson, T. B., J. R. Kaplan, and M. R. Adams. 1985. The role of individual differences in lipoprotein, artery wall, gender, and behavioral responses in the development of atherosclerosis. Pages 28-45 in K. T. Lee, ed. Atherosclerosis. Annals of the New York Academy of Sciences, New York.

Consumer and Food Economics Institute. 1979. Composition of foods - fats and oils. USDA Handbook No. 8-4. SEA, U. S. Department of Agriculture, Washington, D. C.

Dietary goals for the United States. 1977. U. S. Senate Committee on Nutrition and Human Needs, Washington, D. C.

Dietschy, J. M. 1984. Regulation of cholesterol metabolism in man and in other species. Klin. Wochenschr. 62:338-345.

Dodd, N. K., C. E. Sizer, and J. Dupont. 1982. Cholanoic acids and cholesterol 7a-hydroxylase activity in human leucocytes. Biochem. Biophys. Res. Commun. 106(2):385-389.

Dupont, J. 1965. Relationship between utilization of fat and synthesis of cholesterol and total lipid in young female rats. J. Am. Oil Chem. Soc. 42:903-907.

Fanelli, M. T., and M. L. Kaplan. 1978. Effects of high fat and high carbohydrate diets on the body composition and oxygen consumption of ob/ob mice. J. Nutr. 108:1491-1500.

Garcia, P. A., K. B. Hanson, C. Kies, S. Y. Oh, J. A. Story, D. Romsos, and J. Dupont. 1987. Plasma lipoproteins and steroid excretion of women eating diets with different fatty acid composition. J. Am. Coll. Nutr. (Submitted)

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265-275.

Morrisett, J. D., H. S. Kim, J. R. Patsch, S. K. Datta, and J. J. Trentin. 1982. Genetic susceptibility and resistance to diet-induced atherosclerosis and hyperlipoproteinemia. Arteriosclerosis 2:312-324.

Mueller, M. A., M. P. Cleary, and D. Kritchevsky. 1983. Influence of dietary fiber on lipid metabolism in meal-fed rats. J. Nutr. 113:2229-2238.

Mulvihill, B. J., and B. L. Walker. 1984. Dietary fatty acids - cholesterol interaction and serum cholesterol in the hypercholesterolemic mice. Nutr. Res. 4:611-619.

Nestel, P. J., and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metabolism 25:1591-1599.

Nordstrom, J. L., V. W. Rodwell, and J. J. Mitschelen. 1977. Interconversion of active and inactive forms of rat liver hydroxymethylglutaryl-COA reductase. J. Biol. Chem. 252:8924-8934.

Oh, S. Y., and J. Dupont. 1975. Identification and quantitation of cholanoic acids in hepatic and extra-hepatic tissues of rat. Lipids 10:340-347.

Peterson, G. L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal. Biochem. 83:346-356.

Roberts, A., and J. S. Thompson. 1975. Inbred mice and their hybrids as an animal model for atherosclerosis research. Pages 313-327 in C. E. Day, ed. Advances in experimental medicine and biology. Atherosclerosis drug discovery. Vol. 67. Plenum Press, New York.

Roberts, A., and J. S. Thompson. 1977. Genetic factors in the development of atheroma and on serum total cholesterol levels in inbred mice and their hybrids. Prog. Biochem. Pharmacol. 14:298-305.

Salvioli, G., R. Lugli, and J. M. Pradelli. 1985. Cholesterol absorption and sterol balance in normal subjects receiving dietary fiber on ursodeoxycholic acid. Dig. Dis. Sci. 30:301-307.

Seigler, L., and W. T. Wu. 1981. Separation of serum high-density lipoprotein for cholesterol determination: Ultracentrifugation vs precipitation with sodium phosphotungstate and magnesium chloride. Clin. Chem. 27:838-841.

Takeuchi, N., M. Ito, and Y. Yamamura. 1976. Cholesterol metabolism in rats sensitive to high cholesterol diet. Pages 267-288 in C. E. Day, ed. Advances in experimental medicine and biology. Atherosclerosis drug discovery. Vol. 67. Plenum Press, New York.

Walker, B. L. 1984. Plasma cholesterol response to dietary saturated and hydrogenated fats in CBA and C57BR/cdJ mice. Nutr. Res. 4:601-610.

Weber, A. M., L. Chartrand, G. Doyon, S. Gardon, and C. C. Roy. 1972. Determination of faecal bile acids in children by the enzymic method. Clinica Chim. Acta 39(2):524-531.

PART 3. DIETARY FAT EFFECTS ON WHOLE BODY CHOLESTEROL

KINETICS IN CBA/J AND C57BR/CDJ MICE

Dietary fat effects on whole body cholesterol kinetics in CBA/J and C57BR/cdJ mice

Son-iu Kuan[1], Jacqueline Dupont[1],

Richard C. Seagrave[2] and Leah Patterson[2]

Departments of Food and Nutrition[1],

and Chemical Engineering[2],

Iowa State University, Ames, IA 50011, USA

ABSTRACT

Effects of dietary fats on whole body cholesterol kinetics were studied in CBA/J (hyporesponders to diet-induced hypercholesterolemia) and C57BR/cdJ mice (hyperresponders). Six-wk-old male mice were fed either a US74 diet (40 en% fat, 347 mg cholesterol / 1000 Kcal, P/S=0.24) or a modified fat diet (30 en% fat, 46 mg cholesterol / 1000 Kcal, P/S=0.91). After 8 wk of feeding, 0.5 μCi of ^{14}C-cholesterol was injected intraperitoneally into each mouse and mice were killed at intervals over 34 days. The mass and radioactivity of cholesterol in serum, heart, liver and carcass were determined.

Mice fed the US74 diet had more cholesterol in the liver than did those fed the modified fat diet, and the C57BR/cdJ strain had less cholesterol in serum, liver and carcass but more in heart compared to the CBA/J strain. The kinetic data were consistent with the cholesterol mass data as mice fed the US74 diet had lower initial specific radioactivity than those fed the modified fat diet, and CBA/J mice had lower initial specific activity than did C57BR/cdJ mice, reflecting a greater dilution of the radioactive cholesterol by unlabeled cholesterol in the liver.

Computer analysis using a three-compartment model (serum, heart and liver) showed that liver and serum compartments were in rapid balance in mice fed the modified fat diet and in C57BR/cdJ mice fed the US74 diet. The CBA/J mice fed the US74 diet, however, had a delay in the balance between liver and serum compartments. The heart showed an initial buildup of radioactivity, and the radioactivity started to die away after 4 days. The higher cholesterol specific activity in heart than in liver or serum of the C57BR/cdJ strain fed the modified fat diet and the lower cholesterol specific activity in the carcass than in other tissues of both strains fed either diet probably reflected two or more sub-compartments in heart and carcass which were not in rapid balance. Further analysis is in progress to incorporate carcass into the model and to improve the level of confidence in the calculated transfer rates.

INTRODUCTION

Whole body cholesterol metabolism has been customarily studied by following the decline of plasma cholesterol specific activity over time after a single injection of tracer. Each change of slope of the curve is usually interpreted as indicating a different kinetic pool of cholesterol. By the curve peeling technique, Goodman and Noble (1968) described two kinetically distinct pools of cholesterol in normal persons, untreated hyperlipidemic patients and hyperlipidemic patients treated with cholestyramine. Similar to the human, miniature swine (Dupont et al., 1974) also showed a two component disappearance curve in serum. Later, a three component disappearance curve was reported by Goodman et al. in 1973 based on an experiment done on humans over longer periods of time. The pools were postulated to represent tissues having different rates of equilibrium of cholesterol with serum cholesterol. The three major body cholesterol compartments were thought to be: a rapidly miscible pool, a slowly miscible pool, and a very slowly miscible pool. These pools, however, only represented mathematical constructs and did not have precise physical meaning. The curves did not define the characteristics of cholesterol within pools nor the location of the compartments. Studies of tissue

cholesterol metabolism suggested that the rapidly miscible pool contained cholesterol in the liver, plasma, red blood cells and intestine; the slowly miscible pool contained muscle and adipose cholesterol; and the very slowly miscible pool contained cholesterol of brain and nervous tissue (Dupont, 1982).

The three-pool model does not account for what is known about cholesterol metabolism. Mitropoulos et al. (1974) found that the rat liver contained one compartment of cholesterol accessible to cholesterol 7a-hydroxylase (COH) and a separate compartment available for lipoprotein synthesis. Balasubramaniam et al. (1973) reported that the COH-accessible pool consisted of de novo synthesized cholesterol while the other pool represented turnover of serum cholesterol. In other words, in vivo turnover of cholesterol actually involves a large number of metabolically heterogeneous pools of cholesterol in different tissues and within given tissues. Schwartz et al. (1978) used a simulation analysis and modeling (SAAM) process to characterize the hepatic bile acid and biliary cholesterol precursor sites in patients with total bile fistula. A model was designed using current knowledge of cholesterol metabolism and was then tested by a computer simulation program to determine whether the data fit. With

this approach, Schwartz et al. (1978) concluded that 31% and 20%, respectively, of the cholesterol input into the bile acid and biliary cholesterol precursor sites were derived from newly synthesized hepatic cholesterol, 10% of bile acid from plasma esterified cholesterol, and the remainder predominantly from lipoprotein-free cholesterol.

In human studies, blood is almost the only available tissue for analysis except, in a few cases, bile is also available. Animal studies, however, can provide more information as each tissue can be analyzed. A previous study (Kuan and Dupont, 1987) showed that dietary fat composition affected cholesterol metabolism in mice and different strains of mice responded to diet differently. When fed a diet with 40 en% fat (P/S=0.24) and 346 mg cholesterol / 1000 Kcal compared to a diet with 30 en% fat (P/S=0.91) and 46 mg cholesterol / 1000 Kcal, CBA/J mice maintained cholesterol homeostasis by suppressing hepatic cholesterol synthesis while C57BR/cdJ mice increased fecal excretion of cholesterol. The CBA/J strain was described as resistant to and the C57BR/cdJ strain as susceptible to diet-induced hypercholesterolemia and atherosclerosis (Roberts and Thompson, 1975 and 1977).

In the study reported herein a mathematical modeling approach based on a phenomenological model was used to

investigate how diets and genetics affected whole body
cholesterol kinetics in mice.

METHODS

Experimental Design

C57BR/cdJ (susceptible to diet-induced atherosclerosis) and CBA/J (resistant) mice were purchased from the Jackson Laboratory (Maine) and bred in the Animal Care Facility of the Food and Nutrition Department, Iowa State University. The diet used in the breeding colony was Purina Breeder Chow. At 6 wk of age, male mice were caged singly in a room with a reversed light cycle (light: 1700-0500 h; dark: 0500-1700 h) and fed either a US74 or a modified fat diet (Kuan and Dupont, 1987). The two experimental diets were isonutrient and were based upon that devised by Fanelli and Kaplan (1978) with variation in fat composition. The US74 diet had a fat content similar to that of the average American diet in 1974 in accord with the HANES I survey (Abraham and Carroll, 1979) (40% of energy from fat, 600 mg cholesterol / 2000 Kcal, and a P/S ratio of 0.3), while the modified fat diet was designed to meet the Dietary Goals for the United States (1977) (30% of energy from fat, less than 300 mg cholesterol / 2000 Kcal, and a P/S ratio of 1.0). After 8 wk of feeding, 0.5 μCi of $[4]^{14}$C-cholesterol was injected intraperitoneally to each mouse. Mice were killed at intervals over 34 days.

Tissue Analyses

Mice were anesthetized with ether and blood was obtained after decapitation. Serum was prepared by allowing blood to clot at room temperature and then centrifuging at 2,000 x g for 10 min. Liver and heart were excised. Serum, liver, heart and the eviscerated carcass were stored at -80°C until analysis.

Liver, heart and serum were saponified in 10% ethanolic KOH at 60°C for 1 h (Dupont et al., 1982). The nonsaponifiable fraction was extracted twice with petroleum ether. An aliquot of the extract was taken for cholesterol mass determination (Carlson and Goldfarb, 1977). The remaining extract was transferred to a scintillation vial, dried and counted for radioactivity in a Packard TRI-CARB liquid scintillation spectrometer using ScintiLene (Fisher Scientific Co.) as the scintillation fluid. This gave an estimate of radioactive cholesterol present. The carcass was hydrolyzed in 10% KOH in 50% aqueous ethanol at 80°C for 4 h (Dupont et al., 1980). The nonsaponifiable fraction was extracted and analyzed in the same way as the other organs.

Data Analyses

In this experiment, it was assumed that mice were in a steady state of cholesterol metabolism. In other words, the cholesterol masses in different organs and carcass were constant among mice of the same treatment and there should be no time effect on the distribution of cholesterol mass. Any data point having cholesterol mass outside the mean±2SD was omitted from the analysis. Then, an analysis of variance using the SAS (Statistical Analysis System) computer program was carried out to test whether there was a time effect. When there was, outlying points were dropped. The data were then subjected to the analysis of variance to test the diet effect, the strain effect, and the diet by strain interaction.

Specific radioactivity (dpm/mg) was determined as a function of time for cholesterol in each organ and the eviscerated carcass minus the head. Adjustment was made to correct for differences in body weight and radioactive ^{14}C-cholesterol dose. A mathematical model (Figure 1) was constructed based on the exchange of tracers between compartments, and a computer program was written. The differential equations that describe the variation of the specific activity of cholesterol in each compartment with time were then written as follows.

Serum compartment

$$M_S(dY_S/dt)=R_{HS}Y_H+R_{LS}Y_L+R_{TS}Y_T-(R_{ST}+R_{SH}+R_{SL})Y_S$$
$$+(E_cf/(1-f))Y_L$$

Liver compartment

$$M_L(dY_L/dt)=R_{SL}Y_S-((E_c/(1-f))+R_{LS}+T_L)Y_L$$

Heart compartment

$$M_H(dY_H/dt)=R_{SH}Y_S-(R_{HS}+T_H)Y_H$$

Tissue compartment

$$M_T(dY_T/dt)=R_{ST}Y_S-(R_{TS}+T_T)Y_T$$

where the symbols are defined as follows:

M_i=mass of cholesterol in compartment i

R_{ij}=transfer rate of cholesterol from compartment i to j

T_i=rate at which cholesterol is converted

E_c=excretion rate

Y_i=labeled cholesterol/total cholesterol in compartment i

The assumptions underlying this model are as follows.

1) Total cholesterol is at a steady-state

2) Each compartment is well-mixed

Under these conditions, the only variables in these
equations are the Y's. The data from the experiments were
then fit with a 3rd-order spline fit, and the equations were

solved by using a numerical integration scheme on a digital computer. A regression routine was used to adjust the values of the constants representing the transfer rates until a satisfactory fit between the data and the numerical solutions was obtained. This technique has the advantage of producing the flow rates directly, in contrast to curve-peeling techniques. It also allows testing of alternate hypotheses regarding the movement of cholesterol.
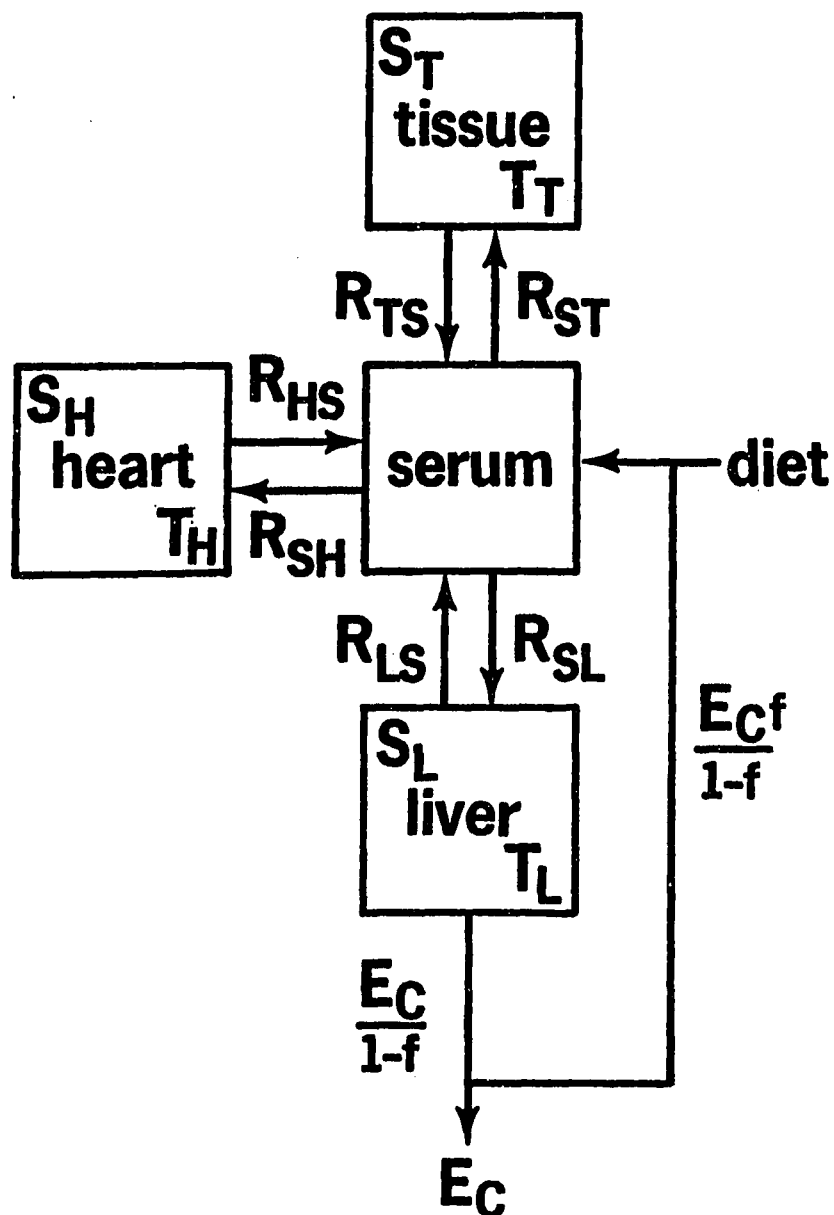
FIGURE 1. Four-compartment model of cholesterol flow in the body (R=rate of cholesterol flow; S=cholesterol synthesis; T=conversion of cholesterol; f=fraction of cholesterol recycled; E_C=cholesterol excretion)

RESULTS

The cholesterol masses in serum, heart, liver and
carcass are shown in Table 15. Strain effect was
significant in all these organs. C57BR/cdJ mice had less
cholesterol in serum, liver and carcass, but more in heart
compared with CBA/J mice. The diet effect was significant
in liver and carcass only. Mice fed the US74 diet had
higher accumulations of cholesterol in liver and carcass
than those fed the modified fat diet.

The computer model described earlier was employed to
analyze the kinetic data. It was found that transformation
of labeled cholesterol (the T_i's) was negligible, and that a
satisfactory fit was best obtained using a three-compartment
model (serum, heart and liver), and ignoring the tissue
(carcass) compartment. The raw data of carcass are shown in
Table 16. Figures 2, 3, and 4 showed the specific activity
time course of experimental data and computer-generated
curves in serum, heart and liver, respectively, using CBA/J
mice fed a US74 diet as an example. The computer-generated
specific activity time course curves to fit the serum, heart
and liver data of CBA/J and C57BR/cdJ mice fed diets with
different fat compositions are shown in Figures 5, 6 and 7,
respectively. Figures 8, 9, 10 and 11 are overlay of
computer-generated serum, heart and liver curves for

TABLE 15. Cholesterol masses in serum, heart, liver and carcass of 2 strains of mice fed diets with different fat compositions (n=30-36 per group)

strain	diet	serum[1,2]	heart[3]	liver[4]	carcass[5]
		mg/dl	mg/50g	body weight	
CBA	US74	161 ± 16^a	0.298 ± 0.028^a	35.5 ± 9.6^a	67.4 ± 11.9^a
CBA	modified	158 ± 22^a	0.295 ± 0.028^a	7.8 ± 2.0^b	66.0 ± 9.4^a
C57BR	US74	$156 \pm 25^{a,c}$	0.323 ± 0.038^b	20.3 ± 3.9^c	57.0 ± 6.2^b
C57BR	modified	144 ± 21^c	0.320 ± 0.057^b	7.4 ± 1.0^b	50.8 ± 5.6^c

[1]Values are means±SD. Values within a column not sharing a common superscript are different (P<0.05).

[2]Significant strain effect (P<0.05).

[3]Significant strain effect (P<0.01).

[4]Significant strain effect (P<0.01), diet effect (P<0.01) and strain x diet interaction (P<0.01).

[5]Significant strain effect (P<0.01) and diet effect (P<0.01).

different groups of mice. Table 17 gives the calculated exchange flows which are consistent with the figures.

TABLE 16. Specific activity time course in carcass of 2
strains of mice fed diets with different fat
compositions after an injection of radioactive
cholesterol (n=2-4 per time point)

strain		CBA		C57BR	
diet	US74	modified	US74	modified
day		10^2dpm/mg		
1	21.4	24.7	27.3	50.9
2	22.3	24.0	18.7	48.2
4	31.2	45.7	29.8	41.7
6	11.6	23.0	17.7	23.8
10	12.0	11.9	14.8	19.3
13	12.4	22.7	12.2	24.0
16	11.0	15.4	14.4	37.3
20	7.4	12.3	7.4	24.3
23	8.4	13.6	10.7	18.5
26	6.2	9.3	8.2	11.9
30	5.5	15.1	10.4	25.4
34	4.4	7.9	7.2	17.8

FIGURE 2. Specific activity time course in serum of CBA/J mice fed a US74
diet after an injection of radioactive cholesterol (stars=actual
values, line=computer-generated curve)

THOUSANDS   CBA/J,US 74 DIET, HEART

FIGURE 3. Specific activity time course in heart of CBA/J mice fed a US74
           diet after an injection of radioactive cholesterol (stars=actual
           values, line=computer-generated curve)
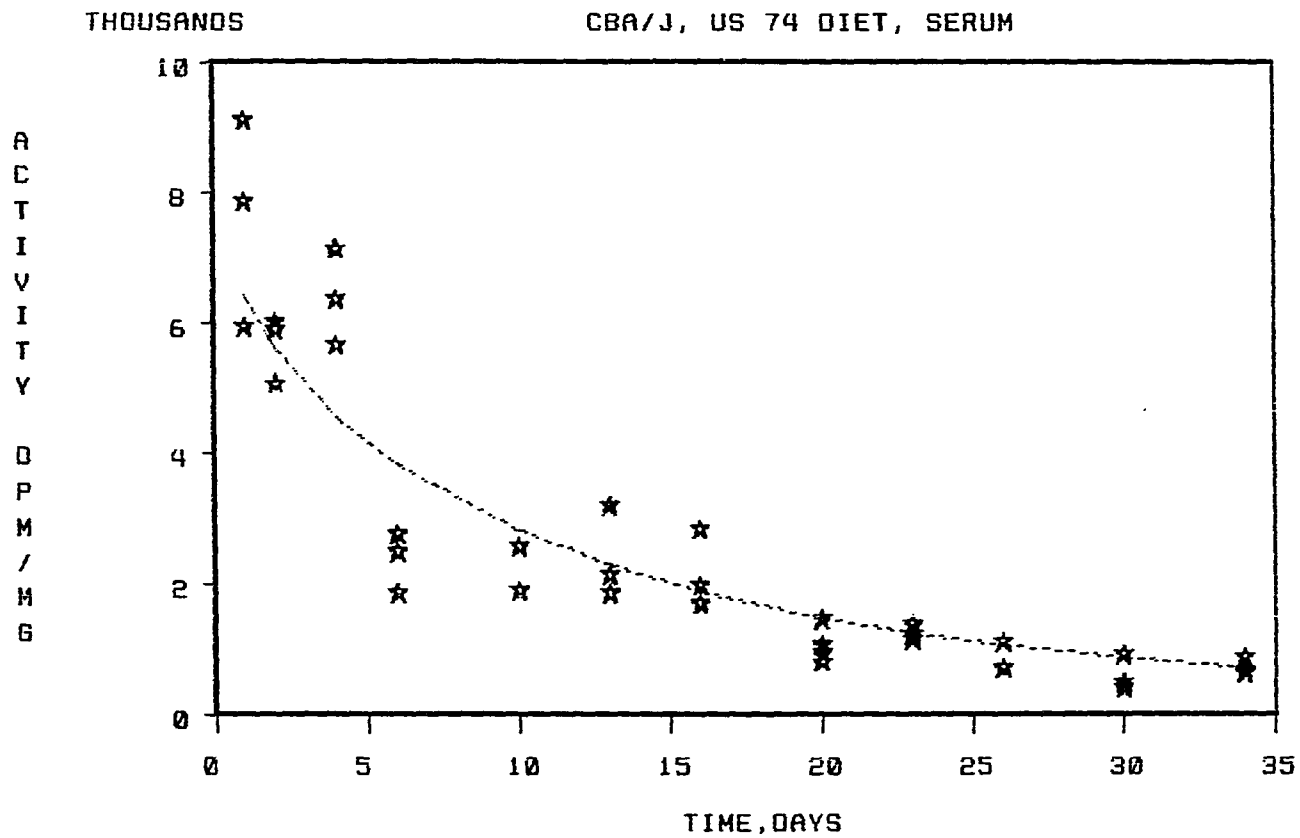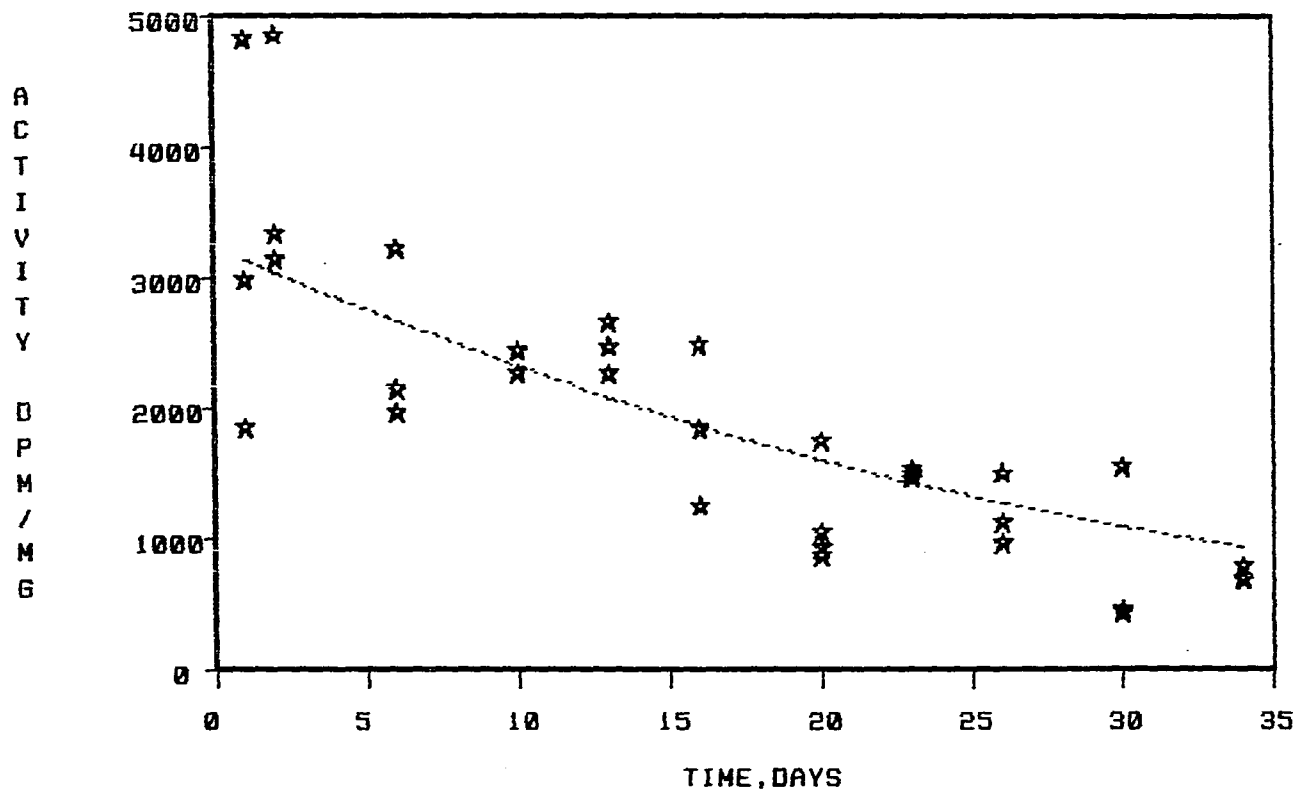
FIGURE 4. Specific activity time course in liver of CBA/J mice fed a US74 diet after an injection of radioactive cholesterol (stars=actual values, line=computer-generated curve)
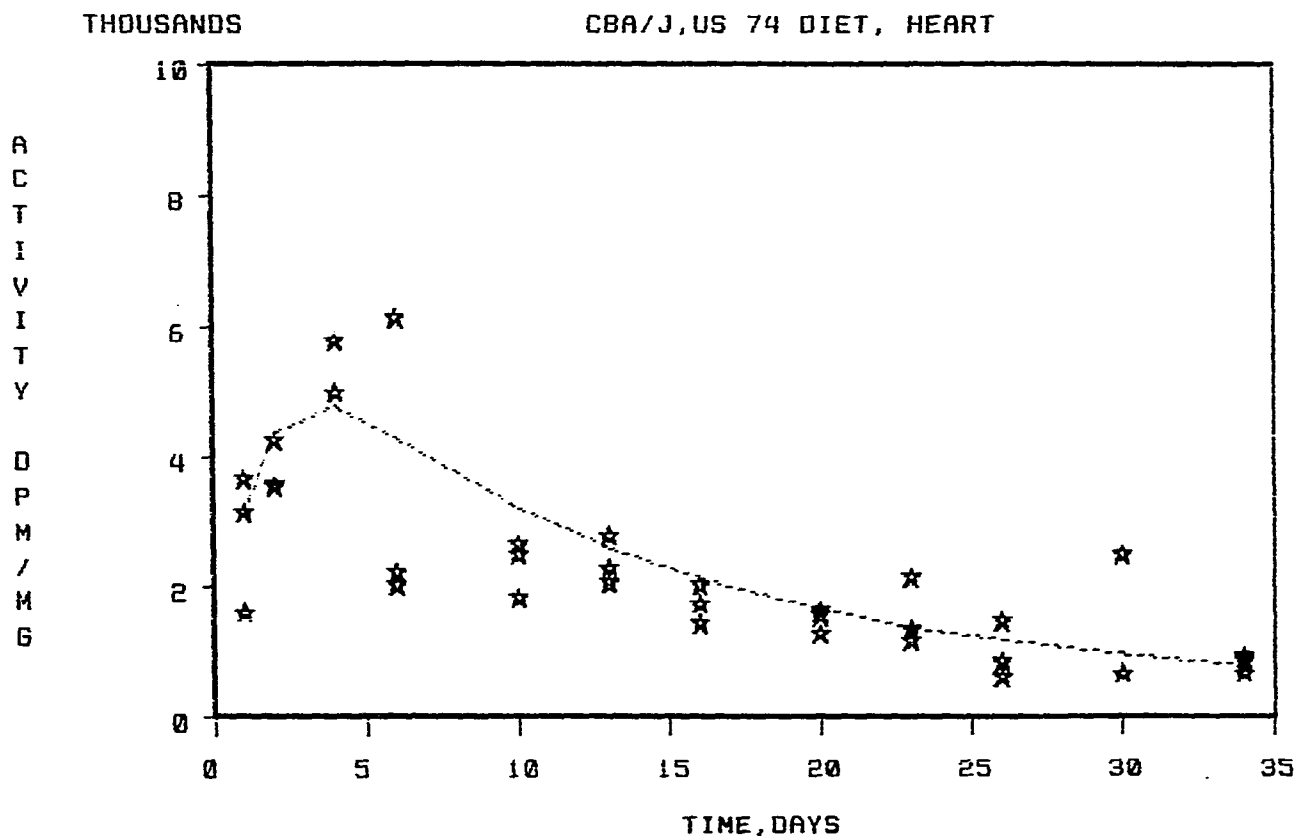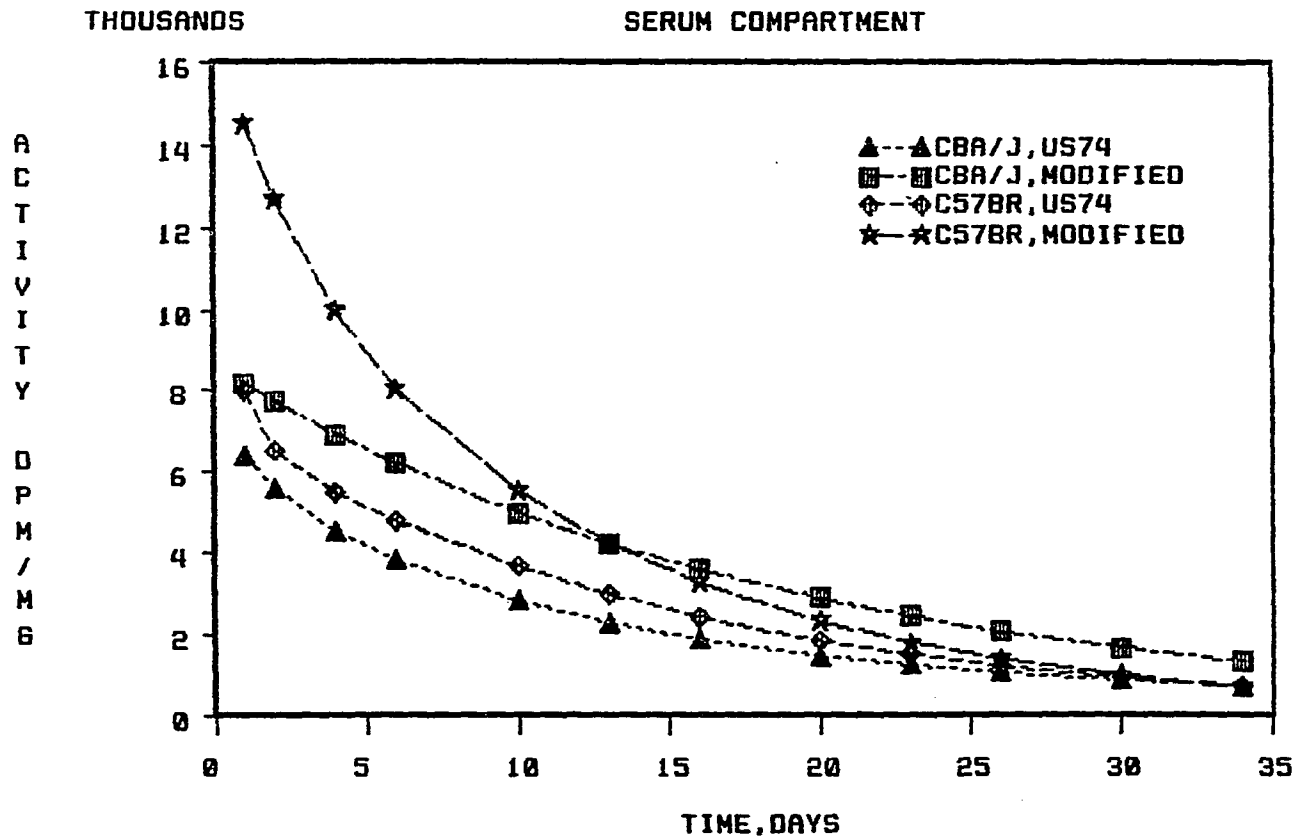
FIGURE 5. Computer-generated specific activity time course curves fitting the serum data of 2 strains of mice fed diets with different fat compositions

THOUSANDS   HEART COMPARTMENT
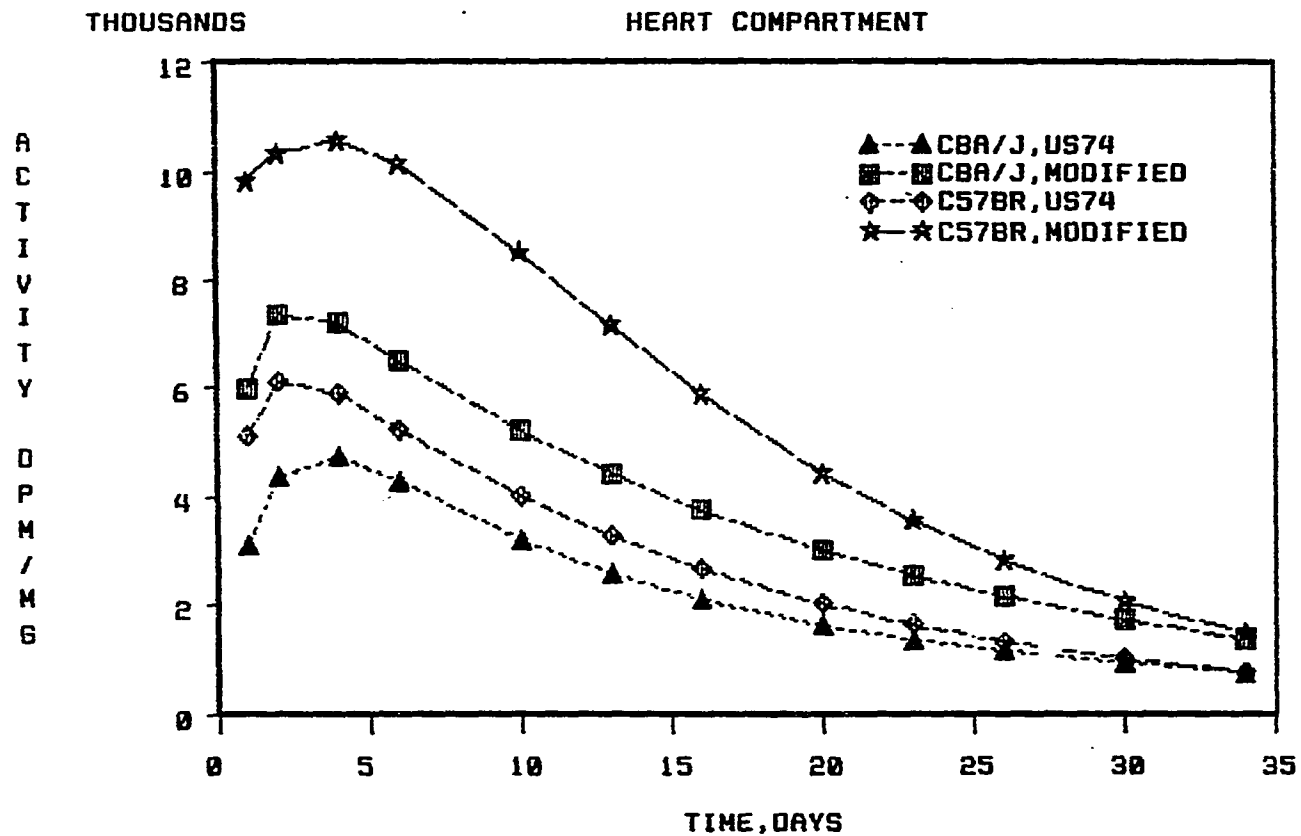
FIGURE 6. Computer-generated specific activity time course curves fitting
the heart data of 2 strains of mice fed diets with different fat
compositions
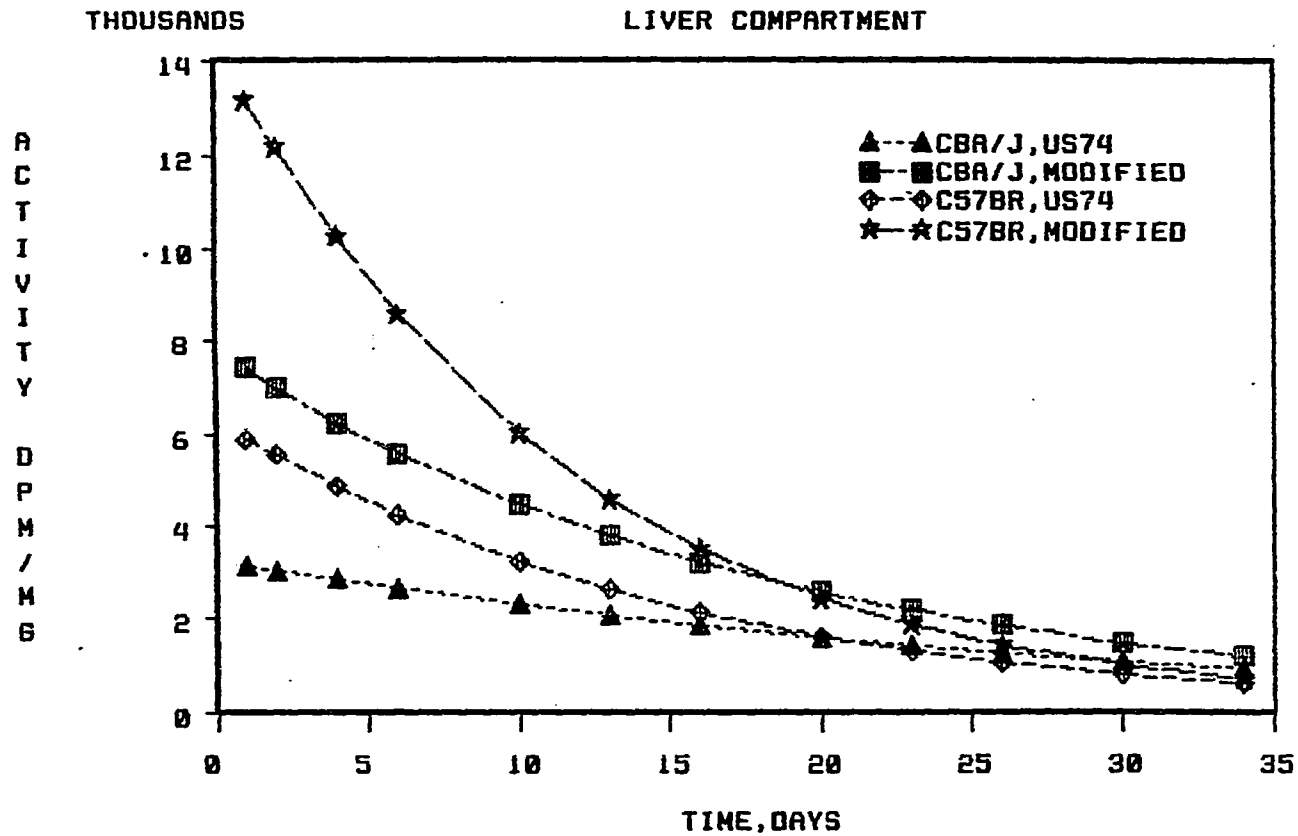
FIGURE 7. Computer-generated specific activity time course curves fitting the liver data of 2 strains of mice fed diets with different fat compositions

CBA/J, US 74, ALL COMPARTMENTS
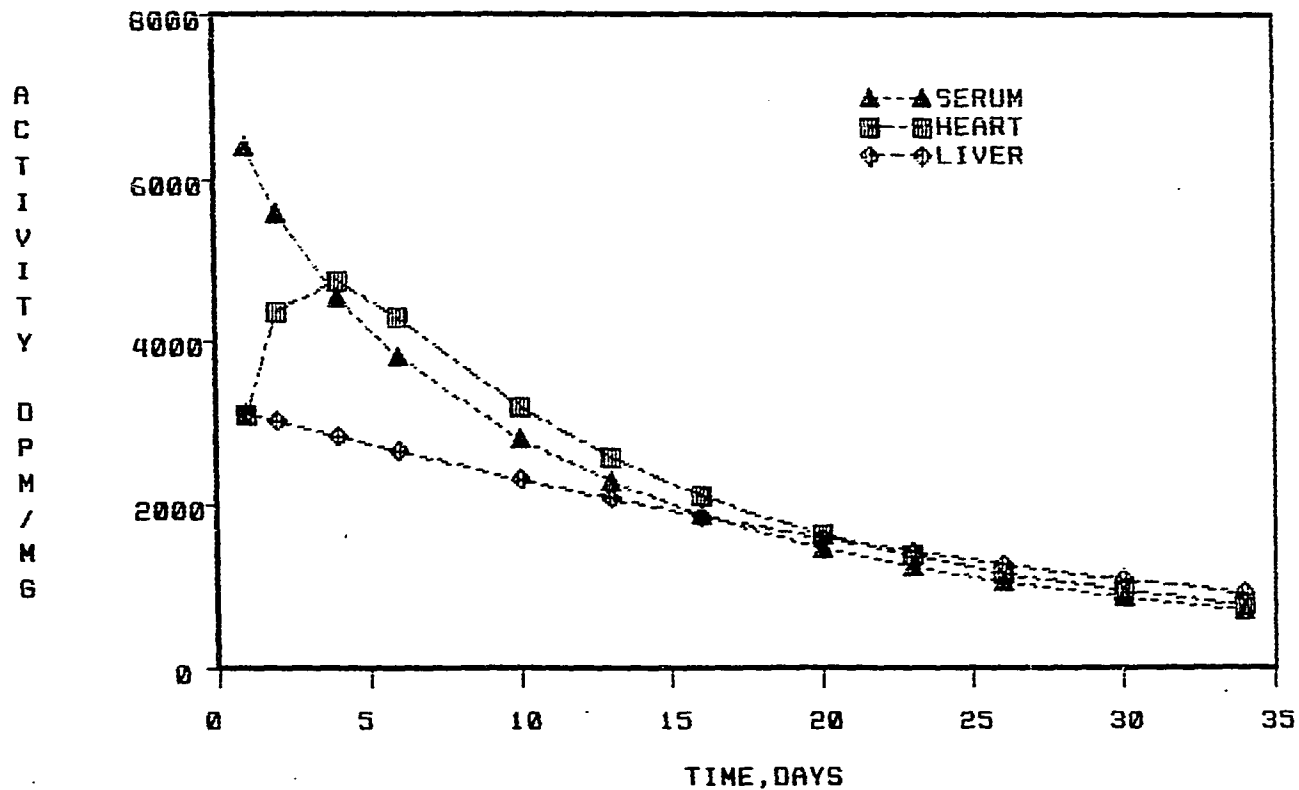
FIGURE 8. Overlay of the computer-generated specific activity time course curves of serum, heart, and liver in CBA/J mice fed a US74 diet

THOUSANDS   CBA/J, MODIFIED DIET, ALL COMPARTMENTS
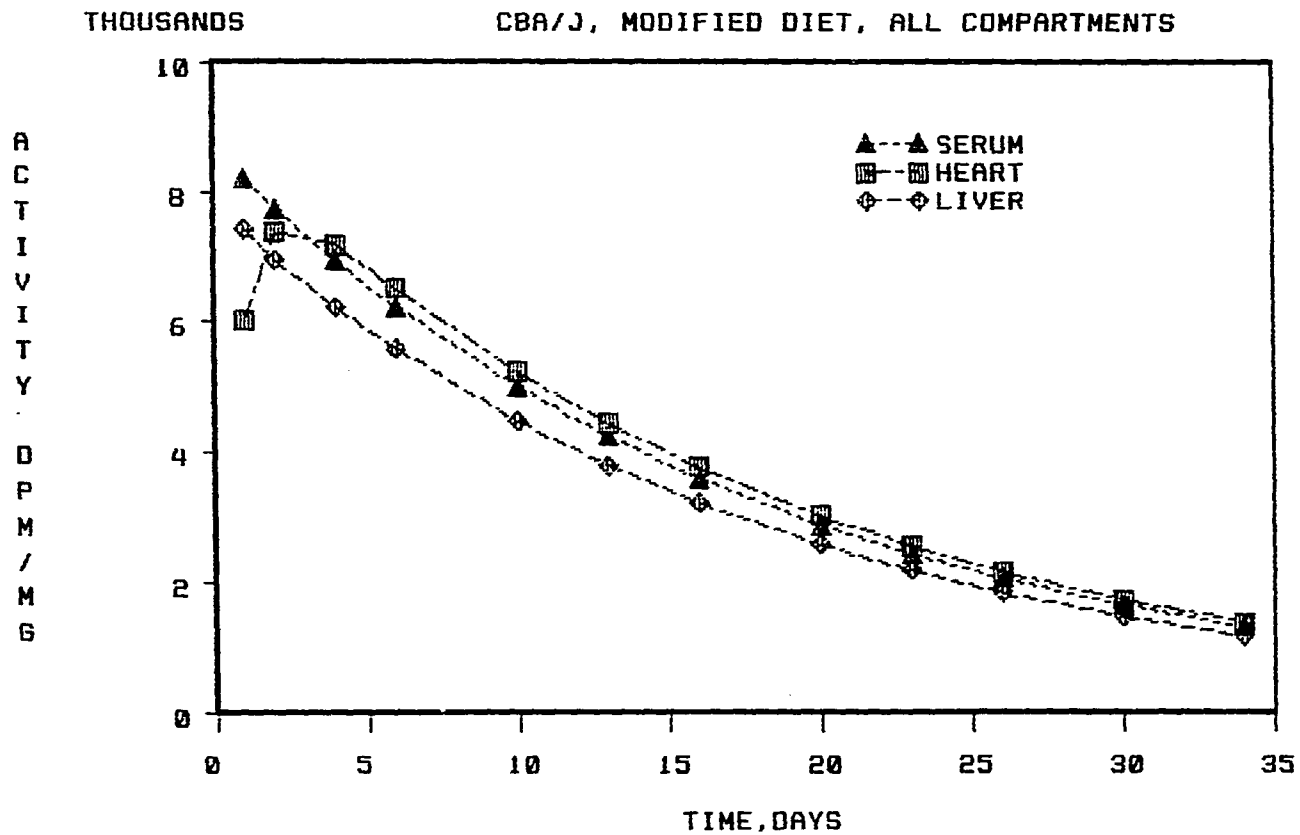
FIGURE 9. Overlay of the computer-generated specific activity time course
curves of serum, heart, and liver in CBA/J mice fed a modified fat
diet

THOUSANDS   C57BR/cdJ, US 74 DIET, ALL COMPARTMENTS

TIME,DAYS
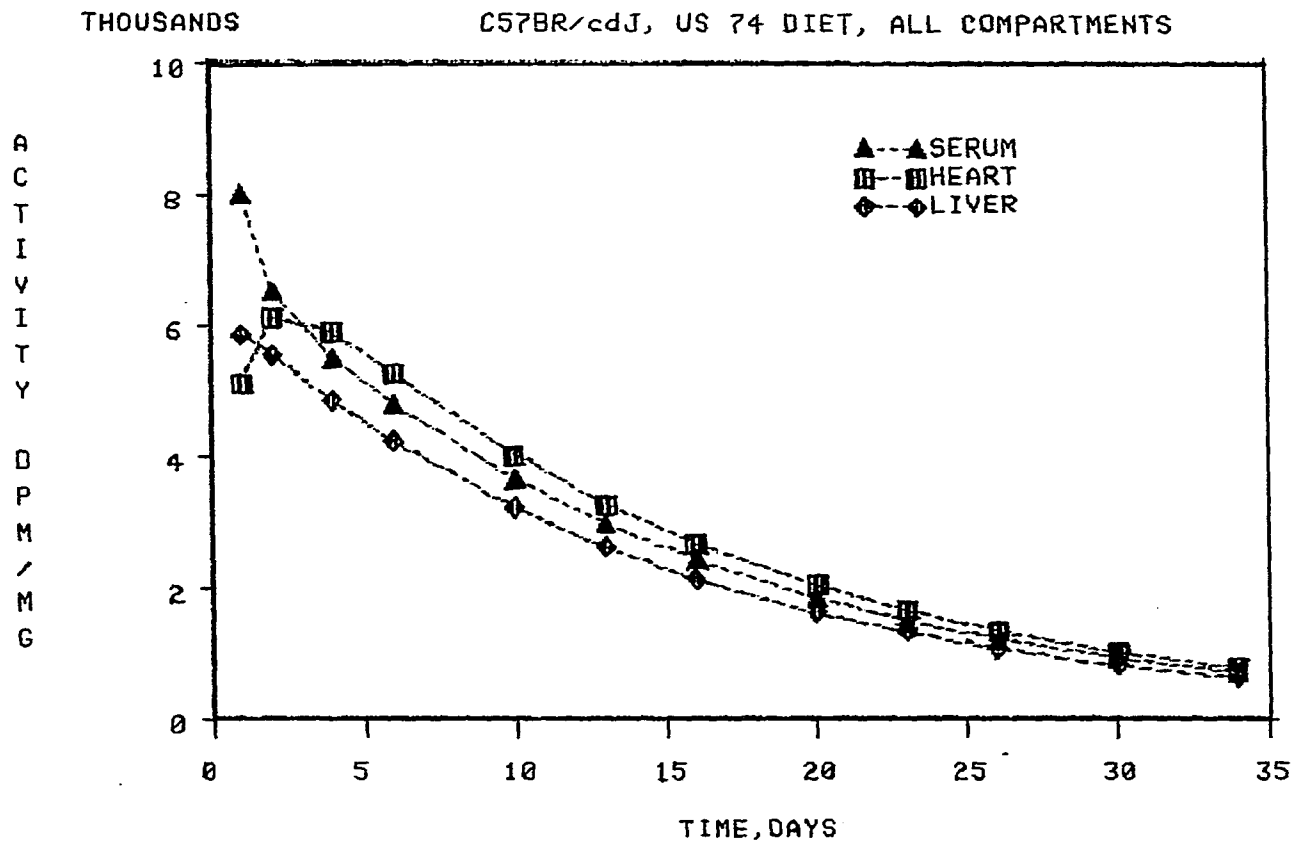
FIGURE 10. Overlay of the computer-generated specific activity time course curves of serum, heart, and liver in C57BR/cdJ mice fed a US74 diet

THOUSANDS   C57BR/cdJ, MODIFIED DIET, ALL COMPARTMENTS
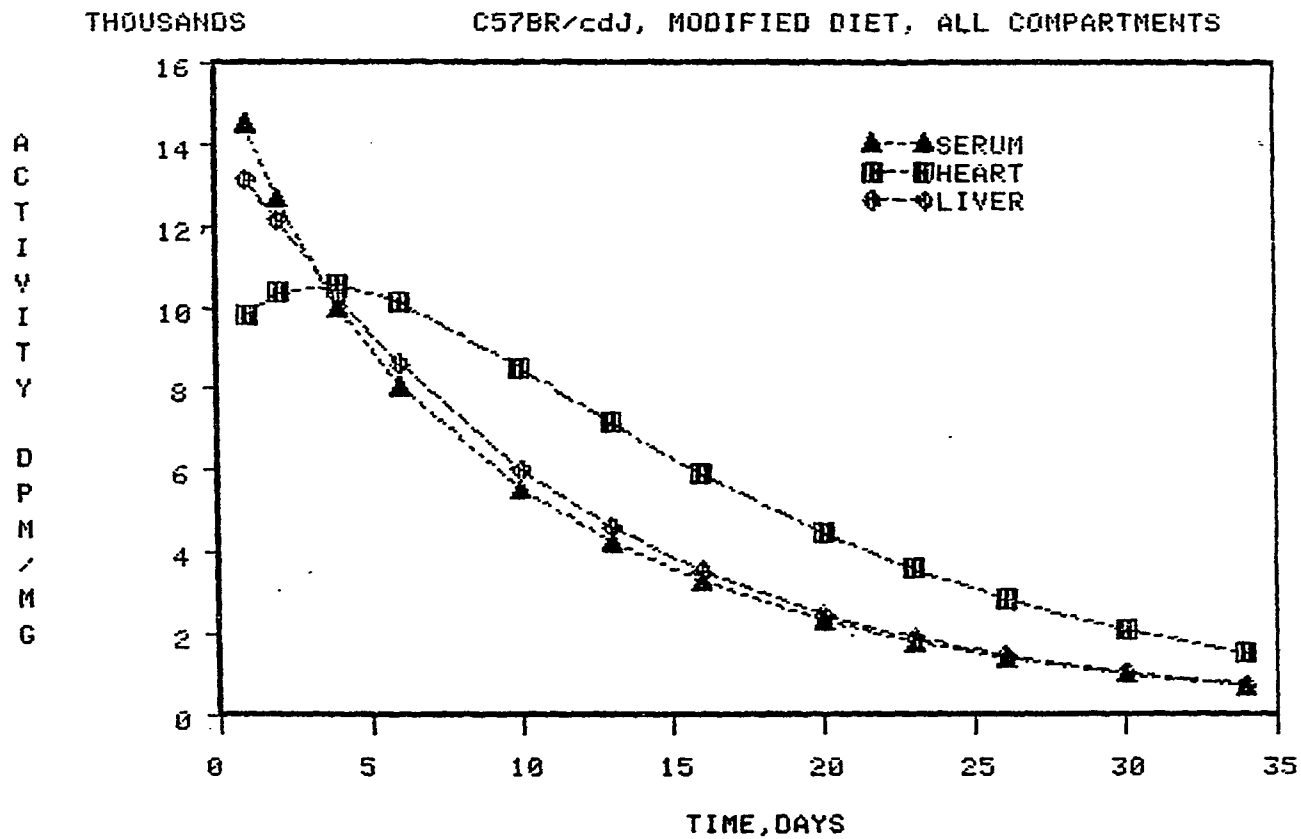
FIGURE 11. Overlay of the computer-generated specific activity time course
curves of serum, heart, and liver in C57BR/cdJ mice fed a
modified fat diet

TABLE 17. Calculated exchange flows of cholesterol between compartments based on a three-compartment model (serum, heart, and liver)

| strain	diet	$R_{HS}(R_{SH})$[a]	R_{SL}[b]	R_{LS}[c]	E_c[d]	f[e]
		mg/h			fraction	
CBA	US74	0.0069	0.011	–	0.059	0.096
CBA	modified	0.0139	0.328	0.166	0.022	0.899
C57BR	US74	0.0077	0.072	0.037	0.050	0.443
C57BR	modified	0.0015	0.017	0.007	0.026	0.084

[a]Transfer rate of cholesterol between heart and serum.

[b]Transfer rate of cholesterol from serum to liver.

[c]Transfer rate of cholesterol from liver to serum.

[d]Excretion rate of cholesterol.

[e]Fraction of cholesterol recycled.

DISCUSSION

The cholesterol mass data showed that CBA/J mice accumulated more cholesterol in the liver and carcass compared to C57BR/cdJ mice. Results are consistent with our previous study (Kuan and Dupont, 1987) showing that CBA/J mice had higher hepatic hydroxymethylglutaryl coenzyme A reductase activity but lower fecal cholesterol excretion than C57BR/cdJ mice. The high capacity of the CBA/J mouse to store cholesterol in the liver and carcass compared with the C57BR/cdJ mouse may be one factor explaining their resistance to diet-induced hypercholesterolemia. On the contrary, C57BR/cdJ mice stored more cholesterol in the heart compared with CBA/J mice. Since atherosclerosis occurs in coronary artery walls, the high accumulation of cholesterol in the heart of the C57BR/cdJ mouse may be one factor predisposing this strain to diet-induced atherosclerosis.

Mice fed the US74 diet had lower initial specific activity than those fed the modified fat diet and CBA/J mice had lower initial specific activity than C57BR/cdJ mice, reflecting a greater dilution of the radioactive cholesterol by unlabeled total cholesterol in the liver. The kinetic results are consistent with the cholesterol mass data which showed that CBA/J mice had more cholesterol in the liver

than C57BR/cdJ mice and mice fed the US74 diet had more cholesterol in the liver than those fed the modified fat diet.

The slope of the curve represents the rate of cholesterol turnover. The greater change in the slope of the serum, liver and heart curves of C57BR/cdJ mice fed the modified fat diet compared to other treatment groups indicates that this group of mice had a faster fractional turnover of cholesterol.

Liver and serum were in rapid balance except in CBA/J mice fed the US74 diet. Study with rats (Oh et al., 1976) demonstrated a rapid balance between serum and liver, but skeletal muscle and kidney had a slow buildup of radioactivity. In this study, mice also showed an initial buildup of radioactivity in the heart, and the radioactivity started to die away after 4 days. The specific activity time course curves of heart in C57BR/cdJ mice fed the US74 diet and in CBA/J mice fed either diet followed a regular precursor-product relationship with serum curves. The C57BR/cdJ mice fed the modified fat diet, however, showed a different result. The heart had higher cholesterol specific activity than did the liver or serum after 4 days, possibly reflecting the presence of two or more different sub-compartments in the heart which were not in rapid balance.

The specific activity in carcass (tissue) was lower than that of other compartments throughout the time course studied. This may suggest that carcass is composed of two cholesterol compartments -- one is in balance with the serum while the other is inaccessible to serum. Further analysis is in progress to incorporate carcass data into the model and to improve the level of confidence in the calculated transfer rates.

BIBLIOGRAPHY

Abraham, S., and M. D. Carroll. 1979. Fats, cholesterol, and sodium intake in the diet of persons 1-74 years: United States. Advance Data, Vital and Health Statistics, No. 54. U. S. Department of Health and Human Services, Washington, D. C.

Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat-liver microsomes. Eur. J. Biochem. 34:77-83.

Carlson, S. E., and S. Goldfarb. 1977. A sensitive enzymatic method for determination of free and esterified tissue cholesterol. Clinica Chim. Acta 79:575-582.

Dupont, J. 1982. Cholesterol balance and whole body kinetics. Pages 117-144 in J. Dupont, ed. Cholesterol systems in insects and animals. CRC Press, Boca Raton, Florida.

Dupont, J., A. W. Nelson, and D. J. Clow. 1982. Cholesterol kinetics in control and hypercholesterolemic foxhounds. Artery 11:15-32.

Dupont, J., S. Y. Oh, L. O'Deen, and M. A. McClellan. 1974. Cholesterol and bile acid turnover in miniature swine. Lipids 9:712-721.

Dupont, J., M. M. Mathias, A. A. Spindler, and P. Janson. 1980. Dietary fat saturation and cholesterol turnover in aging rats. Age 13:19-23.

Fanelli, M. T., and M. L. Kaplan. 1978. Effects of high fat and high carbohydrate diets on the body composition and oxygen consumption of ob/ob mice. J. Nutr. 108:1491-1500.

Goodman, D. S., and R. P. Noble. 1968. Turnover of plasma cholesterol in man. J Clin. Invest. 47:231-241.

Goodman, D. S., R. P. Noble, and R. B. Dell. 1973. Three-pool model of the long-term turnover of plasma cholesterol in man. J. Lipid Res. 14:178-188.

Kuan, S. I., and J. Dupont. 1987. Dietary fat effects on cholesterol metabolism in CBA/J and C57BR/cdJ mice. J. Nutr. (Submitted)

Mitropoulos, K. A., N. B. Myant, G. F. Gibbons, S. Balasubramaniam, and E. A. Reeves. 1974. Cholesterol precursor pools for the synthesis of cholic and chenodeoxycholic acids in rats. J. Biol. Chem. 249:6052-6056.

Oh, S. Y., J. Dupont, and D. J. Clow. 1976. Kinetic analysis of cholesterol turnover in rat tissues. Steroids 27:637-655.

Roberts, A., and J. S. Thompson. 1975. Inbred mice and their hybrids as an animal model for atherosclerosis research. Pages 313-327 in C. E. Day, ed. Advances in experimental medicine and biology. Atherosclerosis drug discovery. Vol. 67. Plenum Press, New York.

Roberts, A., and J. S. Thompson. 1977. Genetic factors in the development of atheroma and on serum total cholesterol levels in inbred mice and their hybrids. Prog. Biochem. Pharmacol. 14:298-305.

Schwartz, C. C., M. Berman, Z. R. Vlahcevic, L. G. Halloran, D. H. Gregory, and L. Swell. 1978. Multicompartmental analysis of cholesterol metabolism in man. J. Clin. Invest. 61:408-423.

DISCUSSION

The activity of cholesterol 7α-hydroxylase (COH) in liver microsomes is usually determined by measuring the percentage conversion of radioactive cholesterol into radioactive 7α-hydroxycholesterol. Since liver microsomes contain appreciable amounts of cholesterol, the radioisotope method is influenced by the mixing of the exogenous radioactive cholesterol and the endogenous cholesterol present in the liver microsome. It is known that the added radioactive cholesterol mixes with the endogenous cholesterol to different extents, depending on the way in which it is added (Mitropoulos and Balasubramaniam 1972). Furthermore, Balasubramaniam et al. (1973) and Bjorkhem and Danielsson (1975) have presented evidence for the compartmentation of cholesterol in rat liver microsomes. They reported that not more than 70% of the cholesterol in rat liver microsomes is immediately accessible to COH. So, a better assay for COH would be one using endogenous cholesterol as the substrate. Bjorkhem and Danielsson (1974) described a combined gas chromatography-mass spectrometry (GC-MS) method to measure 7α-hydroxycholesterol formed from endogenous cholesterol. Sanghvi et al. (1978) simplified the procedure and used it to measure oxygenated derivatives of cholesterol. Galli-Kienle et al. (1984)

applied the method to assaying the activities of hydroxymethylglutaryl coenzyme A reductase and COH.

In order to avoid the problems related to the equilibration between the exogenous and endogenous cholesterol, we tried to adopt the GC-MS method to determine the activity of COH in our samples. Trials using standards indicated that cholesterol and the internal standard 5a-cholestane gave good results with GC (Bendix 2500 gas chromatograph equipped with a flame ionization detector and a 6 ft x 3 mm ID, 6 mm OD glass column packed with 4% SE30/6% QF1, 100-120 mesh; instrument settings were: column temperature 230° and 250°C, and nitrogen flow 80 ml/min). MS analysis with solid or desorption probe at 280°C, or GC-MS (Finigan model 2400 with model 2500 source, and TEK-N-VENT model 3000 data system) gave nice spectra. However, problems arose during the determination of 7a-hydroxycholesterol concentration. It could be detected with GC or MS alone, but not GC-MS. Results are shown in Table 18. A number of manipulations were made, including changing the column (3% OV101, 1% OV17, 3% OV17, and 4% SE30/6% QF1) and column temperature (210°, 230°, and 250°C), and preparing different derivatives (trimethylsilyl (TMS) ether and ethyl diazoethane ether; because the TMS derivatives plugged the GC detector after 1-2 samples,

partition was performed to get a cleaner sample).
7a-Hydroxycholesterol still did not show up in GC-MS. We
found that this compound was degraded in the GC column on
MS. Another alternative was to use a capillary or Megabore
column instead of a packed column. Because of the
anticipated lengthy time involved, we turned to search for a
simpler method.

Thin layer chromatography (TLC) for separation,
densitometer for quantitation and MS for identification
seemed to be a suitable compromise. 7a-Hydroxycholesterol
was separated from cholesterol by using TLC plates with a
fluorescent indicator. When viewing under a UV lamp, one
could easily tell which sample had COH activity as
7a-hydroxycholesterol was seen only in samples after
incubation. However, the amount of 7a-hydroxycholesterol
formed, thus the fluorescence was too small to be
quantitated accurately by the densitometry used (Kontes
Scanner model 800). The Rf values of cholesterol and its
oxygenated derivatives are shown in Table 19. We also tried
to determine the intensity of the color formed after
charring the sulfuric acid sprayed plate of a mouse liver
microsome extract. The color was barely visible. So, this
TLC-dens, ,ometry method did not seem to be a suitable method
to detect the small amount of 7a-hydroxycholesterol in the
samples.

TABLE 18. Retention times of 5*a*-cholestane, cholesterol and
oxygenated sterols separated by gas
chromatography (GC) or gas chromatography-mass
spectrometry (GC-MS)

	GC[a]		GC-MS[b]	
		min		
5*a*-cholestane	12[c]	5[d]	2.7[e]	2.8[f]
7*a*-hydroxy-cholesterol	26	11	no peak	no peak
cholesterol	29	11	8	7.7
7*β*-hydroxy-cholesterol	36	14.6	no data	no data
7-keto-cholesterol	no data	27.1	no data	no data

[a]Bendix 2500 gas chromatograph equipped with a flame
ionization detector; 6 ft x 3 mm ID, 6 mm OD glass column
packed with 4% SE30/6% QF1, 100-120 mesh; nitrogen flow 80
ml/min; trimethylsilyl derivatives were used.

[b]GC-MS Finigan model 2400 with model 2500 source, and a
TEK-N-VENT model 3000 data system; column temperature 250°C;
6 ft x 3 mm ID glass column packed with 4% SE30/6% QF1.

[c]Column temperature 230°C.

[d]Column temperature 250°C.

[e]Trimethylsilyl derivatives after partition with
methylene chloride.

[f]Ethyl diazoethane derivatives.

For time's sake, further pursuit on methodology was given up and the original radioisotope method was used. Balasubramaniam et al. (1973) reported that Tween 80 was better than acetone to bring radioactive cholesterol into the substrate pool. So, Tween 80 was used to solubilize radioactive cholesterol in this experiment to maximize the equilibration between exogenous and endogenous cholesterol.

Future studies on measuring COH activity using endogenous cholesterol as the substrate shall focus on the GC-MS method. Our trial with a Megabore column (Megabore DB-5 from J and W Scientific, 15 m x 0.53 mm ID, column temperature 180°C, nitrogen flow 10 ml/min) showed that 7a-hydroxycholesterol could be detected though the sensitivity was low (retention time=3 min, 1% total ion current for 0.8 μg). The advantage of using a Megabore column is that compounds can be eluted faster than with capillary column. Increasing the sensitivity using a Megabore column should be suitable for determining COH activity from endogenous substrate by analyzing for 7a-hydroxycholesterol and cholesterol concentrations.

Levels of plasma cholesterol usually seen in Western industrialized countries are inappropriately high. Diet and heredity are two major factors. A diet rich in saturated fat and cholesterol is known to elevate the plasma

TABLE 19. Rf values of cholesterol, oxygenated sterols and zearalenone separated by thin layer chromatography

	Rf^a
7α-hydroxy-cholesterol	0.19
7β-hydroxy-cholesterol	0.22
7-keto-cholesterol	0.28
cholesterol	0.50
zearalenone	0.55
cholesteryl ester	0.76

[a]Compounds were separated on a silica gel TLC plate (Fisher Scientific, 20 cm x 20 cm, 0.25 mm thickness) using ethyl acetate : benzene (3/2, v/v) as the developing solvent in a filter-paper lined saturated tank. The TLC plate had been activated in a 100°C oven for 1 hr.

cholesterol level, but the level does not rise equally in every person. Brown and Goldstein (1986) developed a model to explain the dietary effect on blood cholesterol. In this model, the major factor determining the plasma LDL-cholesterol concentration is the number and saturation of hepatic LDL receptors. When a diet rich in saturated fat and cholesterol is consumed, excess dietary cholesterol accumulates in the liver which results in reduced production of LDL receptors and increased production of VLDL and IDL. Only a small part of the IDL is removed by LDL receptors owing to the decreased number of LDL receptors. The remainder is converted to LDL. For the same reason, the removal rate of LDL also decreases. As a result of the combined effects of increased production and decreased removal of LDL, the plasma LDL cholesterol level increases.

The individual differences in susceptibility to diet-induced elevation of blood cholesterol suggest that genetics plays a role as well. These studies demonstrated the metabolic differences between CBA/J and C57BR/cdJ mice in the responses to diet. Such differences are consistent with results of animal and human studies on cholesterol metabolism in hypo and hyperresponders to dietary fat. CBA/J mice responded to a high-fat diet by suppressing hepatic cholesterol synthesis while C57BR/cdJ mice increased

fecal cholesterol excretion. No elevation of serum cholesterol was detected in this experiment, not even in the purported susceptible strain, C57BR/cdJ, because of the efficient compensatory mechanisms. However, Roberts and Thompson (1975, 1977) reported a large elevation of blood cholesterol in C57BR/cdJ mice and only a slight elevation in CBA/J mice when they were fed an extremely high saturated fat and cholesterol diet. Walker (1984) found that C57BR/cdJ mice were more responsive than CBA/J mice to a moderate level of dietary fat after 11 wk of feedings of 16- to 18-wk-old mice.

Based on Brown and Goldstein's model, the genetic susceptibility to diet-induced elevation of blood cholesterol can probably be explained by the number of LDL receptors in the liver. In dogs the production of hepatic LDL receptors has been shown to decrease with age (Goldstein et al., 1983). The different results of this study and Walker's study can probably be explained by aging effects on LDL receptors. The C57BR/cdJ mouse may have an efficient LDL receptor activity when young, thus showing a lower serum cholesterol concentration than the CBA/J mouse in the present study. However, the LDL receptors in the C57BR/cdJ mouse may decrease in number much faster than in the CBA/J mouse during the aging process so that the C57BR/cdJ mouse

responds to dietary fat less efficiently than the CBA/J mouse when older. That may be why the C57BR/cdJ mouse was more responsive to the diet than the CBA/J mouse in Walker's study. This model can explain very well the diet and age effects on blood cholesterol level. However, it cannot explain the metabolic differences between these two strains of mice.

Modification of the Brown and Goldstein model is proposed to explain such differences. Like the Brown and Goldstein model, liver is considered a major organ in the control of blood LDL in this model. Its importance is reflected by the fact that most LDL is removed from the plasma by the liver and more than 90% of this clearance process is mediated by LDL receptors (Dietschy, 1984). Two types of receptors are present in the liver -- remnant receptor and LDL receptor. Both receptors recognize apoprotein E. The remnant receptor binds only exogenous lipoproteins (chylomicron remnant), but the LDL receptor binds endogenous lipoproteins (IDL and LDL) as well as exogenous lipoproteins (Brown and Goldstein, 1985). This model proposes that the C57BR/cdJ mouse differs from the CBA/J mouse in the hepatic remnant and LDL receptors -- it has either defective or less remnant receptors, but more LDL receptors.

When a low-fat diet is fed to the CBA/J mouse, chylomicron remnants generated are removed efficiently by remnant receptors and the small amount of IDL and LDL are taken up by LDL receptors, leaving a very low level of LDL in the blood. At ordinary intakes of dietary fat, the major factor that limits the removal of LDL from blood is saturation of the LDL receptor. When a high-fat diet is fed, more VLDL is formed, resulting in more IDL and LDL. LDL receptors become saturated. The cholesterol derived from internalized LDL through the LDL receptor has the properties of suppressing the HMGR activity, down-regulating the LDL receptor and activating the ACAT (Brown and Goldstein, 1979). So, the saturation and suppression of hepatic LDL receptor contributes to the build-up of LDL in plasma.

When the C57BR/cdJ mouse is fed a low-fat diet, the small amount of chylomicron remnants can either be removed by the remnant receptor alone or by a combination of remnant and LDL receptors. Because the C57BR/cdJ mouse has a lower blood LDL level, it must have higher LDL receptor activity than the CBA/J mouse. This model also explains the greater response of the C57BR/cdJ mouse to a high-fat diet compared to the CBA/J mouse. When a high-fat diet is fed, the remnant receptors cannot cope with the large amount of

chylomicron remnants, and some of the remnants compete with IDL and LDL for LDL receptors. Since chylomicron remnants have both apo E and apo B-48 while LDL has apo B-100 only, the LDL receptor has higher affinity towards chylomicron remnants and the chylomicron remnant is the winner in the competition. The high-fat diet also causes increased secretion of VLDL thus IDL and LDL to the blood. As a result of the saturation and suppression of hepatic LDL receptors, increased production of LDL, and the strong competition from chylomicron remnants for LDL receptors, LDL accumulates to a very high level.

This model predicts that the diet-induced elevation of blood cholesterol is in the LDL fraction. Unlike humans, the major lipoprotein in mice is HDL. Breckenridge et al. (1985) reported that for both CBA/J and C57BR/cdJ mice fed a commercial laboratory diet, over 90% of the plasma total cholesterol was in the HDL fraction, with extremely low values in the VLDL and LDL fractions. The present results confirm their findings. Despite this fact, the model is applicable to the CBA/J and C57BR/cdJ mice since for both strains, the atherogenic diet-induced increase in plasma cholesterol is in the VLDL, IDL and LDL fractions (Breckenridge et al., 1985; Morrisett et al., 1982).

This model can also explain the different cholesterol synthesis and excretion rates and cholesterol accumulation in the liver between the two strains of mice. Suppose that the HMGR activity and cholesterol excretion are controlled by the cholesterol internalized through the LDL receptors and the cholesterol accumulation is more affected by the remnant receptors. When a moderate-fat diet is fed, both strains store little cholesterol in the liver. Because only a very small amount of cholesterol enters the liver through LDL receptors in the CBA/J mouse, the CBA/J mouse has high hepatic HMGR activity and low excretion of cholesterol in the feces. The C57BR/cdJ mouse, however, has more cholesterol entering through LDL receptors because of more LDL receptors and the processing of chylomicron remnants in addition to LDL. Consequently, the C57BR/cdJ mouse has suppressed hepatic HMGR activity. When a high-fat diet is fed, the CBA/J mouse accumulates a large amount of cholesterol in the liver due to the efficient remnant receptor system and its nondown-regulation property. An appreciable amount of cholesterol also enters the liver through the LDL receptor, leading to the suppression of HMGR activity and increased cholesterol excretion. The C57BR/cdJ mouse also accumulates an increased amount of cholesterol in the liver compared to when a moderate-fat diet is fed, but

the level is much less than the CBA/J mouse because of the
defective or inefficient remnant receptor system and the
down-regulation of the LDL receptor. Again, the high level
of cholesterol which enters the liver through the LDL
receptor further suppresses the HMGR activity and increases
the cholesterol excretion. The aging effect on cholesterol
metabolism is interpreted as a decreased number of LDL
receptors.

With present knowledge one cannot judge which model is
correct or if both are wrong. The confirmation or
modification of the proposed models or the establishment of
a new model shall await future research. It is also
uncertain whether this model is applicable to humans and
whether the mouse which differs so much in its lipoprotein
profile from the human is the best animal model to study
cholesterol metabolism in comparison to the human. However,
judging from present knowledge, it seems that the responses
to diet in mice are similar to those of humans and that the
CBA/J and C57BR/cdJ mice are good models to study
cholesterol metabolism in hypo- and hyperresponders. All
patients of familial hypercholesterolemia show evidence of
defects in the LDL receptors, but not all defects are the
same (Brown and Goldstein, 1986). It is likely that the
causes of diet-induced hypercholesterolemia are also

multiple and the proposed defect in this report is just one of them.

Evidence has shown that there are indiviual variations in susceptibility to diet-induced hypercholesterolemia. The question arises whether dietary recommendations to hyperresponders should be different from those to hyporesponders. A Consensus Conference (1985) on "Lowering blood cholesterol to prevent heart disease" sponsored by the National Institutes of Health recommended that all Americans above 2 years old should reduce dietary fat to less than 30% of total calories, increase polyunsaturated fat to 10% of calories, and reduce dietary cholesterol to 250-300 mg or less per day. Alfin-Slater (1987) commented that these recommendations could translate into a diet very limited in animal protein and other associated nutrients, and an increase in cereal grains at the expense of animal products might result in a decrease of some protective micronutrients such as vitamins and minerals and thereby pose health risks to children. Furthermore, a low-fat, high-carbohydrate diet may be harmful to some old or carbohydrate intolerant people (Reaven, 1986). Nevertheless, the recommendations for change are quite moderate and unlikely to result in such large effects unless individuals over-respond to the diet. When these possible harmful effects are taken into

consideration, should all Americans follow the recommendations? Should the recommendations be given to hyperresponders only? Can the hyporesponders be free from such dietary modifications? These questions still remain unanswered. Judging from the serum cholesterol level only, it seems that a prudent diet is neither necessary nor beneficial for the hyporesponders. However, this study shows that hyporesponding mice fed a high-fat diet store much cholesterol in the liver. Will it cause damage to the liver? One difficulty in dietary recommendations is that we have to consider the general health of a person. A diet good for a certain organ, however, may not be good for other organs. Recommendations on severe dietary changes to all Americans seem premature at this time. Until more research has been done, the best advice is moderate consumption of dietary fat and choices from a variety of foods. The present study demonstrates that there exist metabolic differences between the CBA/J and C57BR/cdJ mice in the response to diet, and such differences are consistent with the human data (Nestel and Poyser, 1976). This suggests that the CBA/J and C57BR/cdJ mice provide a good animal model for future investigations of dietary effects and may lead to answers to the above questions.

BIBLIOGRAPHY

AHA Committee Report. 1978. Diet and coronary heart disease. Circulation 58:762A-766A.

Alfin-Slater, R. B. 1987. Dietary fat and cholesterol. Nutr. & M.D. 13:4.

Anderson, K. M., W. P. Castelli, and D. Levy. 1987. Cholesterol and Mortality - 30 years of follow-up from the Framingham study. J. Am. Med. Assoc. 257:2176-2180.

Armstrong, B. K., J. I. Mann, A. M. Adelstein, and F. Eskin. 1975. Commodity consumption and ischemic heart disease mortality with special reference to dietary practices. J. Chron. Dis. 28:455-465.

Aubert, R., D. Perdereau, M. Roubiscoul, J. Herzog, and D. Lemonnier. 1987. Genetic variations of serum lipid levels in inbred mice and response to hypercholesterolemic diet. Lipids. (in press)

Avigan, J., C. D. Williams, and J. P. Blass. 1970. Regulation of sterol synthesis in human skin fibroblast cultures. Biochim. Biophys. Acta 218:381-384.

Baker, H. N., D. A. Eggen, G. W. Melchior, P. S. Roheim, G. T. Malcom, and J. P. Strong. 1983. Lipoprotein profiles in rhesus monkeys with divergent responses to dietary cholesterol. Arteriosclerosis 3:223-232.

Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat-liver microsomes. Eur. J. Biochem. 34:77-83.

Beynen, A. C., C. E. West, L. F. M. vanZutphen, and M. B. Katan. 1986a. Relation between the responses of serum cholesterol to dietary cholesterol and to the type of dietary fat in random-bred rabbits. Nutr. Rep. Intl. 33:71-78.

Beynen, A. C., C. E. West, M. B. Katan, and L. F. M. vanZutphen. 1986b. Hyperresponsiveness of plasma cholesterol to dietary cholesterol, saturated fatty acids and casein in inbred rabbits. Nutr. Rep. Intl. 33:65-70.

Beynen, A. C., J. J. Visser, J. A. Schouten, and M. B. Katan. 1987. Cholesterol metabolism in rabbits fed diets containing either corn oil or olive oil. Nutr. Rep. Intl. 35:111-116.

Bhattacharyya, A. K., and D. A. Eggen. 1980. Cholesterol absorption and turnover in rhesus monkeys as measured by two methods. J. Lipid Res. 21:518-524.

Bhattacharyya, A. K., and D. A. Eggen. 1981. Feedback regulation of cholesterol biosynthesis in rhesus monkeys with variable hypercholesterolemic response to dietary cholesterol. J. Lipid Res. 22:16-23.

Bjorkhem, I., and H. Danielsson. 1974. Assay of liver microsomal cholesterol 7a-hydroxylase using deuterated carrier and gas chromatography-mass spectrometry. Anal. Biochem. 59:508-516.

Bjorkhem, I., and H. Danielsson. 1975. 7a-hydroxylation of exogenous and endogenous cholesterol in rat-liver microsomes. Eur. J. Biochem. 53:63-70.

Blaton, V., M. DeBuyzere, B. Declercq, A. Pracetyo, G. Vanderkelen, J. Delanghe, and J. Spincemaille. 1984. Effect of polyunsaturated isocaloric fat diets on plasma lipids, apolipoproteins and fatty acids. Atherosclerosis 53:9-20.

Breckenridge, W. C., A. Roberts, and A. Kuksis. 1985. Lipoprotein levels in genetically selected mice with increased susceptibility to atherosclerosis. Arteriosclerosis 5:256-264.

Brown, M. S., and J. L. Goldstein. 1979. Receptor-mediated endocytosis: Insights from the lipoprotein receptor system. Proc. Natl. Acad. Sci. USA 76:3330-3337.

Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein receptors in the liver: Control signals for plasma cholesterol traffic. J. Clin. Invest. 72:743-747.

Brown, M. S., and J. L. Goldstein. 1985. The receptor model for transport of cholesterol in plasma. Pages 178-182 in K. T. Lee, ed. Atherosclerosis. Annals of the New York Academy of Sciences, New York.

Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. Science 232:34-47.

Brown, M. S., S. E. Dana, and J. L. Goldstein. 1973.
Regulation of 3-hydroxy-3-methylglutaryl coenzyme A
reductase activity in human fibroblasts by lipoproteins.
Proc. Natl. Acad. Sci. USA 70:2162-2166.

Brown, M. S., J. L. Goldstein, and J. M. Dietschy. 1979.
Active and inactive forms of 3-hydroxy-3-methylglutaryl
coenzyme A reductase in the liver of the rat. J. Biol.
Chem. 254:5144-5149.

Brown, W. V., and W. Karmally. 1985. Coronary heart
disease and the consumption of diets high in wheat and
other grains. Am. J. Clin. Nutr. 41:1163-1171.

Bruell, J. H. 1963. Additive inheritance of serum
cholesterol level in mice. Science 142:1664-1666.

Clarkson, T. B., J. R. Kaplan, and M. R. Adams. 1985. The
role of individual differences in lipoprotein, artery
wall, gender, and behavioral responses in the development
of atherosclerosis. Pages 28-45 in K. T. Lee, ed.
Atherosclerosis. Annals of the New York Academy of
Sciences, New York.

Clarkson, T. B., H. B. Lofland, B. C. Bullock, and H. O.
Goodman. 1971. Genetic control of plasma cholesterol:
Studies on squirrel monkeys. Arch. Pathol. 92:37-45.

Clow, D. J., J. Dupont, A. B. Butterfield, W. V. Lumb, L. A.
O'Deen, S. Y. Oh, and T. A. Mahoney. 1987. Dietary fat
and kinetics of cholesterol metabolism in miniature
swine. Pages 821-838 in M. E. Tumbleson, ed. Swine in
biomedical research. Vol. 1. Plenum Press, New York.

Connor, W. E., and D. S. Lin. 1974. The intestinal
absorption of dietary cholesterol by hypercholesterolemic
(Type II) and normocholesterolemic humans. J. Clin.
Invest. 53:1062-1070.

Consensus Conference. 1985. Lowering blood cholesterol to
prevent heart disease. J. Am. Med. Assoc.
253(14):2080-2086.

Dietschy, J. M. Regulation of cholesterol metabolism in man
and in other species. 1984. Klin. Wochenschr.
62:338-345.

Dolecek, T. A., N. C. Milas, L. V. V. Horn, M. E. Farrand, D. D. Gorder, A. G. Duchene, J. R. Dyer, P. A. Stone, and B. L. Randall. 1986. A long-term nutrition intervention experience: Lipid responses and dietary adherence patterns in the Multiple Risk Factor Intervention Trial. J. Am. Diet. Assoc. 86:752-758.

Dupont, J. 1966. Synthesis of cholesterol and total lipid by male and female rats fed beef tallow or corn oil. Lipids 1:409-414.

Dupont, J. 1982. Cholesterol balance and whole body kinetics. Pages 117-144 in J. Dupont, ed. Cholesterol systems in insects and animals. CRC Press, Florida.

Dyerberg, J., and H. O. Bang. 1982. A hypothesis on the development of acute myocardial infarction in Greenlanders. Scand. J. Clin. Lab. Invest. 42:7-13.

Eggen, D. A. 1976. Cholesterol metabolism in groups of rhesus monkeys with high or low response of serum cholesterol to an atherogenic diet. J. Lipid Res. 17:663-673.

Farinaro, E., J. Stamler, M. Upton. 1977. Plasma glucose levels: Long term effects of diet in the Chicago Coronary Prevention Evaluation Program. Ann. Internal Med. 86:146-154.

Field, F. J., E. J. Albright, and S. N. Mathur. 1987. Effect of dietary n-3 fatty acids on HMG-CoA reductase and ACAT activities in liver and intestine of the rabbit. J. Lipid Res. 28:50-58.

Flow, B. L., and G. E. Mott. 1982. Genetic mediation of cholesterol metabolism in the baboon (Papio cynocephalus). Atherosclerosis 41:403-414.

Flow, B. L., and G. E. Mott. 1984. Relationship of high density lipoprotein cholesterol to cholesterol metabolism in the baboon (Papio sp.). J. Lipid Res. 25:469-473.

Flow, B. L., T. C. Cartwright, T. J. Kuehl, G. E. Mott, D. D. C. Kraemer, A. W. Kruski, J. D. Williams, and H. C. McGill. 1981. Genetic effects on serum cholesterol concentrations in baboons. J. Hered. 72:97-103.

Fogelman, A. M., J. Edmond, J. Seager, and G. Popjak. 1975. Abnormal induction of 3-hydroxy-3-methylglutaryl coenzyme A reductase in leukocytes from subjects with heterozygous familial hypercholesterolemia. J. Biol. Chem. 250:2045-2055.

Galli-Kienle, M., G. Galli, E. Bosisio, G. Cighetti, and E. Paoletti. 1984. Evaluation of enzyme activities by gas chromatography-mass spectrometry: HMG-CoA reductase and cholesterol 7a-hydroxylase. J. Chromatogr. 289:267-276.

Gill, J. F., Jr., P. J. Kennelly, and V. W. Rodwell. 1985. Control mechanisms in sterol uptake and biosynthesis. Pages 41-72 in H. Danielsson and J. Sjorvall, eds. Sterols and bile acids. New comprehensive biochemistry. Vol. 12. Elsevier Science, Amsterdam.

Ginsberg, H., N-A. Le, C. Mays, J. Gibson, and W. V. Brown. 1981. Lipoprotein metabolism in nonresponders to increased dietary cholesterol. Arteriosclerosis 1:463-470.

Glueck, C. J. 1979a. Appraisal of dietary fat as a causative factor in atherogenesis. Am. J. Clin. Nutr. 32:2637-2643.

Glueck, C. J. 1979b. Dietary fat and atherosclerosis. Am. J. Clin. Nutr. 32:2703-2711.

Goldstein, J. L., T. Kita, and M. S. Brown. 1983. Defective lipoprotein receptors and atherosclerosis: Lessons from an animal counterpart of familial hypercholesterolemia. N. Eng. J. Med. 308:288-296.

Goodman, D. S., R. P. Noble, and R. B. Dell. 1973. Three-pool model of the long-term turnover of plasma cholesterol in man. J. Lipid Res. 14:178-188.

Goswami, S., and J. Dupont. 1982. Catabolism and bile acid metabolism. pages 97-116 in J. Dupont, ed. Cholesterol systems in insects and animals. CRC Press, Florida.

Grundy, S. M. 1986. Comparsion of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N. Eng. J. Med. 314:745-748.

Haug, A., A. T. Hostmark, and O. Spydevold. 1985. Plasma lipoprotein distribution, faecal cholesterol excretion, and activities of lipoprotein lipase, hepatic lipase and lecithin:cholesterol acyltransferase in rats fed diets rich in sucrose or sunflower oil. Acta Physiol. Scand. 125:609-617.

Hennig, B., and J. Dupont. 1983. Lipoprotein lipid and protein responses to dietary fat and diabetes in rats. J. Nutr. 113:1984-1994.

Herold, P. M., and J. E. Kinsella. 1986. Fish oil consumption and decreased risk of cardiovascular disease: A comparison of findings from animal and human feeding trials. Am. J. Clin. Nutr. 43:566-598.

Hjermann, I., K. Velve-Byre, and I. Holme. 1981. Effect of diet and smoking intervention on the incidence of coronary heart disease. Lancet 2:1303-1310.

Hodgson, P. A., R. D. Ellefson, L. R. Elveback, L. E. Harris, R. A. Nelson, and W. H. Weidman. 1976. Comparison of serum cholesterol in children fed high, moderate, or low cholesterol milk diets during neonatal period. Metabolism 25:739-746.

Huang, Y. S., R. Takahashi, J. Mitchell, and D. F. Horrobin. 1986. Effects of polyunsaturated fats on plasma and liver cholesteryl ester composition in hypercholesterolemic rat. Nutr. Rep. Intl. 34:635-644.

Hulcher, F. H., and R. D. Margolis. 1982. Rate-limiting, diurnal activity of hepatic microsomal cholesterol 7a-hydroxylase in pigeons with high serum cholesterol. Biochim. Biophys. Acta 712:242-249.

Hulcher, F. H., M. J. Steel, and W. Payne. 1976. Regulation and circadian rhythm of the control enzymes of cholesterol metabolism in hyper and hyporesponding pigeons. Circulation 53 and 54(Suppl.II):178. (Abstr.)

Jones, D. C., H. B. Lofland, T. B. Clarkson, and R. W. St. Clair. 1975. Plasma cholesterol concentrations in squirrel monkeys as influenced by diet and phenotype. J. Food Sci. 40:2-7.

Kagan, A., B. R. Harris, and W. Winkelstein, Jr. 1974. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: Demographic, physical, dietary and biochemical characteristics. J. Chron. Dis. 27:345-364.

Keys, A. 1970. Coronary heart disease in seven countries. Circulation 41(Suppl.I):1-211.

Kimura, N. 1967. A farming and a fishing village in Japan: Tanushimaru and Ushibuka. Acta Med. Scand. 460(Suppl.):231-249.

Knapp, H. R., I. A. G. Reilly, P. Alessandrini, and G. A. FitzGerald. 1986. In vivo indexes of platelet and vascular function during fish-oil administration in patients and atherosclerosis. N. Eng. J. Med. 314:937-942.

Kraemer, F. B., M. Greenfield, T. A. Tobey, and G. M. Reaven. 1982. Effects of moderate increases in dietary polyunsaturated:saturated fat on plasma triglyceride and cholesterol levels in man. Br. J. Nutr. 47:259-266.

Kronmal, R. A. 1985. Commentary on the published results of the Lipid Research Clinics Coronary Primary Prevention Trial. J. Am. Med. Assoc. 253:2091-2093.

Levy, R. I. 1981. Declining mortality in coronary heart disease. Arteriosclerosis 1:312-325.

Levy, R. I. 1984. Causes of the decrease in cardiovascular mortality. Am. J. Cardiol. 54:7c-13c.

Lim, H. S., S. H. Oh, and I. K. Han. 1985. Effects of dietary polyunsaturated and saturated (P/S) fat ratio on plasma cholesterol and lipoprotein in rats. Korean J. Anim. Sci. 27:535-539.

The Lipid Research Clinics Coronary Primary Prevention Trial Results. 1984a. I. Reduction in incidence of coronary heart disease. J. Am. Med. Assoc. 251:351-364.

The Lipid Research Clinics Coronary Primary Prevention Trial Results. 1984b. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J. Am. Med. Assoc. 251:365-374.

Lofland, H. B. Jr., T. B. Clarkson, R. W. St. Clair, and N. D. M. Lehner. 1972. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. J. Lipid Res. 13:39-47.

Lopez-S, A., W. A. Krehl, R. E. Hodges, and E. I. Good. 1966. Relationship between food consumption and mortality from atherosclerotic heart disease in Europe. Am. J. Clin. Nutr. 19:361-369.

Marson, L. A., and M. T. Clandinin. 1985. Dietary linoleic acid modulates liver plasma membrane unsaturated fatty acid composition, phosphatidylcholine and cholesterol content, as well as glucagon stimulated adenylate cyclase activity. Nutr. Res. 5:1113-1120.

Masi, I., E. Giani, C. Galli, E. Tremoli, and C. R. Sirtori. 1986. Diets rich in saturated, monounsaturated and polyunsaturated fatty acids differently affect plasma lipids, platelet and arterial wall eicosanoids in rabbits. Ann. Nutr. Metab. 30:66-72.

Mattson, F. M., and S. M. Grundy. 1983. Effects of monounsaturated fatty acids on lipoprotein levels in man. Arteriosclerosis 3:482a. (Abstr.)

Mattson, F. H., B. A. Erickson, and A. M. Kligman. 1972. Effect of dietary cholesterol on serum cholesterol in man. Am. J. Clin. Nutr. 25:589-594.

McGill, H. C. 1979. The relationship of dietary cholesterol to serum cholesterol concentration and to atherosclerosis in man. Am. J. Clin. Nutr. 32:2664-2702.

McGill, H. C., C. A. McMahan, and J. D. Wene. 1981. Unresolved problems in the diet-heart issue. Arteriosclerosis 1:164-176.

Mitropoulos, K. A., and S. Balasubramaniam. 1972. Cholesterol 7α-hydroxylase in rat liver microsomal preparations. Biochem. J. 128:1-9.

Morris, J. N., J. W. Marr, J. A. Heady, G. L. Mills, and T. R. E. Pilkington. 1963. Diet and plasma cholesterol in 99 bank men. Br. Med. J. 1:571-576.

Morrisett, J. D., H-S. Kim, J. R. Patsch, S. K. Datta, and J. J. Trentin. 1982. Genetic susceptibility and resistance to diet-induced atherosclerosis and hyperlipoproteinemia. Arteriosclerosis 2:312-324.

National Center for Health Statistics - National Heart, Lung, and Blood Institute Collaborative Lipid Group. 1987. Trends in serum cholesterol levels among US adults aged 20 to 74 years: Data from the National Health and Nutrition Examination Surveys, 1960 to 1980. J. Am. Med. Assoc. 257:937-942.

Nestel, P. J. 1986. Fish oil attenuates the cholesterol induced rise in lipoprotein cholesterol. Am. J. Clin. Nutr. 43:752-757.

Nestel, P. J., and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metabolism 25:1591-1599.

Norum, K. R., and C. A. Drevon. 1986. Dietary n-3 fatty acids and cardiovascular diseases. Arteriosclerosis 6:352-355.

Oh, S. Y. 1982. Cholesterol transport. Pages 77-95 in J. Dupont, ed. Cholesterol systems in insects and animals. CRC Press, Florida.

Paigen, B., A. Morrow, C. Brandon, D. Mitchell, and P. Holmes. 1985. Variation in susceptibility to atherosclerosis among inbred strains of mice. Atherosclerosis 57:65-73.

Radcliffe, J. D., and K. S. Liebsch. 1985. Dietary induction of hypercholesterolemia and atherosclerosis in Japanese quail of strain SEA. J. Nutr. 115:1154-1161.

Rahimtoola, S. H. 1985. Cholesterol and coronary heart disease: A perspective. J. Am. Med. Assoc. 253:2094-2095.

Reaven, G. M. 1986. Looking at the world through LDL-cholesterol-colored glasses. Am. J. Nutr. 42:1143-1147.

Reiser, R., J. L. Probstfield, A. Silvers, L. W. Scott, M. L. Shorney, R. D. Wood, B. C. O'Brien, A. M. Gotto, Jr., D. Phil, and W. Insull. 1985. Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. Am. J. Clin. Nutr. 42:190-197.

Roberts, A., and J. S. Thompson. 1975. Inbred mice and their hybrids as an animal model for atherosclerosis research. Pages 313-327 in C. E. Day, ed. Advances in experimental medicine and biology. Vol. 67. Plenum Press, New York.

Roberts, A., and J. S. Thompson. 1977. Genetic factors in the development of atheroma and on serum total cholesterol levels in inbred mice and their hybrids. Prog. Biochem. Pharmacol. 14:298-305.

Russell, E. S. 1969. The importance of animal genetics in biomedical research. National Academy of Sciences Publication 1679.

Sacks, F. M., W. P. Castelli, A. Donner, and E. H. Kass. 1975. Plasma lipids and lipoproteins in vegetarians and controls. N. Eng. J. Med. 292:1148-1151.

Sanders, T. A. B., D. R. Sullivan, J. Reeve, and G. R. Thompson. 1985. Triglyceride-lowering effect of marine polyunsaturates in patients with hypertriglyceridaemia. Arteriosclerosis 5:459-465.

Sanghvi, A., M. Galli-Kienle, and G. Galli. 1978. A rapid, sensitive method for simultaneous measurements of tissue levels of oxygenated derivatives of cholesterol. Anal. Biochem. 85:430-436.

Shepherd, J., C. J. Packard, S. M. Grundy, D. Yeshurun, A. M. Gotto, Jr., and O. D. Taunton. 1980. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. J. Lipid Res. 21:91-99.

Shepherd, J., C. J. Packard, and J. R. Patsch. 1978. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apolipoprotein A-I. J. Clin. Invest. 1582-1592.

Simons, L. A., J. C. Gibson, C. Paino, M. Hosking, J. Bullock, and J. Trim. 1978. The influence of a wide range of absorbed cholesterol on plasma cholesterol levels in man. Am. J. Clin. Nutr. 31:1334-1339.

Spady, D. K., D. W. Bilkeimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. Proc. Natl. Acad. Sci. USA 80:3499-3503.

St. Clair, R. W., R. K. Randolph, M. P. Jokinen, T. B. Clarkson, and H. A. Barakat. 1986. Relationship of plasma lipoproteins and the monocyte-macrophage system to atherosclerosis severity in cholesterol-fed pigeons. Arteriosclerosis 6:614-628.

Stamler, J., D. Wentworth, and J. Weaton. 1986. Is the relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? J. Am. Med. Assoc. 256:2823-2828.

Sugano, M., T. Ide, T. Ishida, and K. Yoshida. 1986. Hypocholesterolemic effect of gamma-linolenic acid as evening primrose oil in rats. Ann. Nutr. Metab. 30:289-299.

Sullivan, D. R., T. A. B. Sanders, I. M. Trayner, and G. R. Thompson. 1986. Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridaemic patients and normal subjects ingesting fish oil. Atherosclerosis 61:129-134.

Truswell, A. S. 1985. ABC of Nutrition: Reducing the risk of coronary heart disease. Br. Med. J. 291:34-37.

Vega, G. L., E. Groszek, R. Wolf, and S. M. Grundy. 1982. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. J. Lipid Res. 23:811-822.

Wagner, W. D., and T. B. Clarkson. 1974. Mechanisms of the genetic control of plasma cholesterol in selected lines of Show Racer Pigeons. Proc. Soc. Exp. Biol. Med. 145:1050-1057.

Wagner, W. D., T. B. Clarkson, M. A. Feldner, and R. W. Prichard. 1973. The development of pigeon strains with selected atherosclerosis characteristics. Exp. Mol. Pathol. 19:304-319.

Walker, B. L. 1984. Plasma cholesterol response to dietary saturated and hydrogenated fats in CBA and C57BR/cdJ mice. Nutr. Res. 4:601-610.

Walsh-Hentges, L. S., D. C. Beitz, N. L. Jacobson, A. D. McGilliard, and J. J. Veenhuizen. 1985a. Cholesterol transport and uptake in miniature swine fed vegetable and animal fats and proteins. 1. Plasma lipoproteins and LDL clearance. Lipids 20:735-742.

Walsh-Hentges, L. S., D. C. Beitz, N. L. Jacobson, and A. D. McGilliard. 1985b. Cholesterol transport and uptake in miniature swine fed vegetable and animal fats and proteins. 2. LDL uptake and cholesterol distribution in tissues. Lipids 20:757-764.

Weibust, R. S. 1973. Inheritance of plasma cholesterol levels in mice. Genetics 73:303-313.

Wood, P. D. S., R. Shioda, and L. W. Kinsell. 1966. Dietary regulation of cholesterol metabolism. Lancet 2:604-607.

Worth, R. M., H. Kato, G. G. Rhoads, A. Kagan, and S. L. Syme. 1975. Epidemiologic studies of coronary heart disease and stroke in Japanese living in Japan, Hawaii and California: Mortality. Am. J. Epid. 102:481-490.

Worthington, R. E., and J Miller. 1986. Effects of dietary peanut and corn oils on blood serum LDL/HDL ratios in rats: A crossover study. Nutr. Rep. Intl. 33:679-684.

Yamamoto, R. S., L. B. Crittenden, L. Sokoloff, and G. E. Jay, Jr. 1963. Genetic variations in plasma lipid content in mice. J. Lipid Res. 4:413-418.

APPENDIX A: PREPARATION OF LIVER MICROSOMES

Materials

Homogenizing buffer: 0.1 M potassium phosphate buffer (KPi) (pH 7.4) containing 0.25 M sucrose, 0.03 M nicotinamide, 0.001 M dithiothreitol and 0.001 M disodium EDTA

Procedure

The liver sample was held on ice throughout the whole procedure. The liver was minced, homogenized in a Potter-Elvehjem tissue grinder with 4 volumes of homogenizing buffer, and centrifuged at 20,000 x g in a J2-21 Beckman high speed refrigerated centrifuge for 30 min. The supernatant was transferred to an ultracentrifuge tube for centrifugation at 100,000 x g in a Ti 50 rotor in a Beckman model L3-50 ultracentrifuge for 60 min. The pellet was the microsomal fraction and it was resuspended using a Kontes tube in one volume of the same buffer. The microsomal suspension was then stored at −80°C until analysis.

APPENDIX B: CHOLESTEROL DETERMINATION

Materials

Assay solution:

sodium cholate	3.0	mmol/l
4-aminoantipyrine	0.82	mmol/l
phenol	14.0	mmol/l
Na_2HPO_4	50.0	mmol/l
NaH_2PO_4	50.0	mmol/l
carbowax-6000	0.17	mmol/l
cholesterol ester hydrolase	33	U/l
cholesterol oxidase	117	U/l
peroxidase	67000	U/l
10% triton X-100	20	ml/l

pH 7.0

0.9% NaCl

Cholesterol standard (50 and 200 mg/dl)

Procedure

One milliliter of assay solution was added to 20 μl of serum or sample. Blank was prepared by substituting water for sample. The resulting solution was incubated at 37°C for 15 min. Then, 1 ml of 0.9% NaCl was added and the

spectrophotometric reading at 500 nm was recorded using a Beckman quartz spectrophotometer, model DU. The amount of cholesterol present was calculated using the standard as the reference. Free cholesterol was determined using the same procedure except that cholesterol ester hydrolase was omitted from the assay solution.

APPENDIX C: HYDROXYMETHYLGLUTARYL COENZYME A REDUCTASE

ASSAY

Materials

0.05 M potassium phosphate buffer (pH 7.5) containing 0.3 M

KCl, 1.0 mM EDTA and 5.0 mM dithiothreitol

NADPH generating system: 4.5 μmol of glucose-6-phosphate,

0.3 IU of glucose-6-phosphate dehydrogenase, 0.45 μmol of

NADP$^+$ and 3.6 μmol of EDTA in 30 μl of water

^{14}C-HMG CoA: 1.38 μCi/μmol, 50 nmol/20 μl of water

^{3}H-mevalonolactone: 6.7 Ci/mmol, 2 x 10^4 dpm/10 μl

of benzene

Silica gel thin layer chromatography (TLC) sheet (0.1 mm

thickness, 20 x 20 cm, Eastman Kodak Company, Rochester,

NY)

Bray's solution (Research Products International, Mount

Prospect, IL)

Procedure

To 100 μl of sample which had been properly diluted

with potassium phosphate buffer, 20 μl of ^{14}C-HMG CoA and 30

μl of NADPH generating system were added. Blank was

prepared by replacing sample with potassium phosphate

buffer. After incubation at 37°C for 30 min, the reaction was stopped by addition of 15 μl of 6 N HCl. Ten microliters of ^{3}H-mevalonolactone was added as an internal standard and the sample was incubated again at 37°C for 30 min for complete lactonization. The sample was centrifuged in a Beckman microfuge for 1 min to sediment denatured protein. The supernatant was applied to an activated silica gel TLC sheet which then was developed in benzene/acetone (1:1, v/v). The mevalonolactone region (Rf 0.5-0.9) was scraped off, Bray's solution was added and the radioactivity was counted in a double channel (^{3}H and ^{14}C) scintillation counter (Packard TRI-CARB 2425 liquid scintillation spectrometer). The enzyme activity was calculated as amount of mevalonate formed.

APPENDIX D: CHOLESTEROL 7a-HYDROXYLASE ASSAY

Materials

[14]C-cholesterol: pre-purified by thin layer chromatography

using benzene/ethyl acetate (2:3, v/v) as the developing

solvent, 2 x 10^{5} cpm/150 μl water, contained 0.36 mg

of Tween 80

NADPH generating system: 0.75 mM NADP^{+}, 3 mM glucose-6-

phosphate, and 1 U/ml of glucose-6-phosphate

dehydrogenase

Homogenizing buffer: 0.1 M potassium phosphate buffer

(pH 7.4) containing 0.25 M sucrose, 0.03 M nicotinamide,

0.001 M dithiothreitol and 0.001 M disodium EDTA

Assay buffer: 0.1 M potassium phosphate buffer (pH 7.4)

containing 35 mM cysteamine

4.5 mM $MgCl_2$

Silica gel TLC plates (Fisher Scientific Company, 0.25 mm

in thickness, 20 x 20 cm)

7a-hydroxycholesterol (Steraloid Company)

Scintisol (Isolab, Akron, OH)

Procedure

The assay solution containing 300 μl of assay buffer, 150 μl of ^{14}C-cholesterol suspension, 300 μl of sample in homogenizing buffer and 150 μl of NADPH generating system was incubated at 37°C for 40 min. Two milliliters of ethanol was added to stop the reaction and the sterols were extracted twice with 20 ml of petroleum ether. The extract was evaporated to dryness with nitrogen and reconstituted in 0.125 ml of benzene/methanol (4:1, v/v). The solution was pipeted as a streak on an activated TLC plate. 7a-hydroxycholesterol standard was added to each streak for later identification. The plate was developed in benzene/ethyl acetate (2:3, v/v). 7a-hydroxycholesterol appeared blue and cholesterol pink after spraying with 50% sulfuric acid in water and heating. The 7a-hydroxycholesterol band and the top cholesterol area were scraped off. Eight milliliters of Scintisol were added to each sample and the radioactivity was counted in a Packard TRI-CARB 2425 liquid scintillation spectrometer. The enzyme activity was calculated as percentage conversion of cholesterol to 7a-hydroxycholesterol.

APPENDIX E: PROTEIN DETERMINATION

Materials

10% sodium dodecyl sulfate (SDS)

0.15% deoxycholate (DOC)

72% trichloroacetic acid (TCA)

Copper-tartrate-carbonate (CTC): 0.01% copper sulfate,

    0.2% potassium tartrate and 10% sodium carbonate

0.8 N NaOH

Reagent A:

    CTC:NaOH:SDS:H_2O=1:1:1:1

Reagent B:

    2 N Folin-Ciocalteau phenol:H_2O=1:5

Bovine serum albumin (BSA) (5-100 ug/ml)

Procedure

One tenth milliliter of DOC was added to 1 ml of
properly diluted sample (5-100 μg protein). After 10 min at
room temperature, 0.1 ml of TCA was added. The sample was
centrifuged at 3,000 x $\underline{g}$ for 15 min. The supernatant was
discarded and the pellet was brought to 1 ml with distilled
water. One milliliter of reagent A was added and the
solution was allowed to stand at room temperature for 10

min. Then, 0.5 ml of reagent B was added. The absorbance at 750 nm was recorded after 30 min at room temperature using a Beckman quartz spectrophotometer. A calibration curve was prepared using BSA as the standard, and the concentration of the sample was read from the curve.

APPENDIX F: ENZYMATIC DETERMINATION OF TOTAL BILE ACIDS

Materials

0.01 M potassium phosphate buffer, pH 7.2

0.1 M sodium pyrophosphate, pH 9.5

0.1 M sodium dodecyl sulfate (SDS)

3.2% hydrazine hydrate, pH 9.5

6.8 μmol/ml β-NAD

3α-hydroxysteroid dehydrogenase, 1 U/ml of 0.01 M potassium
   phosphate buffer

sodium cholate standard (5-50 μg/0.1 ml methanol)

Procedure

One and four tenths milliliters (1.4 ml) of
pyrophosphate buffer, 1.0 ml of hydrazine hydrate, 0.1 ml of
β-NAD, and 0.3 ml of enzyme solution were added to 0.1 ml of
sample in methanol. After incubation at 26°C for 35 min,
0.1 ml of SDS was added to stop the reaction and clear the
solution. A blank was set up for each sample by adding the
enzyme only after the SDS. Absorbance at 340 nm was
recorded using a Beckman quartz spectrophotometer, model DU.
Since one mole of bile acid forms one mole of NADH in the
presence of 3α-hydroxysteroid dehydrogenase, the amount of
bile acids present is calculated from the following formula:

amount of bile acids (μmol) = (A x 3)/6.22

where   A = increase in extinction

6.22 x 10^6 = molar extinction of NADH at 340 nm

ACKNOWLEDGEMENTS