COMPARISON OF FECAL LIPID PATTERNS IN HUMANS FED BEEF TALLOW AND CORN OIL AS SOLE SOURCES OF DIETARY FAT

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ABSTRACT

The present study was undertaken to determine whether the hypocholesterolemic effect reported for beef tallow is due to increased fecal steroid excretion as has been reported for corn oil. Since liquid formula diets alter lipid excretion and have a hypocholesterolemic effect regardless of the fat in the forumla, a solid test diet was designed resembling the normal diet but with 40% of the calories from either beef tallow or corn oil. Fecal lipid excretion patterns of six healthy young men fed the tallow and corn oil diets were determined for a 5-day period during each of the two 22-day metabolic studies. The apparent digestibility of corn oil was significantly higher ($P < 0.005$) than that of beef tallow. Lower apparent digestibility of palmitic and stearic acids accounted for the lower digestibility of beef tallow. Non-saponifiable lipid excretion was significantly greater ($P < 0.025$) for corn oil than for tallow, due to a significant increase ($P < 0.005$) in the excretion of plant sterols and neutral steroids derived from endogenous cholesterol. The excretion of major bile acids was appreciably lower than that of neutral steroids, and did not differ significantly between diets. Since cholesterol-derived neutral steroid excretion was significantly lower on a solid diet containing beef tallow than when the same diet contained corn oil, the hypocholesterolemic mechanism of the two fats may differ.

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INTRODUCTION

The effect of dietary fat on cholesterol metabolism has assumed increasing importance because of the correlation between elevated serum cholesterol and atherosclerosis. There have been numerous reports confirming the observations that saturated fats in the diet elevate, whereas unsaturated fats lower, the serum cholesterol concentration in man. However, there has not been a definitive explanation of the mechanism of the hypocholesterolemic effect of polyunsaturated fat in the diet. Theoretically, this action could result from any one or a combination of the following alterations in cholesterol metabolism: (a) a decrease in endogenous cholesterol synthesis; (b) a reduction in absorption of cholesterol; (c) an increase in fecal excretion of cholesterol and/or bile acids; and (d) a redistribution of blood cholesterol between plasma and other tissues. Although the mechanism responsible for the hypocholesterolemic action of dietary polyunsaturated fat remains unresolved, there seems to be considerable evidence that these fats promote increased excretion of fecal steroids.

Beef tallow, one of the major sources of saturated fat in the North American diet, has been found to lack the cholesterol-elevating effect expected of a saturated fat. It has been suggested that the failure to observe an increase in serum cholesterol in response to beef tallow is associated with the relatively high proportion of stearic acid in this

fat. Since increased fecal steroid excretion has been shown to accompany the cholesterol-lowering effect of corn oil, it was considered of interest to compare the fecal lipid excretion patterns of young men fed tallow or corn oil as the sole source of dietary fat. In addition since liquid formulae test diets have been associated with alterations in steroid excretion patterns irrespective of the fat contained in the formula, it was considered necessary to formulate a solid test diet of customary foods which would permit the testing of a single saturated or unsaturated fat source.

REVIEW OF LITERATURE

A. INTRODUCTION

In spite of the high correlation among dietary fat, elevated serum cholesterol levels and the incidence of atherosclerosis (Stamler et al. 1966), there is still a definite lack of understanding regarding the detailed mechanisms whereby cholesterol metabolism is regulated. Polyunsaturated fatty acids have been shown to lower the plasma cholesterol when substituted for saturated fatty acids in the diet (Ahrens et al. 1957; Hegsted et al. 1965), whereas long chain, saturated fatty acids tend to elevate blood cholesterol levels. However, several reports (Ahrens et al. 1957; Connor et al. 1964; Erickson et al. 1964 and Keys et al. 1965c) have indicated that, in man, stearic acid tends to lack the cholesterol-elevating effect of saturated fatty acids. Cocoa butter, a fat rich in stearic acid, did not produce the cholesterol-raising effect expected on the basis of its total content of long-chain saturated fatty acids (Grande et al. 1970). The mechanism whereby unsaturated fats, or saturated fats high in stearic acid, may alter plasma cholesterol is still unknown.

Total body cholesterol is normally regulated by the interaction of three processes: absorption, synthesis and excretion. Hence the following modes of action have been proposed (Spritz et al. 1965) to explain the cholesterol-lowering effects of polyunsaturated fatty acids. (1) An increased excretion of cholesterol as neutral steroid could occur. This could be due to decreased absorption of cholesterol from the diet and

intestinal lumen; (2) An increase in bile acid formation and excretion is a possible mechanism. This could be achieved by decreased bile reabsorption or increased conversion of cholesterol to bile acids; (3) Cholesterol synthesis could be decreased. In addition, a redistribution of cholesterol from plasma into tissues has been suggested as a fourth possible mechanism. A redistribution of cholesterol into tissues, however, has not been documented in man. A brief review of cholesterol metabolism is necessary in order to understand the possible effect of these mechanisms in lowering plasma cholesterol.

B. CHOLESTEROL ABSORPTION

Absorbed cholesterol is derived from two sources, exogenous and endogenous. Exogenous cholesterol is derived from the diet, which supplies from 0.5 to 2 gm. of cholesterol in Western countries (Keys et al. 1965b). Endogenous cholesterol is mainly derived from bile, which supplies about 1 gm. cholesterol per day (Phillips 1960), and cholesterol synthesized by the cells of the ileum. A substantial fraction of the cholesterol from the latter source is discharged into the lumen (Wilson and Reinke 1968) but since this occurs mainly in the terminal ileum, which is beyond the site of maximal cholesterol absorption (Borgstrom 1960; Simmonds et al. 1967), it may not be a major source of reabsorbed cholesterol.

The rate and extent of cholesterol absorption is postulated to be determined by the level of fat and the physical form of cholesterol in the diet, the size of the bile acid pool, the relative activity of cholesterol

esterase and the rate of chylomicron formation (Dietschy and Wilson 1970).

While a high fat diet has been shown to enhance cholesterol absorption (Kim and Ivy 1952; Wilson 1962), the exact mechanism of this enhancement has not been elucidated. The process may involve the promotion of increased bile flow, the provision of additional amphipathic substances (such as monoglycerides), or the increased rate of cholesterol absorption may reflect an increased rate of chylomicron formation after fat feeding.

Diversion of bile acids from the intestinal lumen essentially stops cholesterol absorption (Siperstein et al. 1952), while expansion of the bile acid pool by feeding exogenous bile acids results in a greater than normal rate of cholesterol absorption (Duel 1955). This effect of bile acids on cholesterol absorption has been attributed to their role in promoting micellar solubilization of sterols in the intestinal lumen, and stimulation of pancreatic cholesterol esterase (Vahouny et al. 1959). In addition, it has been suggested (Dietschy and Wilson 1970; Sylven and Borgstrom 1968) that bile acids may facilitate movement of cholesterol from the mucosal cell into the lymph.

Level of cholesterol in the diet has been shown to alter the percent of cholesterol absorbed. The human intestine has a limited ability to absorb cholesterol. Grundy and Ahrens (1969) found that about half of the dietary cholesterol was absorbed when the total intake was between 290 and 452 mg./day. However, Grundy et al. (1969) demonstrated that repeated ingestion of high levels of cholesterol resulted in a marked decline in the proportion of cholesterol absorbed. In fact, Grundy et al. (1969) estimated,

on the basis of studies with two subjects, that maximal dietary cholesterol absorption was about 320 and 340 mg./day. The limited ability of man to absorb more than about 500 mg. of dietary cholesterol daily had previously been suggested by Taylor and Ho (1967).

Dietary cholesterol does not appear to contribute much more than one third of the circulating cholesterol on an average diet. Wilson and Lindsey (1965) demonstrated that no more than 38% of the circulating cholesterol was derived from dietary cholesterol in studies on two subjects. Grundy and Ahrens (1969) confirmed these observations. The contribution from dietary cholesterol ranged from 10 - 36% when 290 - 1600 mg. was eaten daily by 5 subjects.

Because of the limited ability of the human to absorb cholesterol and the minor contribution of absorbed dietary cholesterol to plasma cholesterol levels, alterations in cholesterol absorption are not considered the primary control on serum cholesterol levels. The percentage absorption of cholesterol remains fairly constant under normal conditions. An alteration in bile acid metabolism would, however, affect cholesterol absorption.

C. CHOLESTEROL SYNTHESIS

Cholesterol is synthesized de novo from acetate. Every mammalian tissue is capable of some degree of de novo cholesterol synthesis, but the primary sites of synthesis are the liver and the ileum of the gastro-intestinal tract. Together they account for about 95% of total endogenous cholesterol synthesis (Dietschy and Wilson 1968; Lindsey and Wilson 1965),

with the rate of sterol synthesis in the human intestine being similar to that of the liver (Dietschy and Wilson 1970).

Rate of cholesterol biosynthesis is regulated by three principal physiologic variables: the amount of cholesterol in the diet; the caloric intake of the animal; and the functional integrity of the enterohepatic circulation of bile acids. Cholesterol feeding has been shown to produce both a prompt and a marked suppression of cholesterol synthesis by liver (Dietschy and Wilson 1968). No tissue other than liver, however, shows such marked suppression of sterol synthetic activity (Dietschy and Siperstein 1967). Thus it follows that the gastrointestinal tract, in particular the ileum, becomes the major site for endogenous sterol synthesis when high levels of cholesterol are ingested.

Bile acids have been observed to affect cholesterogenesis. Dietschy and Wilson (1968, 1970) have demonstrated that biliary diversion, in the rat and monkey, enhanced cholesterol synthesis in the liver by 2½ - 3 fold and in the small intestine by as much as 10 fold. Similar measurements on the rate of cholesterogenesis following biliary diversion have not been reported for man. However, using balance techniques in man, Grundy et al. (1966) have presented evidence which suggests that expansion of the bile acid pool decreases the rate of endogenous cholesterol synthesis, whereas interruption of the bile acid enterohepatic circulation enhances cholesterol synthesis.

The primary effect of polyunsaturated fats does not appear to be a direct effect on cholesterol synthesis. The bulk of the evidence suggests

that the primary effect of polyunsaturated fats is on excretion of cholesterol as neutral sterols and/or bile acids, although an alteration in excretion would affect total cholesterol.

D. CHOLESTEROL DEGRADATION AND EXCRETION

Cholesterol excretion and degradation take place via the gastrointestinal tract predominantly by two mechanisms - excretion of cholesterol itself and catabolism to bile acids. These moieties and their bacterial transformation products are thought to account for the vast majority of cholesterol turnover in the normal state.

D.1. Bile Acids. The converson of cholesterol to bile acids serves as a major pathway for the degradation of cholesterol in mammals. This process occurs in the liver exclusively, the primary bile acids formed being cholic acid, chenodeoxycholic acid, and lithocholic acid. Bile acids are conjugated to glycine or taurine and secreted into the bile as sodium or potassium salts. Most of the bile salts that enter the intestinal lumen are subsequently reabsorbed via the enterohepatic circulation although 150-350 mg./day is lost in the feces (Rosenfeld and Hellman 1962). Deoxycholic acid, a product of bacterial action on cholate, also is present in appreciable quantities in intestinal bile. In fact, the bile acids excreted in the feces constitute a complex mixture of compounds formed by the action of intestinal microorganisms.

D.2. Neutral Sterols. In general, there are two sources of fecal neutral sterols: diet and endogenous production. Endogenous sterols (primarily cholesterol) enter the intestine in bile secretion, as intestinal secretion and from sloughed intestinal cells. Some fecal sterols are identical to the sterols present in animal tissues, for example, cholesterol; cholestanol; lathosterol; 7- dehydrocholestanol and methosternol. Other fecal neutral sterols are the products of the action of bacteria in the colon on cholesterol and other sterols of tissue origin. The most abundant bacterial conversion product in feces is coprostanol but others such as epicoprostanol, cholestanone and coprostanone also are present (Masoro 1968).

Dietary sterols are composed primarily of cholesterol and various plant sterols. Plant sterols are poorly absorbed in man and almost all enter the feces as neutral sterols. As previously mentioned, only about 50% of dietary cholesterol is absorbed under normal dietary conditions. Therefore about half the dietary cholesterol is excreted as neutral sterol although most of this is probably modified by the colonic bacteria to compounds such as coprostanol.

The biliary and intestinal secretions plus sloughed mucosal cells deliver about 2 gm. of cholesterol per day to the intestinal lumen (Masoro 1968). However only about half the cholesterol that enters the intestine from endogenous sources is lost in the feces; the remainder, like the bile acids, is reabsorbed.

D.3. Dietary Factors Affecting Steroid Excretion

D.3.a. Carbohydrates. Dietary carbohydrate has been shown to affect neutral sterol excretion. Recovery of dietary plant sterol was found to be complete when a diet of mixed general foods was consumed, whereas only about 25-58% of the ingested plant sterols were recovered when liquid formula diets were fed (Denbesten et al. 1970). The addition of fresh celery or pulverized cellulose to the formula diet partially corrected the plant sterol loss in the feces (80% being recovered). Addition of both cellulose and lactose to the formulae led to complete recovery of ingested plant sterols in the feces. Denbesten et al. (1970) hypothesized that the metabolism of intestinal tract bacteria is altered when certain constituents were not present in the diet, and that these bacteria may degrade the sterol nucleus. Different dietary carbohydrates have been found to affect the half-life and pool size of cholic acid in rats (Portman and Murphy 1958). The half-life of cholic acid was longer on diets containing sucrose than those containing starch or sucrose plus Celluflour. Wells et al. (1960) have shown that feeding of lactose-containing diets induces an increased absorption of cholesterol and an increased excretion of bile acids in rats. The changes in half-life and pool of cholic acid in rats induced by different carbohydrates may be secondary to effects of carbohydrates on intestinal flora (Portman 1960). No data are available on the type of bile acids present in human feces on different carbohydrate diets.

Addition of nondigestible cellulosic residue to the diet increases cholic acid production (Portman and Murphy 1958; Tennent et al. 1960).

In a variety of animals, supplementing the diet with alfalfa, pectin, artichoke roots and mucillagenous polysaccharides reduces serum cholesterol (Keys et al. 1961; Eastwood 1969), presumably by increasing bile acid excretion. Lignin, a major component of vegetable fiber, adsorbs bile acids (Eastwood and Hamilton 1968).

D.3.b. Plant Sterols. In addition to contributing to the total fecal neutral sterol, plant sterols may cause an increase in cholesterol excretion. The sitosterols present in vegetable oils such as corn oil have been shown to have an appreciable cholesterol-lowering effect in man (Beveridge et al. 1956). Grundy et al. (1969) have shown that consumption of large amounts of plant sterols stimulates the excretion of endogenous neutral sterol.

D.3.c. Fat. Substitution of polyunsaturated fats for saturated fats in the diets of man and animals has been shown by numerous investigators to increase fecal steroid excretion (Spritz et al. 1965; Nestel 1970).

There is considerable controversy in the literature as to whether the increase in steroid excretion is due to an increase in bile acid excretion, and an increase in neutral steroid excretion, or an increase in the excretion of both of these fecal steroid components.

Wilson (1961) demonstrated that in rats, addition of 20% linoleic acid to a fat-free diet results in increased excretion of neutral sterols, while addition of 20% palmitic acid does not influence fecal sterol excretion. Gerson et al. (1961) found an increase in coprostanol excretion

when rats were transferred from a fat-free diet to one containing crude or refined corn oil, while excretion of cholesterol remained unchanged.

Cholesterol excretion studies in man indicate that bile acid excretion increased when polyunsaturated fatty acids were substituted for saturated fatty acids in the diet (Lewis 1958; Roels and Hashim 1962; Haust and Beveridge 1958; Haust and Beveridge 1963). However, other investigators have reported an increase in neutral sterol excretion with polyunsaturated fats such as corn oil (Rosenfeld and Hellman 1959; Antonis and Bersohn 1962). And still other studies have demonstrated an increased excretion of both neutral and acidic (bile acid) sterols on substitution of polyunsaturated fats for saturated fats in the human diet (Gordon et al. 1957; Kaleta et al. 1959; Goldsmith et al. 1960; Wood et al. 1966; Moore et al. 1968; Connor et al. 1969).

Numerous investigators have suggested that increased fecal cholesterol losses account for the fate of cholesterol observed leaving the plasma when polyunsaturated fats are substituted for saturated fats (Hellman et al. 1957; Grundy and Ahrens 1970). However several groups of investigators have found no significant differences in the fecal excretion of bile acids by exchanging dietary fats (Lindstedt et al. 1965; Hellstrom and Lindstedt 1966; Ali et al. 1966). Eneroth et al. (1964) noted no significant change in the excretion of neutral steroids with polyunsaturated fats. In some studies, excretion of both bile acids and neutral steroids was not significantly altered by polyunsaturated fats (Avigan and Steinberg 1965;

Spritz et al. 1965).

No adequate reason has yet been advanced that reconciles the inconsistencies in the above studies. Part of the problem may be differences in methodology. Early techniques for measuring sterol balance and identification of fecal lipids were inadequate. These methods and their inadequacies have been well reviewed by Danielsson (1963) and Nestel (1970) and will not be discussed here. Grundy and Ahrens (1969) and Quintao et al. (1971) have recently reviewed the major techniques used to measure cholesterol turnover and have compared their relative merits. Probably the two most reliable methods for fecal steroid analysis are the isotope balance technique and chemical balance techniques using chromatographic procedures including gas-liquid chromatography to purify, partition and quantitate dietary and fecal steroids (Miettinen et al. 1965; Grundy et al. 1965). These methods quantify accurately individual neutral steroids and bile acids and they distinguish between plant sterol and cholesterol excretion products. Nevertheless, differences in technique do not completely account for the variation in results. Isotope balance techniques have both shown (Moore et al. 1968) and failed to show conclusively (Grundy and Ahrens 1970) changes with polyunsaturated fats. Connor et al. (1969) using the chromatographic analysis procedures of Miettinen et al. (1965) and Grundy et al. (1965) obtained results which were at variance with those obtained by Spritz et al. (1965) using the same techniques.

Choice of subjects may be another factor in the different results. Conner et al. (1969) used normal subjects while Spritz et al. (1965) used hypercholesteremic subjects. However, both increased steroid excretion (Goldsmith et al. 1960; Wood et al. 1966; Grundy and Ahrens 1966; Moore et al. 1968), and unaltered steroid excretion (Lindstedt et al. 1965; Avigan and Steinberg 1965; Ali et al. 1966; Grundy and Ahrens 1970), have been observed with both normal (Lindstedt et al. 1965; Wood et al. 1966; Ali et al. 1966; Moore et al. 1968) and hyperlipemic subjects (Goldsmith et al. 1960; Avigan and Steinberg 1965; Grundy and Ahrens 1966; Grundy and Ahrens 1970) when polyunsaturated fats were substituted for saturated fats in the diet.

It may be that some hypercholesterolemic subjects do not respond to alterations in dietary fat source in the same manner as normolipemic individuals. It has been noted, for example, that subjects suffering from Type II hypercholesterolemia have diminished bile acid turnover and low cholic acid excretion on normal diets when compared to individuals with normal blood cholesterol levels (Hellstrom and Lindstedt 1966; Nestel 1970). Kottke (1969) reported a difference in cholic acid turnover between Type II and Type IV hypercholsterolemic patients. Lofland et al. (1968) found that the fecal excretion of steroids and the turnover of cholesterol were consistently greater among monkeys who maintained normal serum cholesterol levels with saturated fat diets as compared to those who developed hypercholesterolemia. It is hypothesized that faulty cholesterol excretion may characterize some hypercholesterolemic states.

The kind of test diet may be an important factor in steroid excretion patterns. As previously mentioned, liquid formula diets devoid of vegetable fiber result in increased bile acid excretion and incomplete plant sterol recovery when compared to a solid diet of mixed foods. In addition, there have been reports in the literature suggestive of a hypocholesterolemic effect associated with the transition from mixed foods to liquid formula diets (Hashim et al. 1959; Ahrens et al. 1957; McOsker et al. 1962). The effects due to formula diets cannot be accounted for by changes in body weight or by fatty acid and cholesterol composition of the formula diets; in some of these studies, the initial effect overshadowed subsequent responses to manipulation of fatty acid composition. Whether these changes are the result of more frequent, spaced feedings, of alterations in intestinal flora, or of other confounding variables are unanswered questions. Lindstedt et al. (1965) noted alterations in bile acid turnover following transition from solid to liquid formula diets. The relative contribution of chenodeoxycholic acid found in bile samples was lower on liquid formula diets than on solid diets. In two additional subjects, transition from a solid diet to a formula diet was associated with large changes in serum cholesterol level with parallel rather than reciprocal changes in cholic acid turnover. The effect of formula diets on cholesterol metaboliam remains to be deliniated.

Digestibility of the fat in the test diet may have an effect on the steroid excretion patterns. A level of 40% of total calories from fat is in agreement with the usual North American intake (Scheig 1969). However,

Haust and Beveridge (1963) and Avigan and Steinberg (1965) used formulae containing 60% of the total calories from fat. Since it has been shown that high fat intakes cause an increased fecal fat excretion (Wollaeger et al. 1947) it is conceivable that larger amounts of sterols and bile acids are carried along in the intestines when the ingested fat is not fully absorbed. Amounts of fecal solids excreted daily by the subjects studied by Avigan and Steinberg (1965) significantly exceeded the range of 13.6 to 13.9 gm. per day found in the feces of subjects on a standard test diet containing some non-digestible solids (Wollaeger et al. 1947). This suggests that there was a higher than usual fat content in the fecal samples of the former study, possibly due to poor absorption of the high fat intakes. Incomplete fat absorption may also be due to decreased digestibility of a test fat, even when the fat is fed at normal levels.

Because of the conflict in results, and the lack of uniformity in methodology, subject selection, and design of test diets in the studies involving dietary fat and cholesterol excretion, considerable controversy still exists concerning the effect of dietary fat on steroid excretion patterns.

E. SUMMARY

Decreased absorption, decreased synthesis and increased excretion of cholesterol have been proposed as mechanisms whereby polyunsaturated fatty acids bring about a reduction in plasma cholesterol. The amount of cholesterol absorbed (exogenous and endogenous) is dependent on the

level of fat in the diet and the size and turnover of the bile acid pool. Cholesterol synthesis is regulated by the level of dietary cholesterol, amount of cholesterol absorbed and the amount of bile reabsorbed via the enterohepatic circulation. Cholesterol excretion, as bile acids or neutral steroids, is affected by the type of carbohydrates in the diet, the dietary levels of cholesterol and plant sterols, and the fatty acid composition of the dietary fat.

A number of studies have demonstrated that substitution of polyunsaturated fats for saturated fats in the diet of man and animals promotes an increase in fecal cholesterol excretion as bile acids and neutral steroids. Several other investigators have failed to observe any increase in cholesterol excretion with polyunsaturated fats. Variations in methodology, subject selection and design of test diets may partially account for the discrepancies in the literature. However, there is considerable recent support for the concept that increased excretion of bile acids and neutral steroids is one of the primary effects of polyunsaturated fatty acids and the mechanism by which these fats produce their serum cholesterol-lowering effect. Since stearic acid, one of the principal saturated fatty acids in beef tallow, lacks the cholesterol-elevating effect generally attributed to saturated fatty acids, the effect of this fat on steroid metabolism remains to be elucidated.

OBJECT OF RESEARCH

The objectives of the study herein described were: (1) to formulate a nutritious and palatable solid diet which would permit the incorporation of beef tallow or corn oil as the sole source of dietary fat, and (2) to investigate the effect of beef tallow compared to that of corn oil on lipid, in particular cholesterol, excretion patterns in the feces of healthy young men.

EXPERIMENTAL METHODS

A. EXPERIMENTAL DESIGN

Six subjects were fed test diets containing either edible beef tallow[1] (Period I) or corn oil[2] (Period II) as the sole source of dietary fat. The test diets, with a 2-day menu rotation for each diet were fed for 22 days. The two test periods were preceded by a 6-day stabilization period in which subjects were fed a controlled mixed fat diet composed of customary foods. Between Period I (beef tallow diet) and the stabilization period preceding Period II (corn oil diet), subjects resumed normal eating habits for 15 days.

Feces were collected for 5 consecutive days (Days 18-22 inclusive) during each of the 22-day test periods.

Duplicates of the total daily food intakes for each menu in both Period I and Period II were prepared for each subject. These food samples were taken on randomly selected days throughout the fecal collection periods. Duplicates of total daily food intakes were also taken for 2 subjects during the stabilization period.

B. SUBJECTS

The subjects, seven healthy male graduate students ranging in age from 22-34 years of age, were selected from a group of volunteers. The subjects

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[1] Canada Packers Limited, St. Boniface, Man.

[2] Mazola Corn Oil, Best Foods Div., Canada Starch Co. Ltd., Montreal, Quebec.

were the same in both periods with one exception. One subject, M.T., had to be replaced by R.H. in Period II. Height, weight and age data for subjects are given in Table 1. During the conduct of the metabolic study the subjects reported daily for breakfast, lunch and dinner, but otherwise were encouraged to maintain their normal activities. Subjects were instructed to weigh themselves daily before breakfast and their caloric intakes were regulated to maintain constant body weight.

C. DIETS

Caloric intakes of the experimental subjects were standardized during the initial pre-experimental stabilization period. The stabilization diet (Table 2) provided approximately 40% of the total daily calories from mixed fats. Adjustments were made in caloric content to meet the needs of individual subjects by addition or deletion of sucrose, jelly, bread or potato. Corresponding adjustments in the butter or cream allowance maintained the percentage of fat calories at approximately 40%. Nutrient composition of the stabilization diet is given in Table 3.

Following each stabilization period, subjects were placed on the test diet, which provided 40% of the total daily calories from fat, for a period of 22 days. The sole fat source in Period I was edible beef tallow and in Period II, corn oil.

The test diet was designed to readily incorporate either a saturated fat, beef tallow, or an unsaturated fat, corn oil. Textured soybean pro-

Table 1

Physical Data of Subjects

Subject	Age (yr)	Height (cm)	Weight (Kg)		
			Initial	Period I (Beef Tallow Diet)	Period II (Corn Oil Diet)
C.W.	22	177	75.6	76.0±0.3[1]	76.4±0.4
L.R.	23	183	82.5	86.0±0.7	86.8±0.6
P.B.	34	175	82.8	82.6±0.4	83.5±0.4
C.B.	27	149	63.5	64.0±0.3	63.8±0.6
V.M.	22	171	95.0	94.7±0.4	94.1±0.3
M.T.[2]	25	180	79.6	80.3±0.8	
R.H.[3]	32	182	76.9		76.8±0.3

[1]Mean ± S.D. for 28 daily weighings.

[2]Participated in Period I only.

[3]Participated in Period II only.

Table 2

Composition of Basic Stabilization Diets

Menu I	Menu II
Breakfast[1]	
90 gm. apple or pineapple juice	120 gm. grapefruit or orange juice
2 A-lg. eggs scrambled in 5 gm. butter	35 gms. cornflakes or branflakes
2 strips crisp bacon	14 gm. brown sugar
2 sl. white toast	1 boiled egg A-lg.
1 pkg. (20 gm.) jelly	2 sl. white toast
8 oz. whole milk	1 pkg. (20 gm.) jelly
25 gm. butter (for day)	8 oz. whole milk
	25 gm. butter (for day)
Lunch[1]	
1 hamburger pattie[2]	Sandwich:
30 gm. cheese slice	25 gm. ham + 30 gm. cheese
1 hamburger bun/relishes	2 slices bread, 5 gm. butter
tossed salad (50 gm. lettuce, 25 gm. tomato)	Celery & carrot sticks
	Cream of Tomato Soup[2]
15 gm. French dressing	100 gm. cnd. fruit + 10 gm. jce.[3]
100 gm. cnd. fruit + 10 gm. jce.[3]	8 oz. whole milk
8 oz. whole milk	
Dinner[1]	
2 pork chops (loin)	120 gm. roast turkey (roll)
100 gm. inst. mashed potato	100 gm. inst. mashed potato
100 gm. vegetable[4]	100 gm. vegetable[4]
1 sl. whole wheat bread	1 sl. whole wheat bread
100 gm. cnd. fruit + 10 gm. jce.[3]	100 gm. cnd. fruit + 10 gm. jce.[3]
8 oz. whole milk	8 oz. whole milk
Snacks[1]	
2 oatmeal cookies, 3 gingersnaps, 2 Oreo cookies.	

[1] Coffee and tea allowed ad lib. Alcohol and other beverages prohibited.
[2] See recipes Appendix Tables 7 and 15.
[3] Fruit cocktail, apricots, pears, pineapple or peaches.
[4] Frozen green beans, peas and carrots were rotated.

Table 3

Nutrient Composition of Basic Stabilization Diets[1]

	Calories	Protein (g)	Fat (g)	Carbo-hydrate (g)	Calcium (mg)	Phos-phorous (mg)	Iron (mg)	Vit.A (I.U.)	Thia-min (mg)	Ribo-flavin (mg)	Niacin (mg)	Vit.C (mg)
Menu I	2832	145.8	140.6	247.9	1365	1893	15.2	10977	2.6	2.7	24.7	49.7
Menu II	2205	105.2	96.4	229.2	1255	1470	10.0	10242	2.3	2.2	21.1	75.4
Average	2519	124.7	118.6	238.6	1310	1682	12.6	10616	2.5	2.5	22.9	62.6

[1] Calculated values using USDA Handbook #8 Composition of Foods (Watt and Merrill 1963).

tein (TVP)[1], skim milk, and spray-dried egg albumin[2] were utilized as the primary protein sources. Essential amino acid intakes (Table 4) were in excess of those recommended by Hegsted (1968). Three different physical forms of TVP, Beef Flavor Strips #10, Pork Flavor Chunks #10 and Beef Flavor Chunks #15, were incorporated into four main entrees: meatballs/tomato sauce; meatloaf/gravy; sweet and sour pork; and beef stew (Appendix Tables 8 to 13). Two of these main entrees per day provided approximately 53-54% of the total daily fat intake (Table 5). Addition of tallow or corn oil to cooked cereals, scrambled egg albumin, cookies, squares and muffins, provided approximately another 35% of the total daily fat. The remaining fat was supplied by corn oil margarine[3] or a specially prepared tallow margarine[4].

Each daily menu was designed to include all food groups and provide variety in texture and flavor. Itemized composition of each menu of the two-day menu rotation is given in Table 6. Calculated nutrient values for each menu is given in Table 7. All nutrient allowances recommended by the Canadian Dietary Standard (1963) were met or exceeded.

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[1]Trade name for Textured Vegetable Protein. Archer Daniels Midlands Co., 733 Marquette Ave., Minneapolis, Minn. 55440.

[2]Cham Foods Ltd., Jarvis St., Winnipeg, Manitoba.

[3]Fleishmann's Corn Oil Margarine, Standard Brands Ltd., Winnipeg 6, Man.

[4]Canada Packers Ltd., Research and Development Laboratories, 2211 St. Clair Ave. W., Toronto 9, Ontario.

Table 4

Essential Amino Acid Composition of Test Diet[1]

Amino acid	Amino Acid (gm/day)		
	Menu I	Menu II	Recommended[2]
Lysine	6.97	6.62	0.545
Threonine	4.83	4.55	0.375
Methionine	2.34	2.21	0.194
Cystine + Methionine	3.29	3.22	0.700
Valine	5.67	5.39	0.622
Isoleucine	4.66	4.35	0.550
Leucine	9.80	9.37	0.727
Phenylalanine	6.00	5.25	0.258
Phenylalanine + tyrosine	10.26	9.48	0.500

[1] As analyzed by the ion-exchange method of Benson and Patterson (1965) on a Beckman Model 117 Amino Acid Analyzer (Beckman Instruments Inc., Palo Alto, Calif. 94304).

[2] Hegsted (1968).

Table 5

Major Sources of Fat in the Diet

	Fat (g.)		% Total Daily Fat[1]	
	Calc.	Anal.		
BREAKFAST:				
Cooked Cereal	10	-	8	11
Scrambled Egg Albumin	4	-	3	
LUNCH & DINNER:				
Menu I				
Sweet & Sour Pork	28	24	18	54
Meatloaf/	43	38	29	
gravy/	5	-	4	
m. potato	4	-	3	
Menu II				
Meatballs/	15	12	10	53
tomato sauce	17	15	13	
Beef Stew	42	37	31	
SNACKS:				
Muffins	13	12	9	24
Cookies	12	11	9	
Square	9	8	6	
Margarine	13	13	10	

[1] Based on total of analyzed items: Menu I = 129 g., Menu II = 122 g.

Table 6

Composition of Basic Test Diets

Menu I	Menu II
Breakfast[1]	
120 gm. grapefruit or organge jce.	90 gm. apple or pineapple jce.
rolled oats[2]	Cream of Wheat[2]
Scrambled egg albumin[2]	Scrambled egg albumin[2]
1 slice white toast	1 slice white toast
20 gm. jelly	20 gm. jelly
14 gm. brown sugar	14 gm. brown sugar
8 oz. skim milk	8 oz. skim milk
15 gm. margarine (for day)	15 gm. margarine (for day)
Lunch[1]	
Sweet and sour pork[2]	2 Meatballs[2]/150 gm. tomato sce.[2]
100 gms. cooked rice	150 gm. cooked spaghetti
tossed salad (50 gm. lettuce, 25 gm. tomato)	tossed salad (50 gm. lettuce, 25 gm. tomato)
100 gm. cnd. fruit + 10 gm. jce.[3]	100 gm. cnd. fruit + 10 gm. jce.[3]
1 slice whole wheat bread	1 slice whole wheat bread
8 oz. skim milk	8 oz. skim milk
Dinner[1]	
Meat loaf[2]/gravy[2]	Beef stew[2]
Instant mashed potato[2]	Tossed salad (50 gm. lettuce, 25 gm. tomato)
100 gm. vegetable[4]	
100 gm. cnd. fruit + 10 gm. jce.[3]	100 gm. cnd. fruit + 10 gm. jce.[3]
1 slice whole wheat bread	1 slice whole wheat bread
8 oz. skim milk	8 oz. skim milk
Snacks[1]	
1 ginger muffin[2], 2 oatmeal cookies[2], 1 fruit square[2]	

[1] Coffee and tea allowed ad lib. Alcohol and other beverages prohibited.

[2] See recipes (Appendix Tables 5 to 18).

[3] Fruit cocktail, apricots, pears, pineapple or peaches.

[4] Frozen green beans, peas and carrots were rotated.

Table 7

Nutrient Composition of Test Diets[1]

	Calories	Protein (g)	Fat (g)	Carbo-hydrate (g)	Calcium (mg)	Phos. (mg)	Iron (mg)	Vit.A (I.U.)	Thia-min (mg)	Ribo-flavin (mg)	Niacin (mg)	Vit.C (mg)
Menu I	3357	123.5	144.1	418.8	1407	1477	15.6	7271	3.1	2.8	11.6	100.0
Menu II	3372	123.9	143.7	429.9	1398	1381	14.8	6520	4.9	2.7	16.6	65.4
Recommended[2]	3100	55.0	_	_	500	_	6.0	3700	0.9	1.5	9.0	30.0

[1]Calculated values using USDA Handbook #8 Composition of Foods (Watt and Merrill 1963).

[2]Based on Dietary Standard for Canada (1963). Values given for males, 176 lb (80 Kg), Activity Level A.

Each allotted serving of the menu items was weighed to the nearest gram on a Sartorius top-loading balance (Model 2254)[1]. All duplicates of daily food intakes were stored at -10°C. Thawed samples were homogenized with 200 ml water using a Waring Commercial Blendor (Model CB-5)[2]. An aliquot of the homogenate was lyophilized on a Virtis Freeze-Mobile Freeze-Drier (Model 10-140BA)[3]. The dried sample was ground with a mortar and pestle, and stored in plastic bags at -10°C for later analysis.

D. FECAL COLLECTION PROCEDURES

Feces were collected on Days 18-22, inclusive, during each of the two test periods. Subjects were instructed to collect all stools for 5 consecutive days in 32 oz. plastic snap-top containers. Samples were stored at -14°C and later lyophilized on a Virtis Freeze-Mobile Freeze-Drier (Model 10-140BA). Dried samples were ground using a Wiley Mill (Model 2)[4]. The dried ground samples, for each subject for the 5-day period, were pooled. A 40 gram portion was taken from each pooled fecal sample, placed in screw-top glass jars and stored at -10°C for later analysis.

E. CHEMICAL ANALYSIS

E.1. Food samples: Total lipid was extracted from lyophilized food samples

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[1] Sartorius-Werke AG, Gottingen, Germany.

[2] Waring Products Co., Winsted, Conn.

[3] Virtis Company Inc., Gardiner, N.Y. 12525.

[4] Arthur H. Thomas Co., Philadelphia.

using the method described by Bligh and Dyer (1959). Methyl esters of the fatty acids were prepared according to the method of Metcalfe et al. (1966). Methyl esters were stored under nitrogen in screw-top glass vials at -10°C prior to gas-liquid chromatographic (GLC) analysis.

The fatty acid methyl esters were separated using an Aerograph gas chromatograph (Model 1740-1)[1] equipped with dual columns, flame ionization detectors, a Varian Aerograph single pen recorder (Model 20)[1] and a Varian Aerograph digital integrator (Model 477)[1]. Helium[2] served as the carrier gas.

Samples were resolved on 2.7m x 3.2mm steel columns packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q[3]. The flow rates were 30 ml/min. for helium[2], 25 ml/min. for hydrogen[2] and 250 ml/min. for air[2]. The columns were operated isothermally at a temperature of 200°C, with injector and detector temperatures maintained at 250°C and 230°C, respectively. The individual fatty acids were identified by comparing retention times with known fatty acid mixtures[4].

E.2. Fecal Samples: Lipid was extracted from lyophilized feces according to Extraction Method B of Van de Kamer et al. (1949). The lipid thus

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[1]Varian Aerograph, 6358 Viscount Rd., Malton, Ontario.

[2]Welder's Supplies, 25 McPhillips St., Winnipeg 3, Manitoba.

[3]Applied Science Laboratories Inc., P.O. Box 440, State College, Pa. 16801.

[4]Hormel Institute, Lipids Preparation Laboratory, 801-16th Ave. N.E., Austin, Minn. 55912.

obtained was partitioned into the fatty acid and non-saponifiable fractions (NSF) utilizing the Hamilton and McDonald (1971) modification of the method of Van de Kamer (1953). Methyl esters of the fecal fatty acids were prepared and the fatty acids resolved as previously described for food.

Fecal steroid was extracted and partitioned into bile acids and neutral sterol components using a modification of the methods described by Grundy et al. (1965) and Miettinen et al. (1965). The following modifications were made: Neutral sterol and bile acid fractions were allowed to separate using separatory funnels instead of centrifugation; Thin layer chromatography, for purposes of purification of samples, was found to be unnecessary; Trimethylsilylation (TMS) ethers were evaporated to dryness under nitrogen and redissolved in dry ethyl acetate prior to GLC analysis instead of subjecting the silylating mixture directly to GLC analysis. Conditions of GLC analysis also differed, from those described in the original methods. Neutral steroid and bile acid TMS ethers were separated on the Varian Aerograph gas chromatograph previously described, using helium as a carrier gas at a flow rate of 20 ml/min. for helium, 25 ml/min. for hydrogen and 250 ml/min. for air. Samples were injected into 6' x 1/8" O.D. columns packed with 2% QF1 on 100/120 mesh Chromosorb W H.P.[1]. The columns were operated isothermally at a temperature of 240°C with injector and detector temperatures maintained at 250°C and 260°C respectively.

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[1]Applied Science Laboratories, P.O. Box 440, State College, Pa. 16801.

Neutral sterols and bile acids were identified by comparison with the retention times of TMS ethers of standard mixtures.[1]

The recovery of radioactive cholesterol-4-C^{14} and cholic-carboxyl-24-C^{14}, added to fecal samples as internal standards, was determined using a Liquid Scintillation Spectrometer (Nuclear-Chicago, Series 720)[2], equipped with a Sample Programmer Module (Model 8260)[2], a Scaler/Timer Module (Model 8251)[2], and an Analyzer Module (Model 811260)[2]. Efficiency of counting was ascertained by the Channels Ratio Method (Wang and Willis 1965).

F. STATISTICAL ANALYSES

Statistical analyses for the differences between group means were performed according to the method of the Paired T test (Steel and Torrie 1960).

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[1]Applied Science Laboratories, P.O. Box 440, State College, Pa. 16801.

[2]Nuclear-Chicago Corp., 33 East Howard Ave., Des Plaines, Illinois.

RESULTS AND DISCUSSION

A. DIETS

The basal test diet (Table 6) with either edible beef tallow (Period I) or corn oil (Period II), as the sole sources of added dietary fat, was well accepted by the six subjects during the two 22-day test periods. Adjustment of caloric intakes was successful, as evidenced by the fact that the subjects maintained body weight within one kilogram (Table 1) throughout both test periods. No digestive upsets were reported, other than increased flatulence which would be expected with soybean protein. The formulation of a solid diet incorporating customary menus and yet permitting the testing of a single fat source (either saturated or unsaturated), represents an improvement in experimental conditions for the study of the effect of a dietary fat source on the excretion of cholesterol and its metabolites. Many investigators (Lindstedt et al. 1965; Spritz et al. 1965; Wood et al. 1966; Connor et al. 1969; Grundy and Ahrens 1970) have utilized liquid formula test diets, despite the fact that liquid formula diets have been found to produce alterations in bile acid excretion, plant sterol recovery and serum lipid levels, independent of fat contained in the formulae. Several investigators (Goldsmith et al. 1960; Eneroth et al. 1964; Moore et al. 1968) also have fed solid diets but the true effect of the test fat on cholesterol metabolism may have been confounded by the presence of other dietary fats in items such as meat and milk. In the diets used by Goldsmith et al.

(1960), for example, 22% of the fat calories were derived from fat sources other than the test fat, and in the study by Eneroth et al. (1964), 33-49% of the dietary fat was derived from fat sources other than the fat being tested. Similarly, Moore et al. (1968), used a basal diet of "3 glasses of milk and two servings of meat" to which they added products made with the test fat. The basal diet thus contributed up to 30% of daily fat calories.

Analyzed values for the fat content of individual menu items compared reasonably well with the fat content calculated from recipes and food composition tables, although the analyzed values were 12-15% lower in every case (Table 5).

Similarly, analysis of aliquots of a composite of the total daily menu gave daily fat intake values that were lower than the calculated intakes. The differences between analyzed fat content and calculations from the amount of fat added to the recipes, could be due to losses on pots, utensils and serving dishes in preparation and transfer.

Fatty acid composition of the two test diets is given in Table 8.

B. FAT DIGESTIBILITY

Apparent digestibility of total fat and individual fatty acids is given in Table 9. Only the five subjects who completed both periods (tallow and corn oil) and thus served as their own controls, have been considered in comparisons to be discussed in this and subsequent sections. Data for subjects M.T. and R.H., who participated in Period I

Table 8

Fatty Acid Composition of Test Diets

Fatty Acid		% of Total Fatty Acids[1]	
		Beef Tallow Diet	Corn Oil Diet
Myristic	(C14:0)[2]	2.7	-
Palmitic	(C16:0)	24.5	12.4
Stearic	(C18:0)	23.5	2.9
Oleic	(C18:1)	39.5	30.4
Linoelic	(C18:2)	3.6	50.7
Linolenic	(C18:3)	-	1.0

[1]Fatty acids contributing less than 1.0% of the total have not been reported.

[2]Carbon number: number of double bonds.

Table 9

Apparent Digestibility of Total Fat and Individual Fatty Acids[1]

Subject	Period	% digestibility						
		Total fat	Individual fatty acids					
			C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
P.B.	I	92.5	97.3	93.1	88.7	98.5	92.4	
L.R.		92.6	98.0	94.5	93.0	97.9	93.0	
C.W.		92.5	96.9	92.8	89.2	98.4	95.3	
C.B.		93.0	98.4	93.5	90.7	98.6	95.1	
V.M.		92.5	98.1	92.5	88.5	98.5	96.4	
Mean		92.6[a]	97.6	93.3[a]	90.0	98.4	94.4[a]	
± S.D.		± 0.2	± 1.0	± 0.8	± 1.9	± 0.3	± 1.7	
P.B.	II	96.5		97.7	91.0	98.4	99.5	98.7
L.R.		97.2		98.3	89.2	99.1	97.7	98.5
C.W.		96.1		97.5	86.7	98.7	99.7	100.0
C.B.		94.1		95.6	93.0	94.1	98.9	99.0
V.M.		96.3		97.8	90.4	99.1	99.6	99.5
Mean		96.0[a]		97.4[a]	90.1	97.9	99.1[a]	99.1
± S.D.		± 1.2		± 1.0	± 2.3	± 2.1	± 0.8	± 0.6

[1] Means within columns marked with the same letter significantly different (a = $P < 0.005$).

and Period II respectively, are given in Appendix Tables 2 to 4. Apparent digestibility of dietary fat was significantly lower ($P < 0.005$) for beef tallow (Period I) than for corn oil (Period II). Lower apparent digestibility of stearic acid (C18:0) and palmitic acid (C16:0), which together comprise 48% of the total fatty acids in edible beef tallow, would appear to be responsible for the lower digestibility of beef tallow. Apparent digestibility of stearic and palmitic acids also was found to be lower than that of other major fatty acids in the diet of pigs fed different dietary fats (Hamilton and McDonald 1969). Similarly, Grande et al. (1970), observed a small increase in total fatty acid excretion when subjects were fed a high proportion of stearic acid. However, they did not consider the increased fat loss associated with stearic acid as being of importance, because it was not associated with any change in body weight. In the present study, a lower digestibility of stearic acid also was noted with the corn oil diet, but the same did not apply to palmitic acid. Young and Garrett (1963) have reported the absorption of palmitic and stearic acids by chicks fed fatty acids was enhanced by the presence of oleic and linoleic acids. In addition, these authors found that the ratio of free palmitic to free stearic acid in the diet also influenced absorption. Palmitic and stearic acids, when fed together as a significant portion of the dietary fat, tended to depress the absorption of each other. This latter phenomenon appears to explain the present observations with beef tallow, where stearic and palmitic acids each supply about 24% of the total fatty acids. In contrast, stearic

acid contributes only about 3% of the total fatty acids in corn oil, and there is a high proportion of long chain unsaturated fatty acids which promote the absorption of palmitic acid.

The low apparent digestibility of linoleic (C18:2) acid for the beef tallow diet could be attributed to the low level (3.6%) of this fatty acid in the diet. Endogenous linoleic would thus contribute more significantly to the linoleic acid recovered in the feces. This phenomenon also may explain the low apparent digestibility of stearic acid as compared to palmitic acid when the corn oil diet was fed.

The mean total fat digestibility of beef tallow (92.6%) was slightly below the range of values (94-98%) reported for the majority of food fats in man (Grande et al. 1970), while corn oil was found to be well digested and absorbed (apparent digestibility of 96.0%). The apparent digestibility for total fat was lower than the digestibility of the individual fatty acids. This low digestibility for total fat can be attributed to the fact that fecal lipids contain a number of minor components of fatty acids not present in the diet or present in very small amounts (less than 1.0%).

The higher digestibility of corn oil would account for the lower percentage of fat in the feces recovered during the corn oil period as compared to the beef tallow period (Table 10). Total lipid excretion on the corn oil diet was found to be approximately half that on the beef tallow diet, while fatty acid excretion decreased to approximately one third when corn oil was substituted for tallow (Table 11). Average

Table 10

Total 5-day Fat Excretion by Subjects Fed Tallow and Corn Oil Diets.

Subject	Period I (beef tallow)		Period II (corn oil)	
	total fat (g)	% of fecal dry wt.	total fat (g)	% of fecal dry wt.
P.B.	35.3	17.7	16.7	12.8
L.R.	42.7	20.5	15.6	13.5
C.W.	43.7	20.6	23.8	11.6
C.B.	42.5	17.3	31.9	13.9
V.M.	45.8	19.1	22.1	10.9
Mean ± S.D.	42.0[a] ± 4.0	19.0 ± 1.5	22.0[a] ± 6.5	12.5 ± 1.3
Average daily fat excretion	8.4 ± 0.8		4.4 ± 1.3	

[a] Means significantly different ($P < 0.005$).

daily fat excretion on the beef tallow diet was not, however, in excess of the average losses of 5-7% of intake observed in normal individuals on an ordinary mixed fat diet (Bollman 1968). Thus with an average analyzed fat intake of 125 gm. fat/day (Table 5), normal fat excretion would be expected to be 6.3-8.8 gms. daily. The average fat excretion during the beef tallow regimen was 8.4 gm./day.

C. EXCRETION OF NON-SAPONIFIABLE LIPID

Fecal fat was separated by liquid/liquid partition (Hamilton and McDonald 1971) into fatty acid and non-saponifiable (NSF) fractions (Table 11). The excretion of NSF was significantly higher ($P < 0.05$) when the test diet contained corn oil than when beef tallow served as the sole source of dietary fat. NSF excretion on the tallow diet averaged 7.6 gm. for the 5-day period which was only 73% of the level (10.4 gm.) excreted during the corn oil period. Increased NSF excretion with polyunsaturated fats has been noted by many other investigators. Haust and Beveridge (1963) reported that NSF excretion on a corn oil diet was approximately eighteen times the NSF excretion on a fat-free diet. Wood et al. (1966) and Moore et al. (1968), found NSF excretion with polyunsaturated fats to be approximately 1¼ to 1½ times the NSF excretion with a saturated fat source. In contrast, Spritz et al. (1965) found no significant changes in the excretion of total fecal steroids in studies on five subjects fed diets of varying fatty acid composition. These conflicting observations may be due to the type of subject used in the study.

Table 11

Percent Contribution of Fatty Acids and NSF to Total Fecal Lipid[1]

Subject	Period	Total fat exc./5 days (g)	Total FA exc./5 days (g)	% FA in fecal fat	Total NSF exc./5 days (g)	% NSF in fecal fat
P.B.	I	35.3	26.4	74.8	5.9	16.7
L.R.		42.7	28.7	67.1	8.2	19.2
C.W.		43.7	33.1	75.6	9.0	20.5
C.B.		42.5	30.4	70.7	7.2	17.0
V.M.		45.8	33.8	73.4	7.8	17.0
Mean ± S.D.		42.0 ± 4.0[a]	30.5 ± 3.1[a]	72.3 ± 3.5[a]	7.6 ±1.2[b]	18.1 ± 1.7[a]
P.B.	II	16.7	6.3	37.5	9.1	54.3
L.R.		15.6	6.7	43.1	7.2	46.0
C.W.		23.8	10.7	44.9	12.8	53.8
C.B.		31.9	17.4	54.4	11.4	35.9
V.M.		22.1	7.8	35.4	11.6	52.3
Mean ± S.D.		22.0 ± 6.5[a]	9.8 ± 4.6[a]	43.1 ± 7.4[a]	10.4 2.2[b]	48.5 ± 7.8[a]

[1] Means within a column marked with the same letter significantly different (a = $P < 0.005$; b = $P < 0.025$).

Spritz et al. (1965) used individuals who were hypercholesterolemic, whereas the subjects in the present study, and the three studies cited above, had normal lipid patterns. It may be that hypercholesterolemic subjects do not respond normally to alterations in dietary fat.

A portion of the increased NSF excreted with the corn oil diet can be attributed to the higher intake of unabsorbable plant sterols during this period (Table 12).

D. PARTITION OF NON-SAPONIFIABLE LIPID

D.1. Neutral Steroids

Neutral sterols separated from the non-saponifiable fraction (NSF) of dietary lipid were further resolved by gas-liquid chromatography (Miettinen et al. 1965). The main dietary sterols identified were cholesterol and the three plant sterols, β-sitosterol, and campesterol plus stigmasterol (Figures I-III). Gas-liquid chromatography of neutral steroid standards[1] revealed that campesterol and stigmasterol were not resolved under the conditions used in the present study (Figures I and III). Hence these two components were calculated together for the purpose of quantitation.

Table 12 lists the 5-day cholesterol and plant sterol (campesterol plus stigmasterol and β-sitosterol) intakes. There was a relatively small amount of cholesterol in both test diets, with the level in the beef tallow diet being nearly twice that of the corn oil diet. The intake of

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

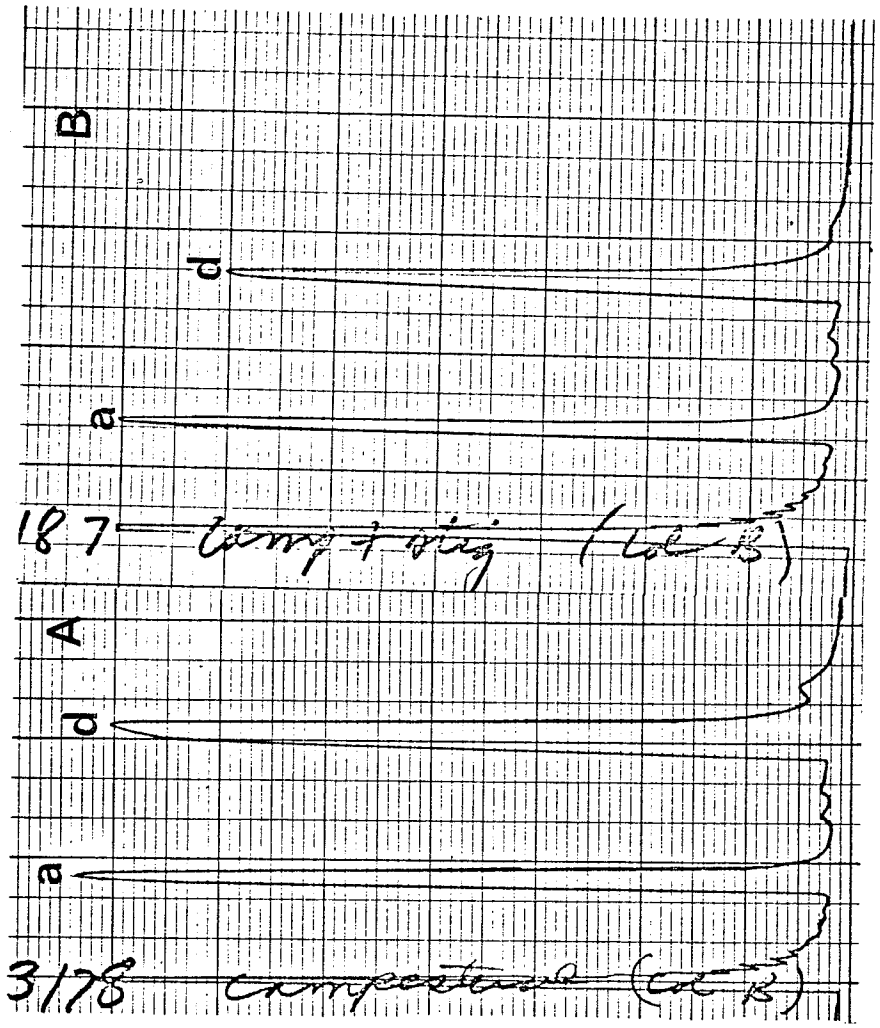

Figure I. GLC chromatograms of TMS ethers of neutral steroid standards.

A: 5-α-cholestane (a) and campesterol (d).
B: 5-α-cholestane (a) campesterol plus stigmasterol (d).

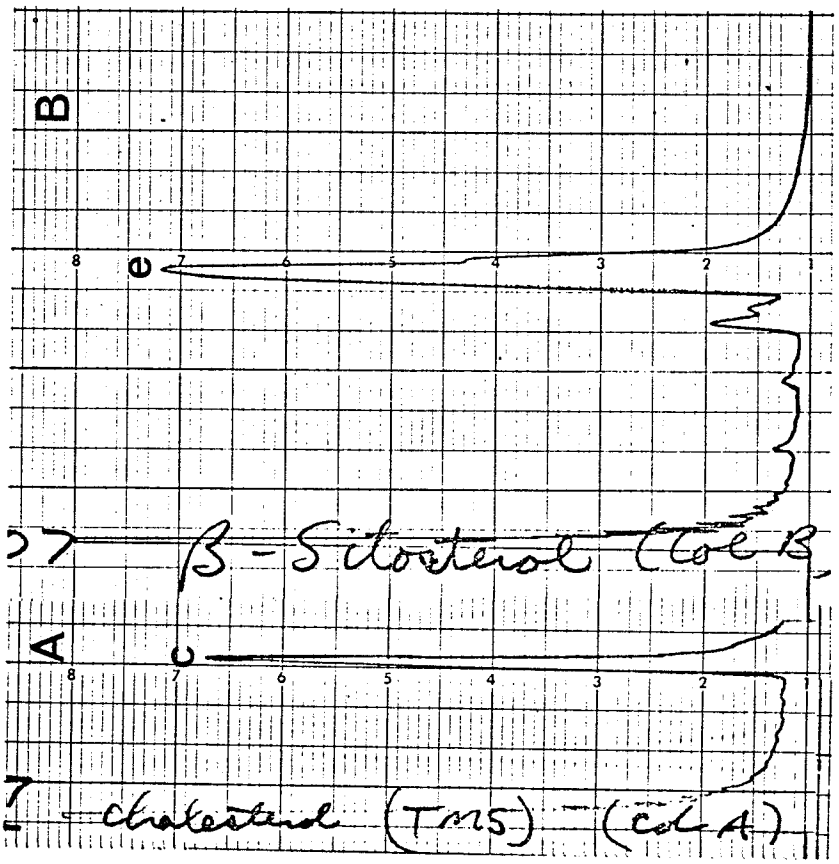

Figure II. GLC Chromatograms of TMS ethers of neutral steroid standards.

A: Cholesterol (c).
B: β-sitosterol (e).

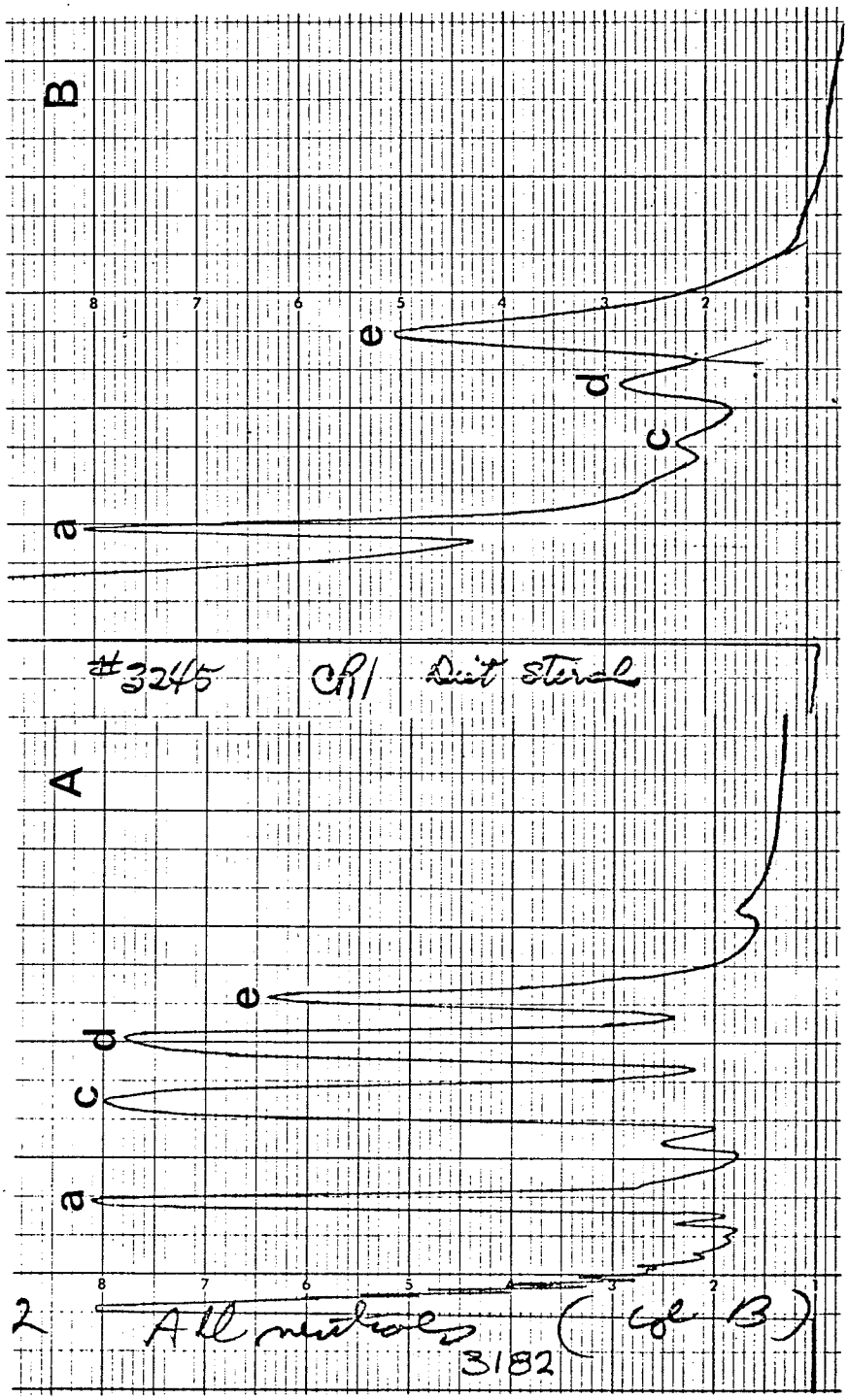

Figure III: GLC chromatograms of TMS ethers of neutral steroid standards and dietary sterols.

A. Standards.

B. Sterols from a diet containing corn oil.

Key: 5-α-cholestane (a), cholesterol (c), campesterol + stigmasterol (d), and β-sitosterol (e).

Table 12

Average Neutral Sterol Intake During a 5-Day Period for Subjects Fed Beef Tallow and Corn Oil

Period	cholesterol (mg)	Plant sterols (mg.)		
		campesterol + stigmasterol	β-sitosterol	total plant sterol
I (Beef Tallow)	474	139	510	649
II (Corn oil)	240	1035	3957	4992

plant sterols, however, was much higher on the corn oil diet. The origin of the plant sterols in the beef tallow diet is uncertain. Eneroth et al. (1964) also reported encountering plant sterols in the feces of subjects on a butter diet and attributed the source of these sterols to the bread, fruit and marmalade consumed by their subjects. The ratio of campesterol plus stigmasterol/ β-Sitosterol was 21:79 for both the tallow and corn oil diets which corresponds very closely to the 18:7:75 ratio for campesterol/stigmasterol/ β-sitosterol in the corn oil diet used by Miettinen et al. (1965).

The NSF of fecal lipid was partitioned into neutral and acidic (bile acid) steroids according to the method of Miettinen et al. (1965). Each of these fractions were then further resolved by GLC chromatography. The main neutral steroids identified were cholesterol, coprostanol (the primary intestinal breakdown product of cholesterol), and β-sitosterol. A representative chromatogram of fecal neutral steroids is shown in Figure IV. The retention time for component "d" was essentially identical with that of campesterol and stigmasterol (Figure I) and the peak was initially identified as campesterol plus stigmasterol. However, it should be noted that the level of campesterol plus stigmasterol in the feces was considerably greater than that of β-sitosterol for all subjects, in both Period I and Period II, whereas the reverse situation prevailed in the diets.

Since campesterol and stigmasterol are not absorbed, their recovery should parallel that of β-sitosterol. Eneroth et al. (1964) compared

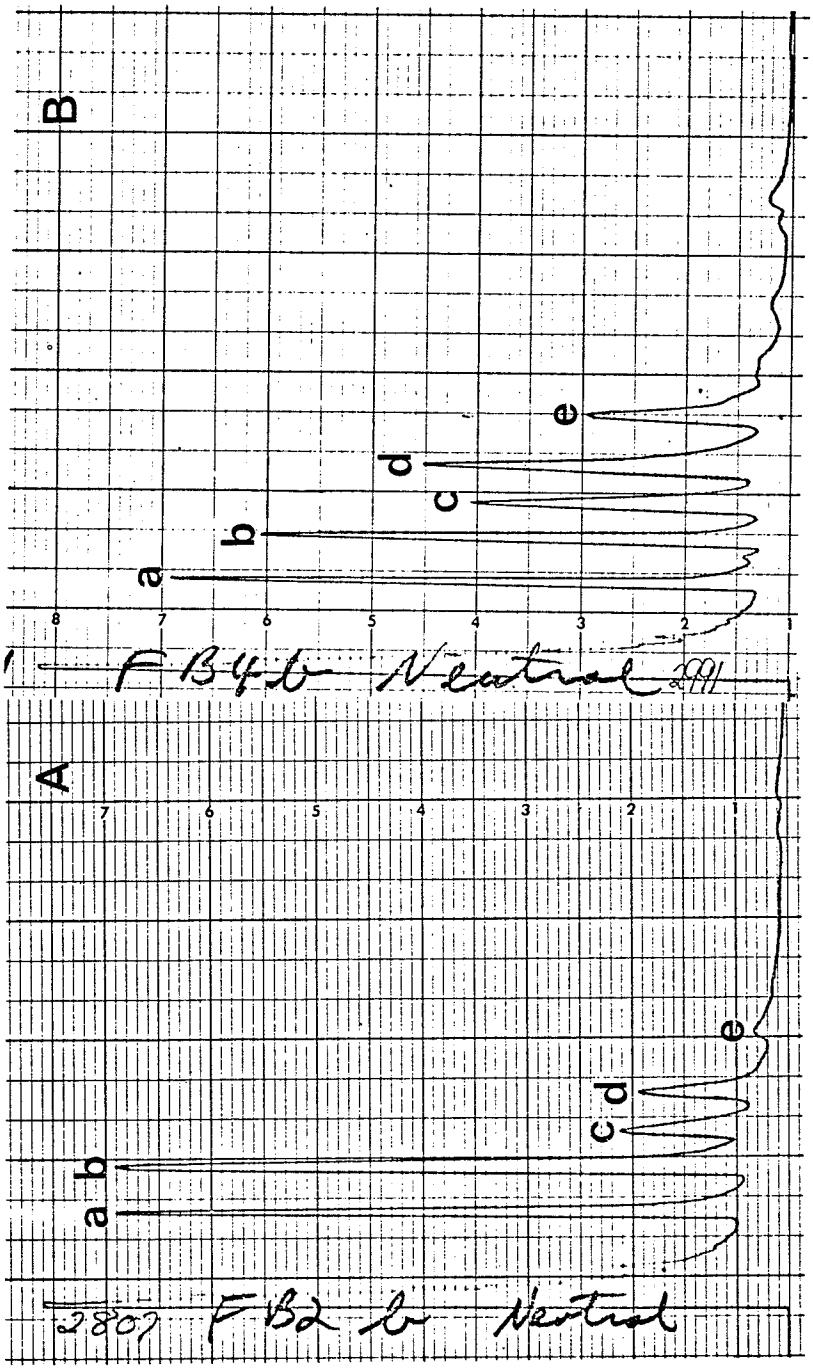

Figure IV. GLC chromatograms of TMS ethers of fecal neutral steroids
A. Beef tallow - Period I.
B. Corn oil - Period II.
Key: 5-α-cholestane (a), coprostanol (b), cholesterol (c), campesterol plus stigmasterol (d), and β-sitosterol (e).

the excretion of neutral steroid in subjects fed butter and corn oil and found the excretion of campesterol (methylcholesterol) was less than the excretion of β-sitosterol in all cases. No stigmasterol was found in the feces of these subjects. Unfortunately, the authors did not report the plant sterol intake for the butter diet, nor the total plant sterol intake for either diets. In addition, Miettinen et al. (1965), in studies on a single subject, found that the ratio of the three plant sterols (and their conversion products) in the feces was the same as that of the diet (campesterol/stigmasterol/ β-sitosterol, 18:7:75). As mentioned previously, the ratio of campesterol plus stigmasterol/ β-sitosterol was 21:79 in the test diets of the present study. The ratio of the three sterols was noticeably altered in the feces, with the component or components identified as campesterol plus stigmasterol contributing the higher proportion of the fecal plant sterols (Figure III,B versus Figures IV,A & B).

The explanation for the discrepancy in campesterol and stigmasterol excretion in the present study is not immediately clear. Miettinen et al. (1965) found that preliminary separation of neutral steroids by thin layer chromatography is necessary to prevent the overlapping of the 3-keto and 5- β -saturated derivatives of plant sterol with campesterol and stigmasterol. This separation was not performed in the present study. Hence it is suggested that the unidentified components contributing to the area of the campesterol plus stigmasterol peak (Figure IV) are the 3-keto and saturated 5- β -homologs of the three dietary plant sterols.

Since Miettinen et al. (1965) reported that the ring-saturated homologs of the major plant sterols appeared in the feces in about the same proportion as in the diet, the ring-saturated homolog of β-sitosterol would be expected to be a major contributor to the peak identified as campesterol plus stigmasterol. If this can be assumed, then the total area of the peaks representing β-sitosterol and campesterol plus stigmasterol would include their conversion products and thus represent the total recovery of plant sterols.

It may be, however, that an unidentified fecal steroid of cholesterol origin chromatographs with the same retention time as campesterol plus stigmasterol, thus contributing to the increased concentration of these components. It is apparent from the representative chromatograms (Figure IV) that the larger campesterol plus stigmasterol peak cannot be explained by tailing error.

Observed excretion data for a five-day period are given in Table 13. The mean excretion of cholesterol was significantly higher ($P < 0.025$) on the corn oil diet than on the beef tallow diet, while there was no significant change in mean coprostanol excretion between the two test periods. However, the excretion of total neutral steroid derived from cholesterol (cholesterol plus coprostanol) was not significantly different ($0.05 < P < 0.10$) for the five subjects between Period I and Period II. In spite of the lack of significance at the 5% level between Period I and Period II for the excretion of cholesterol-derived steroids, excretion of the combined products on the corn oil diet was 1.4 times that on the tallow diet,

Table 13

Total 5-Day Excretion of Major Neutral Steroids by Subjects Fed Tallow and Corn Oil Diets[1]

Subject	Period	Neutral steroids (g)						% recovery total plant sterol
		coprostanol (1)	cholesterol (2)	Total (1 + 2)	campesterol + stigmasterol (3)	β-sitosterol (4)	Total plant sterol (3 + 4)	
P.B.	I (Tallow)	1.772	0.696	2.468	0.590	0.259	0.849	130.8
L.R.		3.729	0.714	4.443	0.909	0.370	1.279	197.1
C.W.		3.293	0.808	4.101	0.806	0.250	1.056	162.7
C.B.		1.730	0.990	2.720	0.522	0.176	0.698	107.6
V.M.		4.651	1.043	5.694	0.886	0.226	1.112	171.3
Mean		3.035	0.850[a]	3.885[c]	0.743[b]	0.256[b]	0.999[b]	153.9[b]
± S.D.		±1.271	±0.158	±1.322	±0.176	±0.073	±0.228	± 35.1
P.B.	II (Corn Oil)	2.033	1.476	3.509	1.902	1.497	3.399	68.1
L.R.		2.168	1.102	3.270	1.933	0.718	2.651	53.1
C.W.		3.481	3.146	6.627	5.140	2.710	7.850	157.3
C.B.		3.788	2.989	6.777	3.857	2.164	6.021	120.6
V.M.		4.358	2.412	6.770	3.514	1.351	4.865	97.5
Mean		3.166	2.225[a]	5.391[c]	3.269[b]	1.688[b]	4.957[b]	99.3[b]
± S.D.		±1.023	±0.907	±1.830	±1.375	±0.768	±2.077	± 41.6

[1] Means within a column marked with the same letter differ significantly (a = $P < 0.025$); b = $P < 0.01$; c= $P < 0.10$).

and cholesterol excretion on the corn oil diet was 2.5 times that on the tallow diet. Furthermore, all subjects, except L.R., excreted more neutral steroids derived from cholesterol during Period II than Period I. The fact that the total dry weight of feces excreted by L.R. during Period II was only 56% that excreted during Period I may account for the failure of this subject to follow the pattern observed for the other subjects. The apparent incomplete collection of feces for this subject also might account for the failure to find a statistically significant difference in the mean excretion of neutral steroids derived from cholesterol between Period I and Period II.

Increased plant sterol excretion during the corn oil period was due to higher levels of these sterols in the corn oil diet. Of interest is the fact that the recovery of plant sterols for L.R. (Table 13) is lower that for the other subjects, although recovery for two subjects in Period II and all subjects in Period I was greater than 100%. The reason that the recoveries of total plant sterol are greater than the levels ingested is not immediately clear. Grundy and Ahrens (1970) have reported β-sitosterol recoveries of greater than 100% for three of 11 subjects studied for periods ranging from 29 - 128 days. Connor et al. (1969) also observed total plant sterol recoveries ranging up to 170% for a 3-week stool collection period.

In spite of the lack of an explanation for the fact that recoveries of plant sterol may exceed that ingested, even during long collection periods, Grundy et al. (1968) have suggested that the fractional recovery

of β-sitosterol (and fecal steroids derived from it) can be used as a marker of fecal flow and cholesterol recovery. They state that losses of labelled cholesterol have been shown to be quantitatively identical to the observed losses of β-sitosterol, and attributed the losses of β-sitosterol during intestinal transit to destruction of the steroid ring by intestinal microorganisms (Grundy et al. 1969).

In the present study, the total recovery of β-sitosterol could not be accurately assessed due to the overlap of plant sterol degradation products with campesterol and stigmasterol (Figure IV). However, Connor et al. (1969) apparently used total plant sterol as an internal recovery standard. Since the recovery of total plant sterol should parallel that of β-sitosterol (Miettinen et al. 1965), the fractional recovery of total plant sterol was used as a correction factor for the recovery of cholesterol and coprostanol in the present study. Corrected neutral steroid excretion values, adjusted using the intake of total dietary plant sterols as an internal standard, are presented in Table 14. It is evident that when this correction is applied, there is much less deviation among individual values within a test period. Furthermore, when the data are adjusted on the basis of plant sterol excretion, the excretion of neutral steroids derived from cholesterol is significantly greater ($P < 0.005$) on the corn oil than on the tallow diet.

D.2. Bile Acids

The major bile acids in the acidic sterol portion of fecal NSF were identified as lithocholic and chenodeoxycholic and/or cholic acids.

Table 14

Corrected Total 5-day Excretion of Cholesterol and Coprostanol by Subjects Fed Beef Tallow and Corn Oil Diets[1]

Subject	Period	Neutral steroids of Cholesterol and Derivatives (g)			% recovery total plant sterol
		Coprostanol (1)	Cholesterol (2)	Total (1 + 2)	
P.B.	I (Tallow)	1.355	0.532	1.887	130.8
L.R.		1.892	0.362	2.254	197.1
C.W.		2.024	0.497	2.521	162.7
C.B.		1.608	0.920	2.528	107.6
V.M.		2.715	0.609	3.324	171.3
Mean		1.919[a]	0.584[b]	2.503[b]	153.9[c]
± S.D.		±0.514	±0.208	±0.528	± 35.1
P.B.	II (Corn oil)	2.985	2.167	5.152	68.1
L.R.		3.878	2.094	6.045	53.1
C.W.		2.213	2.000	4.213	157.3
C.B.		3.141	2.478	5.619	120.6
V.M.		4.471	2.474	6.945	97.5
Mean		3.338[a]	2.242[b]	5.595[b]	99.3[c]
± S.D.		±0.867	±0.221	±1.016	± 41.6

[1] Means within a column marked with the same letter differ significantly ($a = P < 0.025$; $b = P < 0.005$; $c = P < 0.05$).

Representative chromatograms giving relative GLC retention times are shown in Figures V-VII. TMS ethers of cholic and chenodeoxycholic acid standards were found to chromatograph with similar retention times (Figure V) and hence have been considered together in the quantitative data. Minor components shown in the fecal chromatograms (Figure VIII) were not identified.

There was no significant difference between Period I and Period II in the mean excretion of bile acids (Table 15). However there was considerable individual variation in bile acid excretion, with three of the subjects excreting more of the major bile acids on the beef tallow diet than on the corn oil diet, while the reverse situation prevailed for the other two subjects.

The appreciable variation in bile acid excretion observed among subjects in the present study is similar to that observed by Avigan and Steinberg (1965). Similarly, Avigan and Steinberg (1965) did not find a significant increase in mean bile acid excretion when corn oil replaced coconut oil in the diet. Bile acid excretion with coconut oil was slightly higher for two subjects and lower in three subjects when compared to the corn oil diet. A sixth subject in the same study showed no alterations in bile acid excretion on either diet. Ali et al. (1966) also studied changes in bile acid excretion in three normocholesterolemic subjects and found no significant differences in bile acid excretion among subjects fed butter, hydrogenated corn oil, and corn oil diets. Ali et al. (1966) also reported marked variation among subjects in response to the various dietary fats.

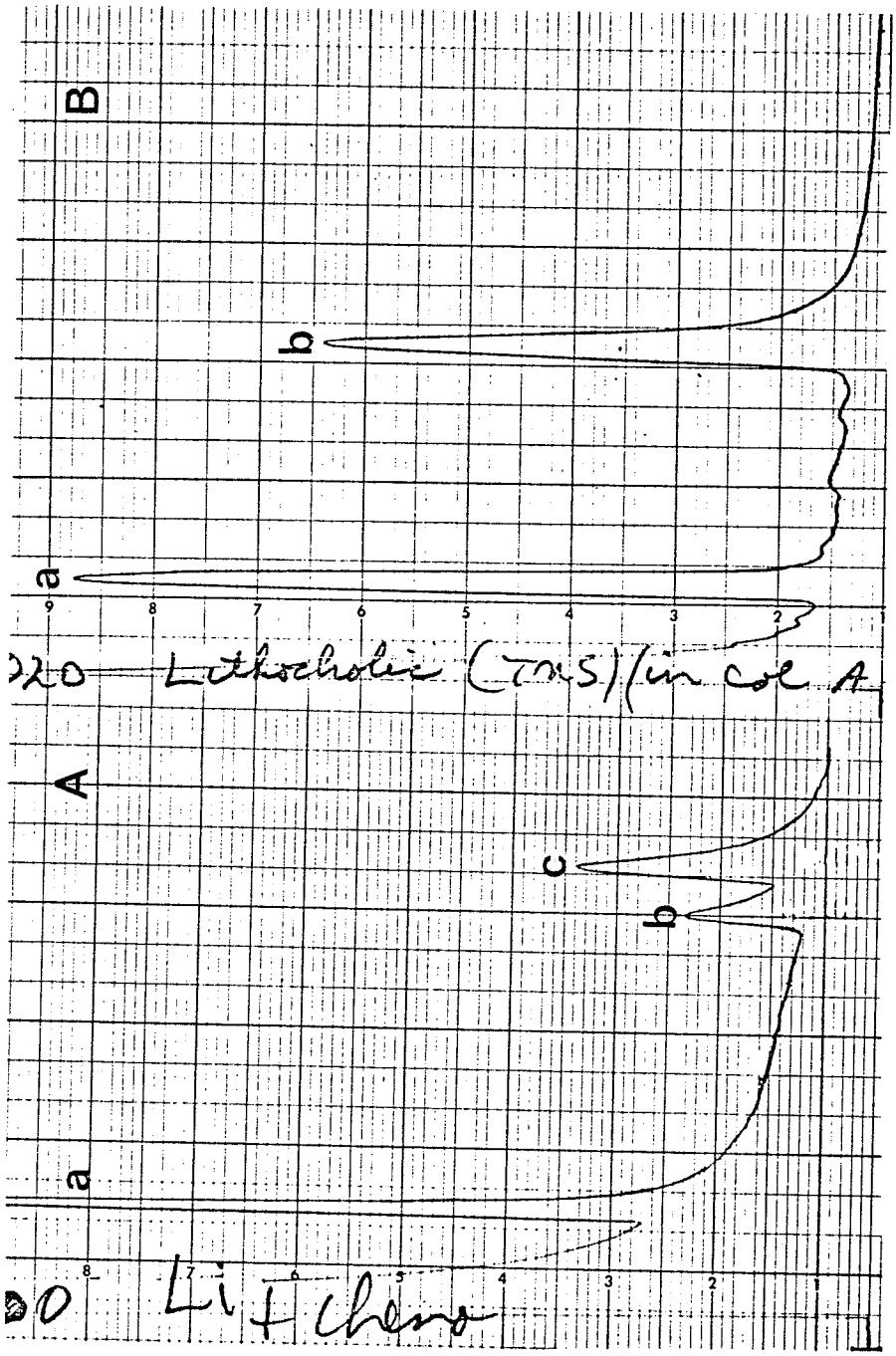

Figure V. GLC chromatograms of TMS ethers of bile acid standards.
A: 5-α-cholestane (a), lithocholic acid (b), and chenodeoxycholic acid (c).
B: 5-α-cholestane (a), lithocholic acid (b).

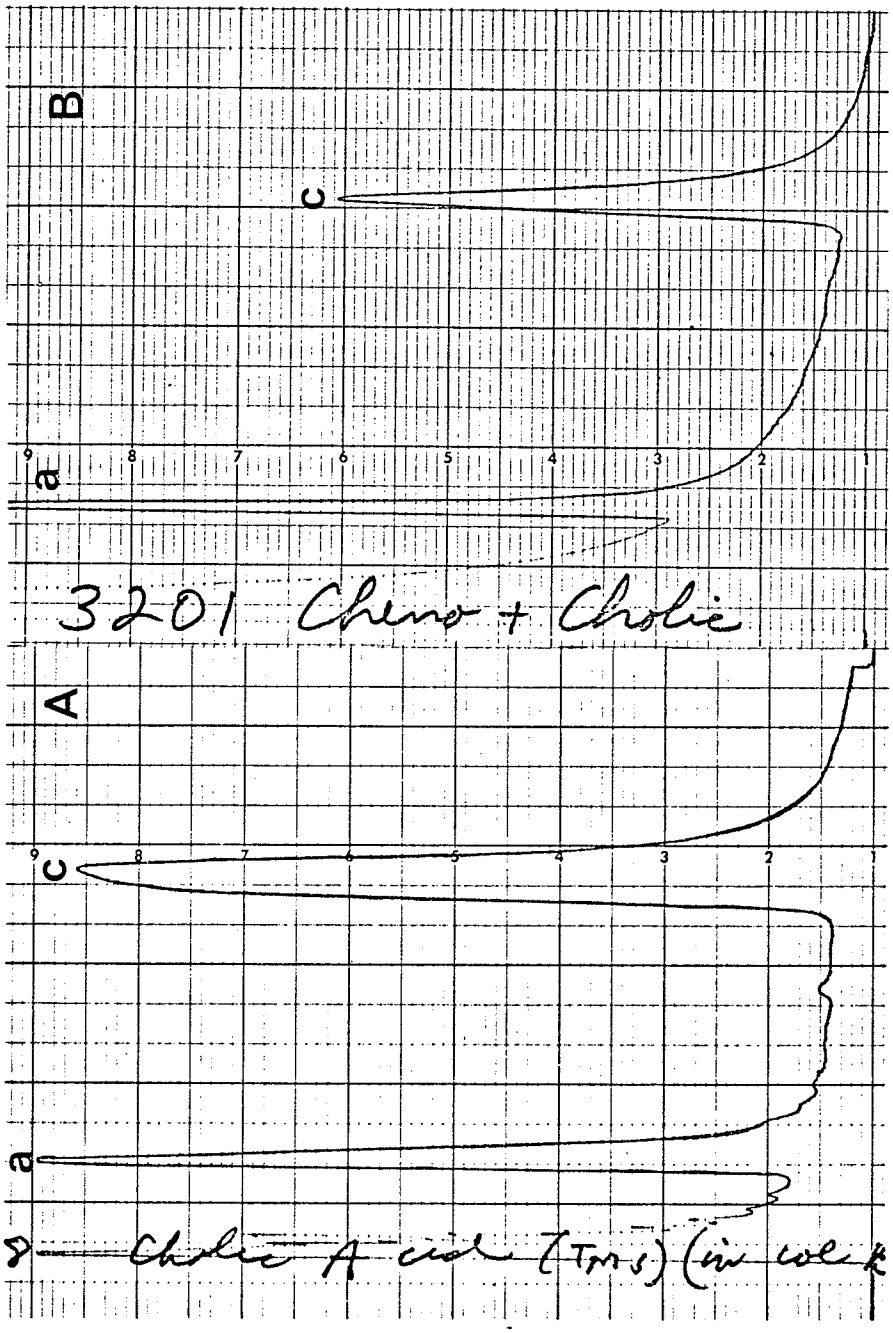

Figure VI. GLC chromatograms of TMS ethers of bile acid standards.
A: 5-α-cholestane (a) and cholic acid (c).
B: 5-α-cholestane (a), and cholic acid + chenodeoxycholic acid (c).

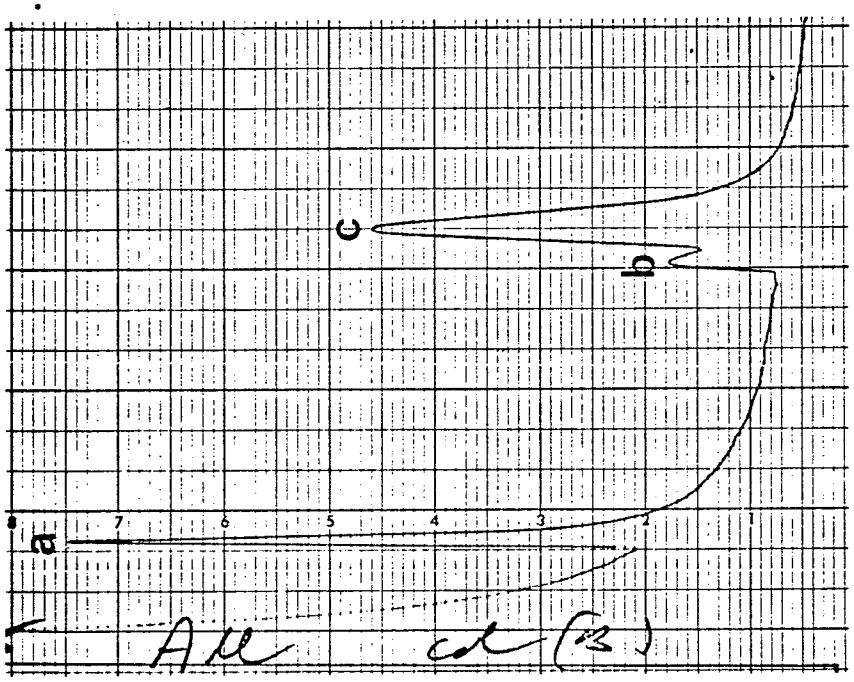

Figure VII. GLC chromatograms of TMS ethers of bile acid standards: 5-α-cholestane (a), lithocholic acid (b), and cholic + chenodeoxycholic acids (c).

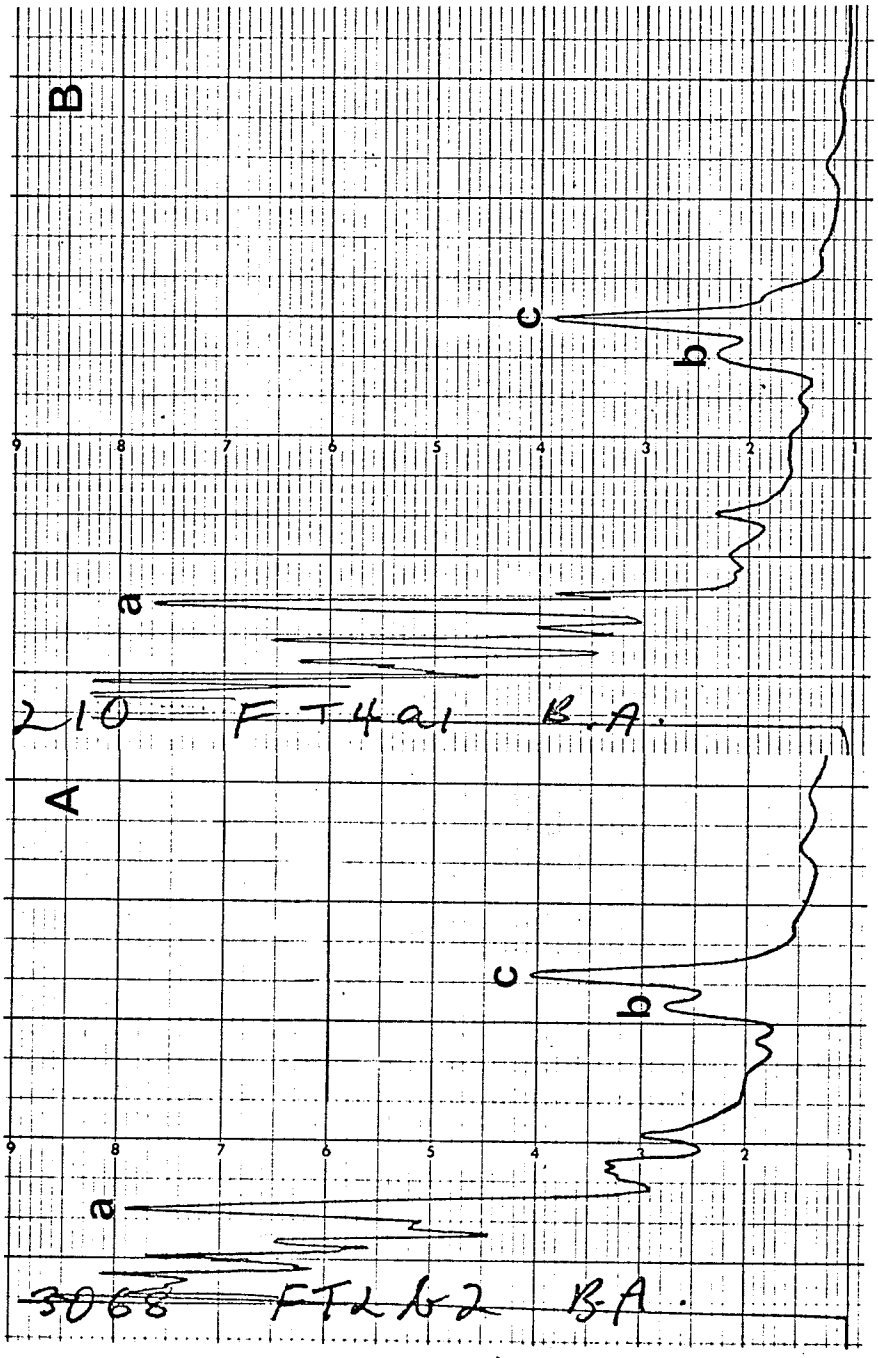

Figure VIII. GLC chromatograms of TMS ethers of fecal bile acids.
A. Beef tallow - Period I.
B. Corn oil - Period II.
Key: 5-α-cholestane (a), lithocholic acid (b) and cholic + chenodeoxycholic acids (c).

Table 15

Total 5-day Excretion of Major Bile Acids by Subjects Fed Tallow and Corn Oil Diets

Subject	Period	Bile acids (mg.)	
		Lithocholic	cholic + chenodeoxycholic
P.B.	I (Tallow)	51.6	107.6
L.R.		134.3	187.0
C.W.		67.0	106.1
C.B.		29.6	72.0
V.M.		98.0	223.0
Mean		76.1	139.1
± S.D.		± 41.0	± 63.1
P.B.	II (Corn Oil)	37.3	87.3
L.R.		86.9	159.5
C.W.		50.2	91.5
C.B.		64.8	159.4
V.M.		126.0	261.5
Mean		73.0	151.8
± S.D.		± 34.9	± 70.6

The average excretion of total bile acids for five of the six subjects studied by Avigan and Steinberg (1965) was 794 mg./day for the saturated fat period and 544 mg./day for the unsaturated fat period. These values are substantially higher than the present findings of 43 mg./day and 45 mg./day excretion of the three major bile acids for beef tallow and corn oil respectively, although minor bile acids would be expected to raise the daily total by a small amount. Moore et al. (1968) have also reported higher daily excretion values for total bile acids, with an average of 482 mg./day being excreted on a butter diet as compared to 548 mg./day for a safflower oil diet.

The reason for the lower excretion of bile acids in the present study is not immediately apparent. The recovery of cholic acid-C^{14}, added to the feces as an internal standard, was essentially complete. The possibility exists, however, that the conjugated bile acids were not completely hydrolyzed, although saponification in ethanolic NaOH was carried out under refluxing for one hour, followed by further saponification in an autoclave for 3 hours.

E. GENERAL DISCUSSION

Partition of non-saponifiable (NSF) lipid from feces into bile acids and neutral steroids, and resolution of major components in each of these fractions, indicated that the greater excretion of NSF lipid by subjects on the corn oil diet compared to the beef tallow diet was due not only to the higher levels of plant sterol in the corn oil diet, but also to

increased excretion of neutral steroids derived from cholesterol (cholesterol and coprostanol).

The correction of neutral steroid excretion data on the basis of plant sterol recovery was found to appreciably reduce the variation among subjects, and when this adjustment was made, the difference in total cholesterol excretion between the two periods was highly significant ($P < 0.005$). However, no explanation can be advanced to account for the fact that recovery of plant sterols in the feces was greater in several cases than the amount ingested during the 5-day collection period. Similar anomalies have been noted by Connor et al. (1969) and Grundy and Ahrens (1970), although, in general, the Rockefeller group (Grundy and Ahrens 1970; Grundy et al. 1968) have found that recovery of β-sitosterol is less than 100 per cent. In fact they propose that the recovery of β-sitosterol can be used to correct for losses of cholesterol. It is possible that the losses observed by these workers represent a phenomenon peculiar to formula-fed hypercholesterolemic subjects because it remains to be demonstrated that similar losses of the sterol nucleus occur in normal subjects on solid diets.

Since the diets contained very small amounts of cholesterol (Table 12), most of the excreted cholesterol must have come from endogenous sources. Increased excretion of endogenously-derived cholesterol in response to the feeding of polyunsaturated fat also has been noted by other investigators (Haust and Beveridge 1963; Eneroth et al. 1964; Wood et al. 1966; Moore et al. 1968; Connor et al. 1969).

Wood et al. (1966) have attributed the increased excretion of cholesterol in response to polyunsaturated fatty acids to a lowered reabsorption of endogenous cholesterol. On the other hand, Connor et al. (1969) postulated that polyunsaturated fat brings about a reduction in serum cholesterol level by increasing its excretion into the bile to be excreted as neutral steroids and bile acids. One or both of these mechanisms could account for the increased excretion of cholesterol observed on corn oil in the present study.

Connor et al. (1969) found a significant increase in cholesterol excretion when corn oil replaced cocoa butter in the diet. Cocoa butter resembles beef tallow in that it contains a high proportion of stearic acid (36%), palmitic acid (24%) and oleic acid (34%), and like beef tallow appears to lack the cholesterol-elevating effect usually associated with saturated fats. They found that mean neutral steroid (cholesterol and its metabolites) excretion was 354 mg./day during the third week on the cocoa butter diet compared to 431 mg./day during a similar collection period on the corn oil diet. These values are somewhat lower than those observed in the present study where the mean daily cholesterol (plus coprostanol) excretion during the third week (Days 18-22) with beef tallow was 777 mg. as compared to 1078 mg. on the corn oil diet. Connor et al. (1969) also observed an accompanying increase in bile acid excretion when corn oil replaced cocoa butter in the diets. In the present study, however, mean excretion of the major bile acids was extremely low on both diets and was not altered significantly when corn oil replaced beef tallow

in the test diet. No explanation is apparent for the low excretion of bile acids. Of interest, however, is the fact that total cholesterol excretion (neutral steroids plus bile acids) for the present study was higher (820 mg./day and 1123 mg./day for tallow and corn oil, respectively) than the combined steroid excretion observed by Connor et al. (652 mg./day and 857 mg./day for cocoa butter and corn oil, respectively).

Although Connor et al. (1969) tested similar fat sources and also used normal subjects, the data may differ from that observed in the present study because Connor et al. used formula diets. In the present investigation successful control of dietary fat was achieved using a solid diet incorporating customary foods. It is felt that the lipid excretion data observed in the present study will have more practical application in the assessment of changes that occur when dietary fat is altered under normal dietary conditions. The consumption of liquid diets constitutes an abnormal situation. In addition, the liquid diets per se have been associated with alterations in cholesterol metabolism irrespective of the fat contained in the formula.

Only two studies on cholesterol excretion are readily comparable to the present study (Eneroth et al. 1964, and Moore et al. 1968). Both of these studies used normal subjects, test diets of solid foods, and gas-liquid chromatographic analysis techniques, although Eneroth et al. (1964) studied only neutral steroid excretion. Both groups of investigators found an increase in neutral steroid excretion when polyunsaturated fats were fed. Moore et al. (1968) also reported an increase in bile acid

excretion with polyunsaturated fat. No significant differences were observed in the amount of major bile acids excreted on the different diets in the present study, although the quantities of bile acids excreted in the present study were appreciably less than the values reported by Moore et al. (1968). In addition, the average daily excretion of cholesterol-derived neutral steroid reported by Moore et al. (1968) was less than that observed in the present study. The variation in the average daily excretion of neutral steroid and bile acids observed by Moore et al. (1968) as compared to that observed in the present study, may be due to the fact that Moore et al. did not study a single fat source as was done in the present study. The butter and safflower oil tested by Moore et al. (1968) were added to a basal diet which contained saturated fat from meat and milk sources.

The fact that Moore et al. (1968) used butter as the saturated fat source may be relevant. Butter fat contains a predominance of short-chain fatty acids in contrast to the beef tallow, used in the present study, which is comprised primarily of long-chain saturated fatty acids. It may well be that the long-chain fatty acids do not affect steroid excretion in the same manner as the short-chain fatty acids.

The present study lends support to the contention that an increase in neutral steroid excretion accompanies the feeding of polyunsaturated fats. Neutral steroid excretion with beef tallow was significantly less than with corn oil, suggesting that beef tallow does not resemble corn oil in its effect on neutral steroid excretion. Thus the cholesterol-

lowering effect of beef tallow observed with these subjects (Losier 1972) does not appear to be related to the increased cholesterol excretion that accompanied the cholesterol-lowering effect of corn oil.

Although increased steroid excretion in response to corn oil has been attributed to its polyunsaturated fatty acid content (Connor et al. 1969), plant sterols per se have been found to increase cholesterol excretion (Grundy et al. 1969). It is interesting to speculate whether there would be any significant difference in cholesterol excretion between the two test periods if the plant sterol content of the two test diets had been identical. Plant sterol levels in the beef tallow diet were 1/8 those in the corn oil diet (Table 12).

Myristic and palmitic acids have been shown to be the primary cholesterol-elevating fatty acids in the average North American diet, whereas oleic and stearic acids have no effect on serum cholesterol (Keys et al. 1965a; 1965c). Since the level of myristic acid in beef tallow is low (2.7%), and data from the present study indicate that palmitic acid is not as well absorbed from beef tallow as from corn oil, the effective concentration of cholesterol-elevating agents in beef tallow is actually lower than would be expected on the basis of the fatty acid composition of the diet. Hence the cholesterol-lowering effect of beef tallow may be partially explained by the fact that there is a decrease in the effective concentration of palmitic acid in the presence of high levels of stearic acid.

SUMMARY AND CONCLUSIONS

A solid test diet was designed to closely resemble the normal diet but with the fat derived solely from either beef tallow or corn oil. A two-day rotating menu, which provided 40% of the total calories from fat, was formulated using fat-free textured soy protein, skim milk and egg albumin as the primary protein sources. This diet was used in two 22-day metabolic studies conducted to assess the effect of beef tallow as compared to corn oil on lipid excretion patterns in six healthy young men.

Results of 5-day digestibility trials indicated that the apparent digestibility of triglycerides from corn oil (96.0%) was significantly higher ($P < 0.005$) than the triglyceride digestibility of beef tallow (92.6%). However, fat excretion on the tallow diet did not exceed average values reported for humans fed mixed-fat diets. Apparent digestibility of stearic acid was lower than that of all other fatty acids for both diets. The low apparent digestibility of stearic and palmitic acids accounted for the lower digestibility of beef tallow.

Total non-saponifiable (NSF) lipid in feces was partitioned into neutral steroids and bile acids. The major bile acids (lithocholic, cholic and chenodeoxycholic acids), and the major cholesterol excretion products of neutral steroids (cholesterol plus coprostanol), were quantitated for the 5-day collection periods. Total NSF excretion was found to be significantly greater ($P < 0.05$) for the corn oil diet than for the beef tallow diet, with the increased NSF on the corn oil being due to increased

excretion of the major neutral steroids derived from cholesterol and plant sterols. Excretion of cholesterol was significantly higher ($P < 0.025$) on the corn oil than on the beef tallow diet, but excretion of cholesterol and its major breakdown product, coprostanol, was not significantly higher ($0.05 < P < 0.10$) on corn oil than on tallow, although excretion of cholesterol-derived neutral steroids with corn oil was 1.4 times that for beef tallow. Adjustment of excretion data for cholesterol and coprostanol, using the recovery of total plant sterols, reduced variation among individuals. Furthermore, when this adjustment was made, excretion of cholesterol-derived neutral steroid (cholesterol plus coprostanol) was significantly higher ($P < 0.005$) on corn oil than on beef tallow. Since the level of cholesterol was low in both test diets, most of the cholesterol-derived neutral steroid must have come from endogenous sources.

The excretion of major fecal bile acids was low and there were no significant differences between corn oil and beef tallow.

It was concluded that the excretion of cholesterol-derived neutral steroids associated with the hypocholesterolemic effect of beef tallow was significantly different from the neutral steroid excretion found to accompany the hypocholesterolemic effect of corn oil. The hypocholesterolemic effect of beef tallow may be partially due to depressed absorption of palmitic acid when beef tallow is fed.

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A P P E N D I X

Appendix Table 1

FATTY ACID COMPOSITION OF STABILIZATION DIETS

Fatty acid		% of total fatty acids
Lauric	(C12:0)	3.2
Myristic	(C14:0)	6.7
Palmitic	(C16:0)	24.9
Palmioleic	(C16:1)	3.7
Stearic	(C18:0)	13.3
Oleic	(C18:1)	33.4
Linoleic	(C18:2)	7.3
Linolenic	(C18:3)	trace

Appendix Table 2

APPARENT DIGESTIBILITY OF TOTAL FAT AND INDIVIDUAL FATTY ACIDS FOR SUBJECTS M.T.[1] AND R.H.[2]

Subject	Period	% digestibility						
		Total fat	Individual Fatty Acids					
			C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
M.T.	I (Tallow)	93.7	98.7	95.2	93.1	98.2	91.1	-
R.H.	II (Corn oil)	95.9	-	97.3	84.2	98.6	99.6	99.4

[1] Participated in Period I only.

[2] Participated in Period II only.

Appendix Table 3

PERCENT CONTRIBUTION OF FATTY ACIDS AND NSF TO TOTAL 5-DAY FECAL FAT EXCRETED BY SUBJECTS M.T.[1] AND R.H.[2]

Subject	Period	Total fat (g) exc./5 days	% fecal dry wt.	Total FA (g) exc./5 days	% FA in fecal fat	Total NSF (g) exc./5 days	% NSF in fecal fat
M.T.	I (Tallow)	20.1	17.3	14.7	73.4	4.1	20.6
R.H.	II (Corn oil)	25.6	12.7	10.0	39.1	13.2	51.4

[1]Participated in Period I only.

[2]Participated in Period II only.

Appendix Table 4

TOTAL 5-DAY FECAL STEROID EXCRETION BY SUBJECTS M.T.[1] AND R.H.[2]

Subject	Period	Major Neutral Steroids (g)					
		Coprostanol (1)	Cholesterol (2)	Total (1 + 2)	Campesterol + Stigmasterol (3)	β-sitosterol (4)	Total (3 + 4)
M.T.	I (Tallow)	1.566	0.556	2.122	0.578	0.313	0.891
R.H.	II (Corn oil)	1.878	2.566	4.444	2.507	2.625	5.132

Subject	Period	Major Bile Acids (mg.)		
		Lithocholic (1)	cholic + chenodeoxycholic (2)	Total (1 + 2)
M.T.	I (Tallow)	35.0	63.8	98.8
R.H.	II (Corn oil)	560.0	173.9	733.9

[1] Participated in Period I only.

[2] Participated in Period II only.

Appendix Table 5

CEREAL RECIPES

Rolled Oats (1 serving)

200 ml. boiling water
40 gm. (½ cup) rolled oats
10 gm. tallow (corn oil)
1/8 tsp. salt

Bring water to rapid boil. Add salt. Slowly stir in rolled oats. Add tallow (corn oil), blending well. Cover and simmer 3 minutes.

Cream of Wheat (1 serving)

10 gm. tallow (corn oil)
21 gm. Cream of Wheat
180 gm. (6 oz.) boiling water
1/8 tsp. salt

Bring salted water to boil. Slowly add Cream of Wheat, stirring constantly till mixture begins to thicken (about 3 minutes). Add tallow (corn oil).

Appendix Table 6

SCRAMBLED EGG RECIPE (1 serving)

100 gm. egg albumin (reconstituted 6:1)
1 tsp. skim milk powder
1 drop yellow coloring
5 gm. tallow margarine (or corn oil margarine)

Combine albumin, coloring, and milk powder. Melt margarine in individual fry pans. Add the egg mixture to pans, stirring frequently as it cooks. Sprinkle with a dash of salt and pepper.

Appendix Table 7

HAMBURGER PATTIES RECIPE

Hamburger patties: 160 gm raw beef each
8 lbs ground chuck yields 22 patties

Recipe:

8 lbs ground chuck
12 whole eggs

1 - Divide ground chuck into 160 gm portions in separate bowls.

2 - Place 12 eggs in a blender and process at "stir" or "mix" for 1 second. Add 26 gms of egg mixture to each dish. Mix well.

3 - Add to each bowl and mix well, 1/8 tsp. salt
1/8 tsp. pepper
Few grains garlic powder
1 tsp. rehydrated minced onion

4 - Shape into patties. Cover in tin foil and freeze.

Appendix Table 8

TOMATO SAUCE RECIPE (5 cups or 10 (150 gm) servings)

Combine in large Pot:
3 tsp. dehydrated onion
952 gm (4 cups) canned tomatoes
12 gm (3 tsp.) white sugar
4 tsp. salt
2 tsp. black pepper
2 tsp. oregano
480 gm (2 cups) water
4 bay leaves
128 gm (½ C.) tomato paste
112 gm (½ C.) tallow or corn oil

Bring to boil, reduce heat and simmer ½ hour. (Cover for last 15 min.). Remove bay leaves.

Make paste of: 47 gm cornstarch
56 gm melted tallow or corn oil

Add to above and cook until mixture thickens, stirring frequently.

Appendix Table 9

MEATBALLS RECIPE (32 meatballs)

Combine in a saucepan: 400 gm TVP beef strips #10
560 gm hot water
240 gm tallow or corn oil

Heat to boiling - reduce heat and simmer, still covered for 5 minutes. Run the hydrated TVP through a meat grinder. Cool well. (The above mixture yields about 1000 gm of "ground beef").

Take: 125 gms of ground TVP beef

Add: 50 gm egg albumin (reconstituted)
pinch of pepper, garlic powder
1 tsp. of hydrated instant minced onion

Cool above mixture for 15 minutes in refrigerator. Shape into 4 meatballs (about 36 gm each).

Appendix Table 10

SWEET AND SOUR PORK RECIPE (1 serving)

Combine in a saucepan: 25 gms TVP pork chunks
128 gm (½ cup) pineapple juice (drained from can)
1/2 tsp. minced dehydrated onion
2 Tbsp. water
9 gm diced green pepper
14 gm melted beef tallow or corn oil

Cover and bring to boil. Reduce heat and simmer, covered for 15 minutes.

Combine and add to first mixture:

1 tsp. vinegar
1½ tsp. soy sauce
14 gm brown sugar
pinch of salt (1/8 tsp.)
50 gms pineapple tidbits

Combine to form a paste, and add to above:

1½ tsp. cornstarch
14 gm melted tallow or corn oil

Simmer for 10 minutes, stirring occasionally.

Appendix Table 11

MEATLOAF RECIPE (17 Meatloaves)

I Combine in each of 2 saucepans:

400 gm beef strips
560 ml water
240 gm tallow

Heat and simmer for 10 minutes or until all water is absorbed. Grind.

II Meanwhile, combine in each of 2 blenders:

238 gm dry cottage cheese
120 gm melted tallow
100 gm egg albumin
2 teaspoons kitchen bouquet
25 gm tomato paste

1 - Use only a blender with detachable bottom section and removable blades.

2 - Fill blender bowl with boiling water to heat. Measure eggs, tallow and albumin in warmed containers.

3 - Place albumin, tomato paste, kitchen bouquet in blender. Mix for 1 second.

4 - Add portions of cottage cheese in stages blending until almost all curds have disappeared.

5 - Add hot tallow, pouring while blender is on "Blend".

6 - Add mixture to ground TVP. Add 2 Tbsp. rehydrated minced onion.

Place 180 gms batter in square aluminum foil pan. Bake at 350° for 30-40 minutes.

GRAVY RECIPE

Combine in saucepan: 1½ tsp. bovril
200 ml hot water

Make paste of: 30 gm melted tallow
15 gm flour

Add paste to bovril mixture. Cook slowly until mixture thickens, stirring frequently.

Weigh out 30 gm portions and serve atop individual meatloaf.

Appendix Table 12

BEEF STEW RECIPE (1 serving)

1. Cook frozen carrots and frozen peas sufficient for No. of stew servings to be prepared.

2. Combine in a saucepan: 50 grams TVP beef chunks #15
   70 gm (ml) hot water
   14 gm tallow (or corn oil)

   Bring to boil and simmer covered, for 5-10 minutes (or until all liquid is taken up). Set aside, still covered.

3. Meanwhile, combine in saucepan:

   1½ tsp. BOVRIL
   6 oz hot water

4. Make a paste of: 28 gm melted tallow (or corn oil)
   15 gm flour

   Add to Bovril mixture.

   Cook slowly until mixture thickens, stirring frequently, Season with salt and pepper to taste.

5. In casserole dish, combine:

   50 gms canned cooked potato
   20 gm cooked onion
   20 gm canned diced carrots
   20 gms cooked frozen peas

Add the hydrated beef chunks. Pour gravy over the mixture.

Appendix Table 13

SPAGHETTI RECIPE (6 servings)

For 6 servings, cook together:

400	gm. spaghetti
4000	ml. water
2	tsp. salt
1	tsp. tallow (or corn oil)

Weigh out 6 150-gm. portions.

Appendix Table 14

INSTANT MASHED POTATO RECIPE (1 serving)

22	gm. instant mashed potato
1/3	cup boiling water
2	Tbsp. skim milk
5	gm. margarine
1/8	tsp. salt

Place boiling water and salt in a small casserole dish. Add milk. Add potato and stir. Add margarine and mix well.

Appendix Table 15

CREAM OF TOMATO SOUP RECIPE (1 serving)

128	gm. condensed Cream of Tomato soup
120	gm. whole milk

Combine in saucepan. Heat, stirring.

Appendix Table 16

GINGER MUFFIN RECIPE (12 muffins)

200	gm. pre-sifted AP flour
75	gm. lightly packed brown sugar
½	tsp. baking powder
½	tsp. baking soda
½	tsp. salt
1	tsp. cinnamon
1	tsp. ginger
¼	tsp. ground cloves
125	gm. light molasses
85	gm. skim milk (3/8 cup or 6 Tbsp.)
½	tsp. vinegar
50	gm. egg albumin (reconstituted 6:1)
160	gm. corn oil (or melted tallow)

METHOD:

1. Preheat oven to 350°.
2. Blend or sift together flour, sugar, B.P., baking soda, salt and spices.
3. Combine skim milk and vinegar, add to molasses.
4. Beat albumin and add to molasses mixture; mix well.
5. Add corn oil (or melted tallow) to mixture.
6. Add dry ingredients to molasses mixture a small amount at a time, mixing well between additions.
7. Place 12 56-gm. portions of batter in muffin tin (teflon).
8. Bake at 350° for 25 - 30 minutes.

Appendix Table 17

SPICY FRUIT SQUARES RECIPE (16 squares, 33 gm. each)

112 gm. melted tallow (or corn oil)
200 gm. lightly packed brown sugar
100 gm. egg albumin (reconstituted 6:1)
1½ tsp. vanilla
85 gm. pre-sifted AP flour
1 tsp. baking powder (B.P.)
¼ tsp. salt
½ tsp. cinnamon
40 gm. raisins
40 gm. chopped cherries

METHOD:

TALLOW

1. Preheat oven to 350°F.
2. Toss raisins and cherries in small amount of the allowed flour.
3. Blend or sift together flour, B.P., salt, cinnamon and brown sugar.
4. Add egg elbumin and vanilla. Mix well.
5. Add melted tallow. Mix thoroughly.
6. Stir in fruit.

Bake at 350° for 25 minutes.

CORN OIL

1. Preheat oven to 350°F.
2. Toss raisins and cherries in small amount of the allowed flour.
3. Blend or sift together flour, B.P., salt and cinnamon. Set aside.
4. Cream corn oil and brown sugar.
5. Add egg albumin.
6. Gradually add dry ingredients blending well after each addition.
7. Stir in fruit.

Bake at 350° for 25 minutes.

Icing

(28 gm.) melted tallow or corn oil
¼ tsp. vanilla
pinch of salt
128 gm. (1 cup) sifted icing sugar
15 gm. (1 Tbsp.) skim milk
1 tsp. orange extract

Gradually add icing sugar to tallow, alternating with milk. Add salt, flavorings. Mix well. Spread 9 gm. icing on each 33 gm. square.

Appendix Table 18

OATMEAL COOKIE RECIPE (4 dozen)

230 gm. flour
125 gm. granulated sugar
1 tsp. baking powder
½ tsp. baking soda
2 tsp. salt
1 tsp. vanilla
1 tsp. cinnamon
240 gm. rolled oats
240 gm. melted tallow (not hot) or corn oil
100 gm. egg albumin (reconst. 6:1)
120 gm. skim milk

METHOD:

1. Preheat oven to 400°.
2. Sift together dry ingredients.
3. Add rolled oats and mix thoroughly.
4. Add, in order, egg albumin, milk, fat (oil). Beat until thoroughly blended.
5. Shape into balls (22 gms. batter) to yield 48 cookies. Press down with fork.
6. Bake at 400°F for 10 to 12 minutes.