Seminar on Atherosclerosis

Nutritional Factors and Serum Lipid Levels*

EDWARD H. AHRENS, JR.

New York, New York

The correlation between the amount of dietary fat, the concentration of serum cholesterol and the incidence of ischemic heart disease is widely accepted as a cause and effect relationship by nutritionists, public health authorities, biochemists, practising physicians and by the public itself. Evidence favoring this correlation has been presented in a large number of epidemiologic studies, among which those of Keys et al. [2,3] figure prominently. The validity of the conclusions drawn from these data has been questioned on numerous occasions, most recently by Yudkin [4], Yerushalmy and Hilleboe [5] and Mann [6].

Their warning bears repetition: a direct correlation, no matter how strong, cannot be used as proof of cause and effect. Each of these reports has emphasized the weaknesses inherent in the basic data, in the mortality statistics, in the food consumption data and in the statistical treatment applied to the data. Their evaluations do not indicate that dietary fat has nothing to do with the incidence of ischemic heart disease; they do emphasize that conclusive proof of a specific association is still lacking. Indeed, the correlations between ischemic heart disease and the intake of animal protein or sugar, the number of television sets or automobile licenses, are said to be stronger than those with total fat, animal fat, vegetable fat, butter fat or margarine. Other defects in the postulate that ischemic heart disease is caused by eating too much fat (or too much of certain kinds of fat) have been pointed out by Page [7] and by Ahrens [8] and their colleagues.

Whether this postulate eventually is proved correct or not, it can be said without fear of question that our knowledge of the factors which control serum lipid levels, although still fragmentary, has grown enormously in the last ten years. It is the purpose of this report to summarize current concepts on this subject, on the grounds that a sound basis of understanding of fat metabolism underlies a true evaluation of its role in arteriosclerosis. In 1951 Davidson [9] discussed the effect of lipotropic agents on serum lipids and on experimental atherosclerosis. He concluded that there was “no general agreement that choline or inositol have any specific influence upon arteriosclerosis or the serum cholesterol level in man or the experimental animal.” With certain exceptions, which will be discussed subsequently, this statement still stands, and we shall say little here about lipotropic agents. Neither can we be concerned here with clinical or experimental studies of atherosclerosis or hypertension except insofar as they contribute to an understanding of the relationship of nutrition and serum lipid levels. Finally, the vistas opened up by the recent work on clearing factor and non-esterified fatty acids in serum are too complex to include here; recent views on these topics can be found in the publications of the Third and Fourth International Congresses on Biochemical Problems of Lipids (Brussels, 1956 and Oxford, 1957, respectively), and the summary by Robinson and French [10]. Other reviews which may prove helpful for study in this field include Fat Metabolism [11], edited by Najjar, and The Chemistry of Lipids As Related to Atherosclerosis [12], edited by Page. Recent technical developments in lipid biochemistry are described in the three volumes entitled...
Progress in the Chemistry of Fats and Other Lipids, edited by Holman, Lundberg and Malkin [13]. The encyclopedic reviews of lipid biochemistry by Hilditch [14] and Deuel [15] are essential references, and the small text by Lovern [16] is condensed but highly informative.

This review will include a consideration of calories per se and of total energy balance in relation to serum lipid levels. Dietary fats, protein and carbohydrate will be separately considered, then intestinal bacteria, "bulk" and, finally, trace substances. Current views on fat digestion and absorption cannot be included here; a recent review of this subject by Bergström and Borgström [17] may be of interest.

**GENERAL CONSIDERATIONS**

Although the major part of this review discusses the effects on serum lipids of the major foodstuffs—fat, protein and carbohydrate—a consideration of any one falls out of context unless total energy balance is considered simultaneously. It is not enough to speak of fat intake, either in grams per day or as a percentage of total calories; one must also relate this intake to total body needs. Does the day's diet contain more than, less than, or just enough calories to maintain body weight constant? For simplification we will assume constant physical activity and a fixed metabolic state unaffected by disease, fluctuating hormonal balances or needs for growth.

We must deal with a four component system—total calories, fat calories, protein calories and carbohydrate calories—in which the first is the sum of the other three. For graphic purposes it may be helpful to plot the interrelationships on a triangular phase diagram (Fig. 1) in which the corners of the equilateral triangle represent 100 per cent of the individual caloric sources. The bases opposite each corner represent 0 per cent, and lines drawn parallel to each base define various degrees between 0 and 100 per cent for each component. It is a matter of simple geometry to show that any point within the triangle exactly defines the total dietary mixture; the sum of the three values represented by each point adds to 100.

Within the limits imposed by the availability of foods and by human ingenuity in preparing them and tolerance in accepting them, the human diet might be defined by a point anywhere within the triangle. However, it is already well known that certain minimum requirements for dietary protein must be met if the diet is to be satisfactory over long periods of time. The exact position of this minimum has not yet been established [18], but it is safe to state that under ordinary circumstances the adult human being can thrive if he receives at least 8 per cent of his calories as mixed vegetable and animal proteins of good quality. (Probably, this minimum limit is considerably lower.) A line drawn parallel to the base opposite "protein" at 8 per cent defines this limit. Now, excepting the Eskimo (whose dietary intake has never been adequately defined), the protein intakes of a wide variety of the world's peoples lies between 8 and 15 per cent of total calories [5,19]. Thus, we may think of another line parallel to the protein base at 15 per cent, which defines the maximum protein intake ordinarily eaten.

When natural protein intakes vary over a twofold range from 8 to 15 per cent in various parts of the world, what happens to fat and carbohydrate intakes? Data gathered from the literature by Keys and Anderson [19] have been plotted in Figure 2. It is readily apparent that the Eskimo diets are in a class by themselves. The other data take a linear form: as protein intakes slowly increase, there is a major replacement of carbohydrate by fat calories. Thus, from one extreme to the other, there is a difference of only 5 per cent of calories as protein, whereas fat calories increase from 10 to 45 per

![Diagram of diet composition](image-url)
cent at the expense of carbohydrate. The diets of civilized countries like the United States are found at the left, while the diets of underprivileged areas are at the right. We may ask why these points are not scattered randomly over the entire chart. Is it a matter only of composition of the dietary mixture and the total energy balance. The investigator who asks whether dietary fat affects serum lipid levels must decide whether or not to make reciprocal changes in carbohydrate intake, in which case the effect produced may be due either to the smaller intake of one component or to the larger intake of the other, or to both. Or he may add or subtract fat calories without changing protein and carbohydrate intakes, in which case the effects observed may be due to the change in fat, to the altered energy balance or to the altered food mixture.

The importance of these considerations can be illustrated by two examples. (1) A study by Messinger et al. [27] aimed at learning whether or not an increased cholestrol intake would cause an increase in serum cholesterol concentrations. The cholesterol intake was increased by feeding egg yolk powder, and a marked increase in serum cholesterol levels was obtained. However, the design of the experiment did not permit a clear answer to the alternate possibilities that this rise was due to the increased cholesterol intake (3.5 gm. per day) or to the increased caloric intake (1,000 calories per day), or to both changes. (2) A recent study by Insull et al. [22] has shown that the fatty acid composition of human breast milk was radically affected by the quantity and quality of the mother's dietary fat. Moreover, it was also altered by feeding more, and later less, calories than were required to maintain her body weight constant. This study clearly demonstrated that the response of the human breast is conditioned by total energy balance as well as by the character and amount of fat in the maternal diet. These important interrelationships are frequently overlooked in the design of metabolic experiments in animals as well as in man.

**TOTAL CALORIES**

This section deals with the effects on serum lipid levels of over- and undernutrition, that is, more or less calories than are required to maintain constant body weight at "normal" levels. This definition, at the outset, begs terms, for we cannot define normal weight nor even describe the state of optimal nutrition. What is normal or optimal for one race need not apply to another. Nor in any one race are these values unaffected by age, sex, body build and many other factors.
Nutritional Factors and Serum Lipid Levels—Ahrens

**Caloric Deficit.** The effect on serum lipid levels of prolonged undernutrition has been summarized by Keys et al. [23] in their monograph, The Biology of Human Starvation. In addition to reviewing the literature up to 1948 they contributed original data in their experiment on twenty-three normal, young volunteers who were fed 1,700 calories per day (protein, fat, carbohydrate = 13, 18 and 69 per cent of calories) for 128 days. Small but significant decreases in total cholesterol levels were obtained in eighteen of twenty-three men, with mean levels decreasing from 169 to 151 mg. per 100 ml. serum. Total serum lipids were unchanged. There was no ketosis.

Two studies of the effect of weight reduction on serum cholesterol and lipoprotein levels were reported by Walker et al. in 1953 [24] and in 1957 [25]. Both reports indicated that significant decreases in cholesterol and in the high-density beta-lipoproteins can be accomplished under various conditions of (1) rate of weight loss, (2) initial levels during weight maintenance, and (3) composition of the dietary mixture during weight reduction. The studies are not sufficiently controlled to permit precise conclusions to be drawn.

The results of Moore et al. [26,27] suggest that men and women may respond differently to weight reduction regimens.

Changes in serum lipid levels during total starvation were described by Kartin et al. [28] in 1944. Men, monkeys and dogs were studied; ketosis and hypercholesteremia failed to develop in dogs only. In man there were significant increases in cholesterol, larger rises in phospholipids and equivocal changes in triglycerides. These changes were reversed by administration of carbohydrate. These workers concluded that the existence of "starvation lipemia" is highly questionable. In 1954 Rubin and Aladjem [29] demonstrated by ultracentrifugation technics that a four to five day fast in six healthy volunteers caused (1) no appearance of Sf₁0₀ lipoproteins, and therefore no lipemia, (2) marked increases in Sf₁₂₀ and Sf₂₀₀ groups, (3) less marked increases in Sf₀₁₂ and Sf₁₀₀ groups, and (4) no significant change in high-density lipoproteins (<1.125 and 1.199). These changes were readily reversed twenty-four hours after resumption of normal meals. One of the six volunteers who failed to show these changes had been on a low-fat (<15 gm./day) diet for more than two years, his response resembled that of Kartin's dogs [28] which were acclimated to high carbohydrate diets.

We know of no controlled study of the effects of variously composed diets on serum lipid levels during states of negative energy balance. It seems reasonable to predict that the incorporation of different dietary fats in subcaloric diets may have relatively little specific effect, since the combustion of the semi-starved patient's own adipose tissue comprises a large part of his total energy expenditure. In their study of breast milk fatty acids, Insull et al. [22] showed that during restriction of total calories the fat composition of breast milk closely resembled human adipose tissue in its pattern of fatty acids. It would be of particular interest to examine the closeness of identity of (1) the various groups of esters in the serum, (2) the non-esterified fatty acids in the serum and (3) the adipose tissue fatty acids during periods of weight reduction.

**Caloric Excess.** The effects of excess calories on serum lipid levels have been even less well defined. There has been no systemic study of variations in fat/carbohydrate calories, although profoundly different effects on the serum lipids might be expected. The study of Walker et al. [24] demonstrated in two men that excess calories over a short period caused striking increases in serum cholesterol and lipoprotein levels, even though the diet was low in fat. In an extension of this project, Mann et al. [30] showed that these increases could be prevented if sufficient exercise were taken to dissipate the excess food calories. In both studies mixed natural diets were fed, and all four parameters—calories, fat, protein and carbohydrate—were simultaneously varied.

In a well controlled metabolic study of twenty physically healthy schizophrenic men, Anderson et al. [37] demonstrated that weight gains due to a daily excess of 660 calories caused significant elevations of total cholesterol (20 mg. per 100 ml. serum). These levels reached their peak at five weeks and were maintained unchanged for fifteen more weeks despite continuing gains in weight. On the other hand, increases in Sf₁₂₀ lipoproteins took place from the tenth to twentieth week. (Since initial levels were not reported, it is not known how this class varied in the first ten weeks.) Other lipoprotein groups, triglycerides and phospholipids were not measured, although one might expect significant alterations in these components.

Much attention has been paid by nutritionists to the effects of dietary deficiency. Its counter-
part—dietary excess—has been long neglected. The animal studies of McCay [32], Silberberg [33], Thomasson [34], McCance [35] and others showed that underfed animals live longer than animals fed ad libitum. The relative merits of a Sybaritic or a Spartan existence for human beings can be debated, but it is clear that much fundamental information on fat metabolism must be acquired before the issue will rest on facts instead of emotions.

**FAT**

Prior to World War II Snapper initiated studies of the iodine values of the serum lipids of Occidentals and Orientals in China [36]. He stated in his 1941 text [37] on Chinese medical conditions that "whereas the Westerner depends for his linoleic acid intake on the traces which may be present in some of the ingredients of the diet, the Chinese ingests daily from his early youth considerable amounts of the important unsaturated compounds." He postulated that these differences in diet might be related to the scarcity in China of a number of diseases, among them arteriosclerosis. Regrettably, his efforts were interrupted by the war and were not resumed.

Snapper's idea was based on the demonstration by Burr and Burr [38] in 1929 that linoleic acid was essential for the growth of rats exhibiting a deficiency syndrome characterized by a scaly skin. The earliest attempts to relate essential fatty acid metabolism to clinical disease were made by Hansen and Wiese, colleagues of the Burrs, who considered that infantile eczema might be an expression in man of essential fatty acid deficiency [39]. It has long been known that the feeding of highly unsaturated fats causes a rapid rise in concentration of unsaturated acids in the depot fat of many organisms, but the possibility that the ingestion of fatty acids of certain double-bond structure might affect serum lipid levels had no supporting evidence until 1955, when reports by Kinsell [40] and by Ahrens [41] and their co-workers opened the question. A description of the present status of this problem will form the main part of this section.

In fact, when Schönhheimer [43] in 1933 placed a hypercholesteremic woman on a plant fat diet, he did so in order to feed a cholesterol-free diet. He demonstrated a striking decrease in her serum cholesterol levels, and his study of her fecal sterols indicated that she either converted her serum cholesterol to some other compound or segregated it in another tissue, for it was not excreted. It is now believed that dietary cholesterol does not affect serum cholesterol levels (*vide seg.*). The changes in serum levels which Schönhheimer observed may have been due to the patient's high intake of olive oil and margarine. On the other hand, Sperry and Schick's [44] failure to affect the serum cholesterol concentrations of a child with hypercholesteremia by means of a cholesterol-free diet may have been due to the fact that their diet was almost devoid of fat of any type.

The effects of vegetarian diets were further explored by Hardinge and Stare [45] whose study was initiated in 1950 and reported in 1954, and by Groen et al. [46] whose report appeared in 1952. The former workers demonstrated that strict vegetarians had lower serum cholesterol levels than the partial vegetarians who ate eggs and dairy products, or than non-vegetarians. Groen devised an experiment in which individual responses to three different diets were tested. Serum cholesterol levels were lowest on the vegetarian regimen, even though the total fat intake was high, and were highest on the animal fat diet. While numerous questions remained unanswered, Groen's data demonstrated clearly that serum cholesterol levels could be independent of total fat intake.

In 1952 Kinsell et al. [47], investigating the effects of diet on the response in patients to various endocrine preparations, reported that diets high in vegetable fat produced dramatic decreases in serum cholesterol and phospholipid levels, whereas isocaloric substitution of animal fats in these diets caused the levels to rise promptly. They noted that the addition of cholesterol to the vegetable fat diet did not reverse the effect.

The 1952 reports were viewed with some scepticism at the time, because numerous workers had shown that serum cholesterol levels decrease significantly on low fat diets [2,48–53] and that the addition of vegetable fats to these regimens produced a dramatic rebound of cholesterol concentrations to previous levels [54–57]. However, the results of a four-month
study of six patients by Ahrens et al. [58] carried out under metabolic ward conditions with strict isocaloric substitution of mixed animal by mixed vegetable fats and with constant intakes of the same dietary protein, amply confirmed the conclusions of Kinsell, Groen and Hardinge.

In retrospect, it seems probable that some of the confusion between 1952 and 1954 arose because the importance of maintaining food intakes at eucaloric levels was not appreciated. Perhaps, the fetishism associated with the practice of vegetarianism also may have influenced reactions to the early claims. (An illuminating historical review [59] was published by the eminent nutritionist, L. B. Mendel.) But perhaps a greater handicap to clear thinking was created by the standard industrial practice of naming fats "animal" or "vegetable." As later events have shown, this arbitrary division is chemically meaningless and even misleading. The errors in thinking created by this unfortunate custom were in some instances further compounded by failure to distinguish between hydrogenated and non-hydrogenated fats. In addition, the well regarded studies of Keys et al. [7] showed that serum cholesterol levels could be influenced by adding or withdrawing dietary fat but not by altering the intake of dietary cholesterol. Because Keys made no distinction at that time between fats of specific chemical structure, he became convinced that total dietary fat was the key factor determining serum levels of cholesterol. His recent reports indicate that he has modified this position [60].

Recent experiments in a number of laboratories have shown clearly that isocaloric exchanges of different fats in the diet produce an array of serum lipid changes which can be related to the degree of unsaturation of the fed fat. Kinsell [40] and Ahrens [41] originally suggested this explanation of their experiments in 1955. Their later results [8,61–63] and those of Bronste-Stewart [64], Beveridge [65,66], Keys [60,67], Malmros [68], Eggstein and Schettler [69] and their co-workers are at least consistent with this hypothesis. Thus, the ingestion of highly saturated fats (butter, coconut oil, cocoa butter, palm oil, for example) leads to the highest levels of serum cholesterol and phospholipids, while diets containing isocaloric amounts of highly unsaturated oils (safflower, corn, cottonseed and peanut oils, for example) produce striking decreases in these levels. These changes are produced without altering the ratio of free/total cholesterol, and there is no indication of liver injury or indeed of any other recognized ill effect. Curiously, the concentrations of serum triglycerides are not systematically altered by these dietary fat exchanges. It has been shown [62] that the serum lipid levels produced by a

![Figure 3](image_url)

**Fig. 3.** Twenty-six-week study of serum lipids in a twenty-seven year old man with hypercholesteremia; no apparent vascular disease or xanthomatosis. TC = total cholesterol, FC = free cholesterol, PL = phospholipids, TG = triglycerides, all in mg. per 100 ml. serum. P-F-C = Protein, fat and carbohydrate intakes, as percentage of total calories. Note three-week transition periods after each dietary exchange, before levels became steady.
Nutritional Factors and Serum Lipid Levels—Ahrens

characteristic response of the patient to that dietary fat is not lost.

Experiments that substantiate these statements are shown in Figures 3 to 7. Figure 3 demonstrates that a saturated fat, coconut oil, causes higher lipid levels than an unsaturated oil, corn oil. When corn oil formula is fed repeatedly to the same patient (other feeding periods intervening), the same cholesterol levels are achieved within ±5 per cent. (Fig. 4.) As all patients do not respond to corn oil to the same degree, the serum lipid levels achieved during ingestion of the corn oil formula must be used as control values for each patient. The percentage differences between control levels and those produced by other dietary fats can be calculated. When these percentage differences are related to the iodine values of the various fats tested, a linear relationship is obtained. (Fig. 5.) The ingestion of formulas containing corn oils saturated by hydrogenation to iodine values of 80 and 58 produces successfully higher levels of cholesterol and phospholipids in the serum (Fig. 6), and the removal of 80 per cent of the non-saponifiable materials (such as sitosterols, carotenoids and tocopherols) from corn oil failed to abolish its cholesterol-lowering properties. (Fig. 7.)

It is keenly debated today whether the effects described are due (1) to the presence in all natural fats of trace materials, i.e., plant sterols, vitamins, minerals; (2) to the absence in most oils of short and intermediate chain length fatty acids, i.e., the C_{16-14} acids so richly distributed in coconut oil and butter; (3) to the content of essential fatty acids in most natural fats and oils, i.e., linoleic, arachidonic or others; or (4) to the aggregate unsaturation of the oil, i.e., the number of double bonds per unit weight of carbon.

Jones et al. [70] reported experiments in chicks which suggested that the corn germ contains substances more potent than the oil itself as depressants of serum cholesterol levels. Beveridge et al. [71] reported evidence which suggested

### Table

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1050</td>
<td>325</td>
<td>425</td>
<td>400</td>
<td>400</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 4. Reproducibility of serum cholesterol levels in six patients retested on basic corn oil formula (P-F-C = 15 to 40 to 45 per cent of calories), with other regimens in intervening periods. Bars show mean levels during steady states, hatched areas = one standard deviation, n = number of weekly data during each steady state. Percentage differences along baseline calculated with reference to mean levels of first feeding periods.
to them that the effectiveness of corn oil is due largely to its beta-sitosterol content. We believe that their data permit other interpretations, but readily agree that the importance of the role played by corn oil's sitosterol must be clarified.

Experiments reported by us [62] suggest that the ingestion of fatty acids shorter than C16 may produce higher serum cholesterol and phospholipid levels than the C16 and C18 acids. Thus butter, which is rich in short and intermediate chain length acids, produces higher serum lipid levels than does cocoa butter, which contains predominantly C16 and C18 acids, yet these fats contain the same amounts of oleic and linoleic acids. On the other hand, Keys [72] has seen no rise in cholesterol levels after feeding 10 gm. per day of butyric acid.

Kinsell and Sinclair [61] postulated that the major determining factor in the highly unsaturated oils is their content of linoleic acid, and that hypercholesteremia and atherosclerosis are expressions in man of a deficiency of essential fatty acids. These conclusions are weakened by the following considerations: (1) significant depressions in serum cholesterol, phospholipid and beta-lipoprotein levels have been produced by ingestion of sardine oil [67], whale oil [68] and pilchard oil [64], although the analytical data currently available [73] indicate that these oils are exceedingly poor in essential fatty acids, and rich in other unsaturated fatty acids; and (2) serum lipid levels have also been depressed by feedings of olive oil [62,68] and rapeseed oil [68] which consist mainly of mono-unsaturated “non-essential” fatty acids, and by linseed oil [69] rich in tri-unsaturated non-essential linolenic acid. These considerations lead us to the tentative conclusion that the major factor in dietary fats which produces depressions in levels of cholesterol and phospholipids in the serum is the total mean unsaturation of the fat, that is, its number of double bonds. We disagree with Keys et al. [60] in their statement that mono-unsaturated acids are neutral in effect and have shown elsewhere [63] what we consider to be the fallacy of their arithmetic.

In the last analysis, all these currently debated issues will be settled by adequately designed experiments. We have stated [63] why we believe that future experiments must lean heavily on the use of “synthetic” fats, in which the experimenter has more flexible control of the fatty acid
Nutritional Factors and Serum Lipid Levels—Ahrens

composition of dietary fat than Nature allows him. It is indeed unfortunate that species differences demand that such experiments be performed in the human being, for this path is thorny and the going is expensive and slow.

Mechanisms. Fatty acids: What evidence exists to suggest that different fatty acids are metabolized through different pathways? (1) Saturated and unsaturated fatty acids in a mixed fat meal are equally well absorbed [74]. The acids of chain length C₂₋₁₃ are absorbed mainly via the portal vein, however, while C₁₄₋₁₇ acids are absorbed entirely via the lymphatic system [77]. (2) Unsaturated acids are hydrogenated in part during absorption from the gut, presumably by bacteria [74]. Dehydrogenation has not been demonstrated. (3) The long-chain fatty acids are tightly bound to albumin, but no significant differences in binding of long-chain saturated and unsaturated acids have been found [75]. (4) The essential fatty acids are those with at least two double bonds, 6 and 9 carbon atoms from the terminal (or methyl) end of the chain. Utilizing his “6-9 terminal” hypothesis, Thomason [73] has identified a number of essential acids in addition to linoleic and arachidonic acids. Klenk [76] has demonstrated that the highly unsaturated acids of fish and animal

**Figure 6.** Effect of ingestion of corn oil hydrogenated to iodine values of 58 and 80, compared to unhydrogenated oil. Levels of TC and PL significantly different (p < 0.01) in all three test periods. Fifty year old man with history of myocardial infarction.

**Figure 7.** Lack of dietary effect on serum lipids of sixfold difference in non-saponifiables of corn oil. Molecular distillation enriches non-saponifiables in distillate fraction; residue fraction is poor in this material. Sixty-seven year old man with history of coronary insufficiency.
Nutritional Factors and Serum Lipid Levels—Ahrens 937

sources have the same chemical structure. (5) The turnover of unsaturated acids is said to be slower than that of saturated acids in rat liver and rat carcass [77]. (6) In natural lecithin the unsaturated acids are preferentially esterified at the alpha carbon of glycerol [78], while in most triglycerides these acids are found in the beta position [79]. In Kennedy's classic work on the enzymatic synthesis of triglycerides and phospholipids, the common diglyceride precursors must contain at least one unsaturated fatty acid [80]. This may be only an apparent enzyme requirement, for the insolubility of the fully saturated diglyceride substrate may hinder the approach of the enzyme to it. If the enzyme requirement is specific, there may be a metabolic distinction between saturated and unsaturated acids, residing in their physicochemical properties. (7) The enzymatic esterification of cholesterol in the human intestinal lumen is more rapid with unsaturated fatty acids than with saturated acids [87]. (8) The metabolic role of the essential fatty acids is still unknown. Two laboratories [82,83] found uncoupled oxidative phosphorylation in essential fatty acid deficiency. Deuel [84] has enumerated a large number of physiologic actions of these acids. Holman [85] believes they serve an important transport function for serum cholesterol.

These considerations strongly indicate that the metabolism of saturated and unsaturated acids may be different, and that the accessibility of these acids to enzymes may determine some part of this difference. Any general physicochemical difference, such as solubility or molecular shape, might determine some features of their different metabolic behavior, but the astonishing specificity of the 6–9 terminal structure of essential fatty acids puts these acids in a special category.

Lipsky et al. [86] in 1955 demonstrated in man that the turnover of non-phospholipid fatty acids, (i.e. cholesterol ester or triglyceride fatty acids, or both) is more rapid than that of the phospholipid acids. In 1957 James et al. [87] identified the fatty acids in the same two ester groups (phospholipids and non-phospholipids) in normal subjects and in patients with coronary heart disease by gas-liquid chromatography, without finding significant differences.

Preliminary studies [63] of the individual fatty acids of triglycerides, cholesterol esters and phospholipids have been made in our laboratory. We considered it essential to have rigid control of the dietary intakes of patients whose serum lipids were so fractionated and made the measurements only after the patients had reached a "steady state" on a given dietary mixture [62]. It was clear that (1) the fatty acid distributions of all ester groups were markedly affected by changes in dietary fat, (2) the triglyceride fatty acids were most responsive to dietary manipulations and most closely resembled in fatty acid composition that of the fed fats, (3) the major part of the serum arachidonic acid was found in the phospholipid fraction, and (4) the cholesterol ester fatty acids were the most unsaturated of the three groups when butter was the sole dietary fat, but the triglyceride fatty acids were

Fig. 8. Effect on serum lipids of varying the proportions of fat and carbohydrate calories reciprocally, keeping total calories, protein and body weight constant. Major changes were produced in serum triglycerides, smaller effects on phospholipids, least striking differences in cholesterol. The lowest serum lipid levels occurred on highest intake of corn oil. Fifty-three-year old man with history of coronary insufficiency.
the most unsaturated when corn oil was fed. A more complete description of the changes in these acid groups produced by diet will form the basis for subsequent turnover studies.

**Cholesterol:** It may reasonably be asked what becomes of the cholesterol and phospholipids which decrease in concentration in the serum when certain dietary fats are fed. Are they metabolized more rapidly? excreted more rapidly? synthesized more slowly? sequestered in some tissue other than serum?

An exploration of synthesis rates of cholesterol was made in 1956 with Drs. Hellman and Rosenfeld in an experiment in which the incorporation of 2-C\(^{14}\)-acetate into free and esterified cholesterol was measured. The time-curves were measured during two dietary periods when the serum cholesterol was held at two different levels. The data obtained were not significantly different in the two periods: per cent incorporation into free or ester cholesterol, time of peak incorporation, time of crossover of free and ester cholesterol curves, or slopes of the decay curves. These results do not support the contention that the two dietary fats may have different effects on cholesterol synthesis. However, Hellman et al. [88] previously noted large individual variations in acetate incorporation into cholesterol, in view of which it would be necessary to obtain at least fourfold differences before significance could be claimed.

A second experiment was carried out with Drs. Hellman and Rosenfeld to measure the excretion of cholesterol in different dietary periods. A patient whose serum cholesterol level was 900 mg. per 100 ml. serum on an ad libitum diet was given radioactive cholesterol (4-C\(^{14}\)-cholesterol) intravenously in order to label the body pools of readily exchangeable cholesterol. A "balance study of labelled sterols" was carried out, to tell whether or not the excretion of the body's readily exchangeable cholesterol into the feces was altered by the feeding of different dietary fats. The findings, reported in preliminary form [89], indicated that when serum cholesterol concentrations rose (on butter feedings) the fecal sterols decreased by almost the same amount; when serum cholesterol fell (on corn oil feedings) the fecal sterols increased by almost the same amount. Thus the net gains and losses in serum cholesterol could be accounted for almost completely by the changes in fecal excretion of the labelled sterols. These results indicate that excretory mechanisms may explain the changes in serum levels of cholesterol which are produced by exchanges of dietary fats. It remains to be determined whether this is due to increased excretion of cholesterol into the gut, or to decreased reabsorption of cholesterol from the gut. The data fail to substantiate the possibility that cholesterol is sequestered in some other tissue (arterial intima) when its concentration in the serum decreases in response to a change in dietary fat. The ratio of labelled bile acids/labelled sterols decreased when corn oil feedings caused the serum cholesterol to fall. Thus we have no indication that this conversion was accelerated when corn oil was the sole dietary fat. The results of the two isotope experiments are in agreement in diverting our attention from synthesis rates to excretory mechanisms.

**Bile acids:** Other laboratories have attempted to gain an understanding of the mechanisms underlying alterations in serum cholesterol levels by measuring the output of bile acids in the feces under various dietary conditions. Although serious methodologic difficulties handicap such efforts, the Capetown group [64] has presented preliminary data which suggest that the excretion of fecal bile acids increases when exchanges of dietary fats cause serum cholesterol levels to decrease. Their results are compatible with those of the labelled sterol balance study already described.

**Dietary Cholesterol.** Keys et al. [90] in 1956 summarized a wide experience which led them to the conclusion that dietary cholesterol has no important effect on serum cholesterol levels. This conclusion was based on (1) long term observations of men eating diets low and high in cholesterol, (2) epidemiologic survey data in Minnesota and Sardinia where dietary intakes of cholesterol vary widely, (3) experiments in which men doubled or halved their cholesterol intakes for many months, (4) experiments in which 500 or 600 mg. per day of cholesterol was added to a rice-fruit diet, and (5) experiments which tested threefold variations in cholesterol intake in mixed food diets containing 66 gm. of total fat per day. They concluded that the independence of serum cholesterol levels and cholesterol intakes demonstrated by them in adult men over the whole range of natural human diets probably also applied to infants, children and women.

Other workers have purposely added considerable amounts of cholesterol to diets containing vegetable fats in an effort to demonstrate...
whether or not the depressions in serum cholesterol and phospholipid levels caused by these diets might be due to an absence of dietary cholesterol. Thus Kinsell in 1952 [47] stated that the addition of 30, then 60 gm. per day of crystalline cholesterol to a tube-fed formula diet consisting of 62 gm. of protein and 195 gm. of vegetable fat caused no elevation of serum cholesterol. His experiment was twelve days in duration. Bronte-Stewart et al. [64] added 3 gm. of cholesterol per day to their food mixtures without losing the cholesterol-depressant action of unsaturated fats. Ahrens et al. [62] showed that when serum cholesterol levels were depressed by feeding 40 per cent of calories as corn oil, the addition of 2 gm. per day of cholesterol produced no significant elevation in serum cholesterol levels; however, the administration of 4 and 8 gm. per day led to small but significant increases in cholesterol and phospholipid levels. Test doses of cholesterol (600 mg. per day) in the range of normal human intakes added to formulas containing 40 per cent of calories as lard also failed to evoke a further elevation in serum cholesterol concentration. These various studies confirm Keys’ conclusions and in addition demonstrate that the effectiveness of unsaturated type dietary fats in depressing serum lipid levels is not due solely to absence of cholesterol from those diets.

In view of the low percentage absorption of cholesterol in human beings [97] it is entirely possible that a considerable amount of cholesterol could be absorbed without causing a significant change in serum levels [92,93]. The possible tissue deposition of cholesterol which is slowly absorbed certainly cannot be excluded on the basis of serum studies. A simple test of this possibility is not available. It is not feasible to carry out accurate cholesterol balance studies by comparing dietary intake and fecal output in the usual manner [94]. An unknown amount of cholesterol is synthesized each day by the liver; cholesterol is converted in the gut into other products which are difficult to measure; and in the body, cholesterol is eventually converted to bile acids, the excretion products of which are exceedingly complex [95]. Stanley and Cheng [96] have devised an ingenious method for calculating the intestinal secretion, absorption and excretion of cholesterol which may clarify some of the questions in this area. The chemical methods recently developed for measurement of fecal sterols by Coleman et al. [97] also may prove helpful. Central to this issue is further study of the quantitative aspects of bile acid metabolism which Lindstedt and others in Bergström’s group [98] have recently elucidated most productively. The technic of a labelled sterol balance study described by Hellman et al. [89] also may be expected to add valuable information in an area which is still largely undefined.

Thus, while it can be stated with considerable assurance that serum cholesterol levels ordinarily are independent of dietary cholesterol intake in man, much remains to be learned about the metabolism of ingested cholesterol.

Other Dietary Sterols. Sperry and Bergmann [99] in 1937 showed in mice that the oral administration of sitosterol produced a lowered content of cholesterol in the liver. In 1951 Peterson [100] reported that in chicks the addition of mixed soybean sterols to a cholesterol-rich diet prevented the expected hypercholesteremia. Numerous later studies confirmed these findings in other laboratory animals, and evidence was given that cholesterol-induced atheromatosis could be prevented. It was shown [101-103] that the effect of vegetable sterols in animals was mediated through interference with cholesterol absorption, perhaps due to interference in the esterification of cholesterol prior to its absorption.

Clinical studies performed by Best et al. [104] established that in man the administration of beta-sitosterol caused significant decreases in serum cholesterol levels. The higher the initial level of cholesterol, the greater the decrease during sitosterol administration. Their results were confirmed by Farquhar et al. [105] and Sachs and Weston [106], but Wilkinson et al. [107] found no effect and numerous other authors have had irregular results. No study other than Wilkinson’s has been carried out under metabolic ward conditions, and it remains uncertain whether total food intake and the composition of the food mixture itself are altered by the ingestion of emulsions containing this plant sterol. It has not been explained why it is necessary to administer such large amounts of this sterol (at least 18 gm. per day) to obtain the effects described, since this is at least six times the amount of cholesterol which passes into the gut each day in the diet and via the bile [108]. In animals, 2:1 ratios of beta-sitosterol/cholesterol sufficed to inhibit cholesterol absorption [109], even though cholesterol was fed in large amounts.

Beveridge et al. [77] reported that as little as
2 gm. of beta-sitosterol administered to normal young men on a fat-free diet for eight days caused large decreases in serum cholesterol levels. Since this decrease was as great as that produced by feeding 60 per cent of calories as corn oil (which was stated to contain an equivalent amount of sitosterol, or 2 gm.), they reasoned that the depressant action of corn oil ingestion on serum cholesterol levels was due to its sitosterol content. However, on a low fat diet the enterohepatic circulation of cholesterol is considerably diminished [110], and it may be that on Beveridge's fat-free diet the 2 gm. of sitosterol administered was sufficient to interfere with a greatly reduced secretion of cholesterol into the gut. The effect of the 2 gm. in the 60 per cent corn oil diet may have been quite dissimilar.

A number of other sterols have been fed to animals with the intention of interfering with cholesterol absorption. The rationale is based in part on the original observations by Schonheimer [117] that plant sterols are completely non-absorbable. However, it is now found that dihydrocholesterol is absorbed by rabbits, as is Δ4-cholestenone (lathosterol) and 7-dehydrocholesterol, and all may produce atheromas [112]. Dihydrocholesterol also may cause biliary concrements and inflammatory lesions of the biliary tract in rabbits [113]. Beta-sitosterol was found to be absorbed by rats and by man; in the rat, unlike dihydrocholesterol, it was not stored [114].

In an effort to decrease cholesterol synthesis in liver, Steinberg and Frederickson [115] fed Δ4-cholestenone for long periods to rats. Toxic effects were noted, with marked adrenal hyper trophy and storage of sterol (presumably, its end product, dihydrocholesterol) in the liver. However, it is interesting that cholesterol synthesis was depressed.

It seems fair to state that an effective non-toxic cholesterol "analogue" has not yet been found, either for the suppression of cholesterol synthesis or of cholesterol absorption in the gut. Interesting studies on sterol derivatives which might serve as competitive inhibitors in the growth of German cockroaches have been described by Noland [116]. He noted that thiocholesterol was extremely active in this regard.

Intravenous Fat Emulsions. Parenteral alimentation by means of intravenous fat infusions, reviewed recently by Meng [117], causes considerable elevations in serum triglycerides during and after the infusion. The administered fat is cleared rapidly, and the serum triglyceride levels of normal men return to pre-infusion levels in twenty-four hours. Serum cholesterol levels in normal men are not significantly altered. Geyer et al. [118] showed in dogs that the fat in these emulsions was rapidly metabolized to carbon dioxide.

Lever and Waddell in 1955 [119] observed that the responses to intravenously administered fat emulsions were different in normal men, in hypercholesteremic patients and in hyperlipemic patients. Single infusions of a 500 ml. emulsion containing 50 gm. of oil (cottonseed oil or synthetic triolein), 25 gm. dextrose, 6 gm. soybean phosphatide and 1.5 gm. of a synthetic surfactant, pluronic, were tested. Clearing of the expected triglyceride elevations in the fourteen normal subjects and four hypercholesteremic patients was noted twenty-four hours after the infusion, whereas seven of nine hyperlipemic patients had higher triglyceride levels than before the infusion. Cholesterol levels were not affected in the normal subjects whose initial concentrations were below 300 mg., while those four of fourteen with pre-infusion levels above 300 mg. showed a mean decrease of 70 mg. The cholesterol levels in the hyperlipemic subjects showed decreases averaging 97 gm., with most marked effects in those whose cholesterol levels exceeded 400 gm. before infusion. The four hypercholesteremic subjects had cholesterol levels ranging from 515 to 590 mg. before infusion; twenty-four hours after infusion these levels decreased on the average 120 mg.

Daily infusions were given for one week to two hypercholesteremic patients and three hyperlipemic patients. Cholesterol, phospholipid and triglyceride levels gradually decreased under this management in all patients, and the decreases were uniformly striking. The effects were temporary, and within three weeks levels in all patients had markedly increased.

Lever and Waddell [119], after testing emulsions made up without oil, concluded that the non-fat ingredients were not responsible for the effects observed. Subsequently, however, Waddell [120] has found that infusions of 5 per cent dextrose cause fairly regular decreases in all lipid classes in hyperlipemic subjects, hypercholesteremic subjects and in normal men. He is currently evaluating this interesting interrelationship of carbohydrate and fat metabolism.

AMERICAN JOURNAL OF MEDICINE
His observation recalls the striking effects of carbohydrates on serum levels of non-esterified fatty acids noted by Dole [127] and by Gordon [122]. Waddell also has found decreases in serum lipid levels in some patients with infusions of pluronic in dextrose, or phosphatides-pluronic-dextrose.

There has been no published evidence of sequestration of serum lipids in other tissues, following fat infusions. It is believed on the basis of dog experiments [118] that the infused fat and that which disappears from the blood stream are both rapidly metabolized.

Subsequent to the original report, Herbst, Lever and Waddell [123] showed significant increases in the electrophoretic mobilities of the serum lipoproteins following fat infusions. Since the same effects had been noted after heparin injections, they postulated that the infusion of fat also may trigger a release of clearing factor.

This new approach may aid in understanding the abnormalities which lead to hyperlipemia and hypercholesteremia, but it is not proposed as a practical therapeutic measure.

Hydrogenated Fats. The postulated increase in incidence of ischemic heart disease since World War I is linked by some [124] to the increased use of margarines prepared by partial hydrogenation of vegetable fats. Following the suggestion in 1955 [40,41] that serum cholesterol levels in man might be related to the degree of unsaturation of the dietary fat, it became tempting to think that the destruction of essential fatty acids by hydrogenation, and the formation of so-called “unnatural” isomers, might lead to harmful effects upon ingestion. This reasoning is based on three unquestioned facts: the finding [125] that cis-trans and trans-trans isomers of linoleic acid cannot replace cis-cis linoleic acid in remodeling essential fatty acid deficiency; the presence of heavy concentrations of trans acids in hydrogenated products [126]; and the large consumption of margarines throughout the Western world.

Mann [6] has shown that there is serious question whether or not the incidence of ischemic heart disease has actually increased. Yudkin points out [4] that the incidence of ischemic heart disease in various populations does not parallel the use of margarines. Thus, Norway, in which the per capita consumption of margarine is three times that of the United States, has less than one-third our incidence of this disease.

The ingestion of hydrogenated fats produces somewhat higher serum cholesterol and phospholipid levels in man than the unhydrogenated oil; this has been shown by Bronte-Stewart [64], Ahrens [62] and Malmros [68]. However, these experiments demonstrate only that the effects observed are correlated with the over-all loss of many of the double bonds of the natural fat. The effect of specific isomers of linoleic and oleic acids on human serum lipid levels has never been critically tested. Therefore, there is no direct evidence that the isomers have a different effect on these levels than the parent acids.

The term “unnatural isomer” deserves comment. Shorland and Hansen [127] have shown in numerous studies that all ruminant depot fats contain significant amounts of branched-chain fatty acids as well as odd-numbered acids (perhaps 5 per cent of the total acids). It is believed that bacterial action in the rumen is responsible for the production of these acids. They are then deposited in the animal's depots, and are natural components of the fat of mutton, goat and beef. Their small concentration in these fats suggests that they are metabolized. Current studies of human fatty acid mixtures [128] have demonstrated the normal occurrence of branched-chain and odd-numbered acids, as well as numerous positional isomers of oleic acid [129]. It would be profitable to study the metabolism of these components.

The use of hydrogenated fats as the sole source of dietary fat in the rearing of forty-six successive generations of rats was shown by Alfin-Slater et al. [130] to have produced no demonstrable ill effect on growth, longevity, reproduction, lactation, litter size and other parameters. It may not be justified to extend these findings to man unreservedly, since the rat is relatively resistant to the experimental production of atherosclerosis.

Heated and Oxidized Oils. Under conditions of economic stress after World War II highly unsaturated fish oils, thermally treated to get rid of undesirable tastes, were included in commercial food products, although it was well known that thermal treatment of oils produces polymerization. When it became apparent that “heat-bodied” oils were not acceptable for human consumption, this practice (largely confined to the Scandinavian countries) was rapidly discontinued. Nevertheless, there continues to be an interest in the possible formation of toxic products during the heating of edible oils, as during deep fat frying. Kummerow has
partially polymerized a number of edible oils and has isolated products which were toxic for rats. He and his colleagues have recently proposed [137] that these products exert their toxicity by destruction of pyridoxine and riboflavin, since supplementation with these substances partially counteracted the toxicity of the polymerized fats. They claimed that, although heat polymerization and oxidative polymerization result in different toxic products, both insults may occur in the course of commercial food frying. Melnick [132], in defense of commercial frying practices, noted no change in iodine value of frying oils sampled in a large number of potato chip factories, and considered this to be adequate proof that polymerization did not take place under practical conditions. The experimental studies of Crampton et al. [133], Kaunitz and Slanetz [134], and Kaneda et al. [135] are pertinent.

The practical importance of heat damage to unsaturated oils remains to be established. Nevertheless, the chemical changes in fats which might be produced by various cooking conditions have not been defined. As unsaturated fats are incorporated more widely in everyday diets, it becomes increasingly imperative that such studies be carried out.

Acute Effects after High-Fat Meals. Clotting: Duncan and Waldron [136] in 1949 were among the first to demonstrate that after ingestion of a fat meal the coagulation time of whole blood is significantly shortened. They suggested that this phenomenon might explain the high incidence of coronary heart disease in hyperlipemic states like diabetes. In 1953 Fullerton et al. [137] confirmed this finding with two tests: the clotting of whole blood in silicone tubes, and an accelerated one-stage prothrombin time (Stypven time, using Russell's viper venom as thromboplastin). They discussed at length the concept that hypercoagulability of the blood following fat meals might play an important role in the pathogenesis of thrombosis and complications of atherosclerosis. A vast amount of research has been stimulated by their provocative paper.

In 1955 Poole [138] concluded that chylomicrons hastened the clotting of recalcified citrated plasma. In later studies [139, 140] Poole and Robinson demonstrated that the factor in chylomicrons which caused this phenomenon, as well as increased Stypven times, was its phosphatide. They identified the active component as phosphatidyl ethanolamine, and stated that lecithin, phosphatidyl serine and inositol phosphatidate were inactive. O'Brien [141] in 1955 concluded that chylomicrons activated the Stypven test, but neither he nor, later, Buzina and Keys [142] were able to relate the clotting time changes to the curve of lipemia. In 1956 O'Brien [143] confirmed the finding that phosphatidyl ethanolamine in exceedingly small concentrations activated clotting in vitro. He found in human volunteers that ingestion of 50 gm. of butter, margarine or a "vegetable cooking fat" all produced equal acceleration of the Stypven time, but that one-third as much egg yolk fat caused an even greater acceleration. In 1957 he [144] reported that four fats of widely varying degrees of unsaturation caused equal reductions in clotting times, and that test meals containing equivalent amounts of phospholipids (soybean phospholipids or egg lecithin) had a still greater effect. He postulated that a part of the phospholipids, absorbed intact without hydrolysis [145], might cause these effects. He obtained no clear relationship between total phospholipid levels in the serum and clotting times, but this negative result would be expected if only one of the several serum phosphatides is primarily reactive. Maclagan and Billimoria [146] studied the addition of various foods to the Stypven test system and concluded that milk products had a unique accelerating effect.

The potential importance of these findings demands that further exploration be made of (1) the striking hypercoagulability of blood after meals containing eggs, (2) the site of action of specific phosphatides in the complex chain of events which is termed "clotting," and (3) the relationship between clotting activity as measured outside the body and the phenomenon of thrombosis itself. O'Brien [147] found no demonstrable difference in Stypven times after fat meals in twenty male patients with coronary thrombosis and twenty age-matched male volunteers, and noted that "blood coagulation studied in the test tube may have little relevance to the in vivo formation of a thrombus." However, McDonald and Edgill [148], studying forty-eight patients in each group, found statistical differences in a number of clotting indices. They could not state with certainty whether the increased coagulability in the coronary patients was cause or effect of their disease. When values of individual patients were compared, there were broad overlaps; thus, it was not possible to predict thrombotic tendencies in any given patient.
It is clear that further progress is hampered by technical difficulties in the testing methods, and it is hoped that more direct and meaningful assays may be developed as the mechanisms of clotting are better understood. It may then be possible to obtain a clearer picture of the time-course curve of change in clotting activity after fat meals, and perhaps to relate it to some aspect of phospholipid metabolism.

Fibrinolysis: Greig [149] reported in 1956 that the ingestion of a high fat meal (butter, eggs, bacon) by healthy volunteers caused a significant reduction in fibrin clot lysis in vitro. The degree of inhibition of in vitro fibrinolysis after a fat meal was decreased by exercise and was reversed after intravenous injection of heparin. When corn or peanut oils constituted the test fat meal, there was no inhibition of fibrinolysis.

In a recently reported extension of these findings, Greig and Kunde [150] found that the ingestion of all vegetable oils, regardless of degree of unsaturation, activated fibrinolysis, while egg yolks and butter fat inhibited it. When lipids were removed from the serum by various solvents and the residues tested for fibrinolytic activity, the fibrinolytic system was reactivated. The degree of reactivation was most striking in the sera of patients fed egg yolk and butter fat, least effective in the case of all vegetable fat feedings.

Greig postulates that feedings of butter and of egg yolk lead to the presence of a type of serum lipoprotein which inhibits fibrinolysis. It is tempting to speculate that in vivo fibrinolysis may also be affected by the feeding of different types of fat. In studies of hypercoagulability, cream [146] and egg yolk [144] seemed to show striking differences from the other oils. In both systems (fibrinolysis and coagulation) differences could not be related to the degree of unsaturation of the dietary fat. Qualitative differences in serum lipids seem more determinant in both systems than quantitative differences.

Blood viscosity: Changes in viscosity of the blood following fat meals have been noted by Swank, as well as increased adhesiveness and aggregation and decreased sedimentation rates of red blood cells [151-154]. He also studied the sludging of blood and changes in the capillary bed in the hamster cheek pouch by means of motion picture records of these responses to fat meals. Aggregation of chylomicrons was noted six to nine hours after a heavy fat meal in volunteers; this was much less marked when unsaturated fats were fed in the test meal than in the tests with saturated fats [157]. It is interesting that all these phenomena occurred several hours after the peak of lipemia [153].

The failure of Watson [155] to find any alterations in blood viscosity of patients after ingestion of cream must be reconciled with Swank's findings. It is possible that the lack of sensitivity of Watson's viscosity method masked the effects he was studying.

Angina pectoris: Kuo and Joyner [156] in 1955 reported a detailed study of the response of fourteen patients with coronary insufficiency to a test meal of butter fat (0.6 gm. per pound body weight). In six patients angina pectoris developed postprandially, with pain at the peak of lipemia. Electrocardiographic changes were demonstrated in four. The administration of a non-fatty meal to three of the reactive patients failed to produce angina. The authors concluded that patients with coronary insufficiency may benefit from a low fat diet, since in such patients postprandial lipemia may have a deleterious effect on the myocardium.

In our experience over the last five years, at least thirty patients have been maintained at constant body weight for periods of four to forty months by means of orally administered liquid formulas which contained various proportions and types of fat [62]. Their daily intakes were usually divided into five feedings. They received a maximum of 70 per cent of calories in the form of fat (or about 0.25 gm. fat per pound body weight five times per day), which might be either corn oil or butter oil or other fats. The most commonly used formulas contained 40 per cent of calories as fat (or about 0.14 gm. fat per pound five times per day). We have never seen any pattern of postprandial angina which could be correlated with the peak of lipemia. In fact, as noted and qualified previously [62], our patients have usually displayed much less angina during their management in the hospital on these formula diets. However, the much larger fat load administered by Kuo and Joyner may explain the results they described, since in a single meal they administered 90 gm. of fat to a 150 pound patient. This dose comprised 810 calories, or 34 per cent of the day's calories, taken in one meal. Their experience would suggest that it may be unwise for patients with coronary insufficiency to gorge on fat. The phenomena described are indeed interesting,
and it is hoped that further studies will explain whether or not the changes in viscosity of blood described by Swank [153] may explain this syndrome. It would be valuable to know whether or not all types of fats cause comparable effects.

For reasons to be discussed (see section on Carbohydrate), we believe that low fat diets may be undesirable in some patients.

**PROTEIN**

Our understanding of the effects of dietary protein on serum lipid levels is fragmentary. A considerable part of our confusion has been created by the use of numerous species of animals in countless variations of experimental design. It is compounded by lack of complete understanding of protein requirements of growing and adult organisms, and by the complexities created by the need to achieve a proper balance of amino acids in the diet [74, 157]. Nevertheless, it is clearly important to clarify the relationship of dietary protein to serum lipid levels: Yudkin [4], Yerushalmy and Hilleboe [5] and Olson et al. [75, 8] have pointed out the correlation which can be drawn between animal protein intake and the incidence of ischemic heart disease. It is essential to determine when this correlation is primary, that is, with dietary protein itself, and when secondary, namely, with the type of fat which is an integral part of animal protein foods.

Dietary protein apparently affects serum lipid levels in at least two ways. If there is a deficiency of labile methyl groups in the diet of rats, hypcholesteremia develops as the liver accumulates fat, even when the diet also contains cholesterol [159, 160]. This hypcholesteremia is not affected by type or quantity of dietary fat [158]. Thus, it appears that hypercholesteremia cannot develop with cholesterol feeding unless the diet contains an adequate supply of labile methyl groups. Possibly, the synthesis of phospholipids is limited, since choline is an integral part of lecithin and sphingomyelin. There may be human counterparts of this deficiency state. In areas where dietary protein is inadequate in quantity or quality, or when the rice diet is administered, serum cholesterol levels often are exceedingly low.

Secondly, it appears that the feeding of protein deficient in sulfur (i.e., alpha-protein of soybeans) leads to hypercholesteremia in cholesterol-fed monkeys [161]; here, again, choline also is limiting. The studies of Portman and Mann [162] showed that this type of sulfur deficiency inhibits the production of taurine, and thence of taurine-conjugated bile acids. When the conversion of cholesterol to taurine-conjugated bile acids is limited by sulfur deficiency in the diet, cholesterol accumulates in the plasma. It seems likely that the so-called "protective" effect of dietary protein against hypercholesteremia in cholesterol-fed chicks, reported by Kummerow et al. [163] and Moyer et al. [164], is due to increased sulfur requirements for conversion of cholesterol to bile acids.

This mode of action may also explain the hypercholesteremia in cholesterol-fed rats on low protein, high choline diets, which Jones et al. [165] described.

On the other hand, the hypercholesteremia produced in old rats on high casein diets by Jones and Huffman [166] has not been explained. It may be due to a relative deficiency of some nutrient caused by the excess intake of this unbalanced protein. These experiments deserve confirmation and extension; coronary atheromas developed in one-third of their rats.

Keys and Anderson [19] in 1957 reported experiments dealing with dietary protein and serum cholesterol levels in man. Two experiments were carried out on physically healthy schizophrenic men under metabolic ward conditions for sixteen or more weeks. In the first, two levels of dietary fat (16 and 39 per cent of total calories) were tested at two levels of dietary protein (11 and 20 per cent of total calories). No significant differences in serum cholesterol levels were produced by the two protein intakes at either fat level. In the second experiment, protein intakes of 8 and 18 per cent were compared (with 19 per cent of calories as fat in all periods). Again no significant differences in serum cholesterol levels were detected. These experiments give a clear negative answer to one question: does increasing the intake of protein from a level generally recognized as adequate for maintenance of health in adult man to a still higher level cause any change in serum cholesterol levels? However, it seems unwarranted to extend these findings to diets containing lower protein intakes than they actually tested, especially to ones which may be suboptimal. The statement that "the results of the present study do not afford confirmation to the suggestion that the low cholesterol values in populations living on low fat intakes are in any way related to the amount or kind of protein in
Nutritional Factors and Serum Lipid Levels—Ahrens

their diets may be misleading. Olson et al. [758] commented on the possibility that the rice diet (25 to 30 gm. protein per day), with its well recognized hypocholesteremia, may be suboptimal in protein. In a well controlled experiment in seven men, they were able to produce hypocholesteremia with a low protein, moderate fat diet [167]. Hatch et al. [168] demonstrated abnormalities in bromsulfalein retention and in free-to-total cholesterol ratios in patients on the rice diet.

In short, it is not clear that the low cholesterol levels seen in populations eating diets very low in fat may not be due in part to low intakes of protein.

CARBOHYDRATE

A number of experiments have been carried out in this laboratory [62] which explored the effects of variations in dietary carbohydrate at the expense of dietary fat. Figure 8 demonstrates that when corn oil made up only 10 per cent of total calories, there was a sudden marked increase in serum triglyceride levels as well as a small rise in phospholipid and cholesterol levels. When the corn oil intake was increased to 70 per cent of total calories, there was a prompt fall in triglyceride and other lipid levels. Thus the lowest serum lipid levels occurred on the highest intake of corn oil.

Similar changes have been produced in four other patients. In the oldest of these, a seventy-two year old woman with coronary insufficiency, there was a fourfold increase in the serum triglyceride level when a formula containing 70 per cent of calories from corn oil was replaced by a diet free of fat [63]. In addition, there was marked lipemia on the fat-free diet and a striking increase in Sf20 lipoproteins. It remains to be determined whether or not this response is a function of age; less dramatic effects on triglycerides were produced by these dietary alterations in a thirty-three year old hypercholesteremic man. Hatch and associates [168] also noted a rise in the triglyceride level and abnormal ultracentrifugal patterns in patients fed rice diets low in fat. The brief note of Nichols et al. [169] confirms our findings in part; they noted major elevations in Sf20-400 lipoproteins when a high carbohydrate, low fat diet was eaten. Such experiments demonstrate that the various serum lipid levels need not vary in a parallel manner and that a total cholesterol value may give little indication of the other lipid levels. Since in some patients a diet low in fat produces high levels of serum triglycerides, we are tempted to ask whether or not the lower density lipoproteins are less “atherogenic” than the higher density lipoproteins rich in cholesterol and phospholipids. We know of no solid evidence on this point, and until this is further explored we question the wisdom of prescribing very low fat diets for the general population.

Yudkin’s [4] analysis of factors related to the incidence of ischemic heart disease showed a better relationship with intake of sugar than with any other major foodstuff. The intake of simple sugars and conversely of complex polysaccharides (starch) unquestionably varies from region to region. Those peoples who subsist largely on tubers, cassavas and other starchy foods probably eat less sugar per se than the wealthier, more civilized peoples. Yet, in the latter group, while total carbohydrate intake is much lower, a large proportion of this intake undoubtedly consists of simple sugars. Thus the people who are said to have the lowest incidence of ischemic heart disease eat diets characterized by (1) lowest total protein and animal protein intakes (which might cause low serum cholesterol levels because of suboptimal intakes of labile methyl groups), (2) lowest total fat intakes, and probably a higher ratio of unsaturated to saturated fats (both factors might serve to depress cholesterol and phospholipid levels), and (3) highest intakes of carbohydrates, of which the largest part is starch.

In the light of these considerations, an observation made by Foster, Hooper and Whipple [170] in 1919 assumes some importance. They noted that in dogs the excretion of bile acids was markedly reduced by feeding diets containing simple sugars. Portman, in his analysis of the factors influencing bile acid excretion in rats, has greatly extended the original findings of the Whipple group. In 1955 he showed [771] that when dextrose or sucrose was substituted in the diet isocalorically for corn starch, the total bile acid excretion decreased. The output of cholesterol and of total bile acids in the bile of rats fed a purified diet containing sucrose as the sole carbohydrate was smaller than when Purina Chow was fed. These findings suggested that the feeding of simple sugars affected the conversion of cholesterol to bile acids in some manner, and that the degree of experimental hypercholesteremia might be influenced by the type of
carbohydrate feeding. Later experiments [172] demonstrated that when corn starch replaced sucrose, glucose or fructose isocalorically, the serum cholesterol levels of cholesterol- and cholic acid-fed rats were considerably lower. The addition of sulfasuxidine to the diet to reduce the bacterial flora in the gut abolished the hypocholesteremic effect of starch. Findings contrary to or confirming Portman's data in rats have not appeared, but Grant and Fahrenbach [173] in studies of cholesterol-induced hypercholesteremia in chicks have found that the response to glucose and sucrose were strikingly different; cholesterol levels were higher on the sucrose diets.

Since Portman's data indicate that the intestinal bacteria may play an important role in the phenomenon described by him, one might expect considerable species differences to appear as these studies are extended. In our investigations of serum lipid levels in man, we have noted no significant differences caused by isocaloric exchanges of sucrose, dextrose and dextrins, but we have not carried out tests with starches. In this connection it should be mentioned that the digestibility of starches of various sources may be very different [174].

**Intestinal Bacteria and “Bulk”**

It is appropriate that this review should include a brief consideration of the factors which influence intestinal bacteria, and the possible role that these bacteria may play in affecting serum lipid levels. In the previous section it was noted that the different effects of corn starch and simple sugars on serum cholesterol levels of rats were abolished by administration of sulfasuxidine [172]. Cholesterol is degraded eventually to bile acids [175] and in the gut the bile acids are chemically altered by the bacteria with the production of materials which probably are not reabsorbed. Thus the method for disposal of cholesterol is highly complex and depends finally on the action of the intestinal flora. In germ-free rats this mechanism is non-existent, and the half-life of the bile acids is greatly prolonged [176]. Reabsorption of undegraded bile acids unquestionably affects the rate at which cholesterol is converted to bile acids in the liver.

In addition to these events, bacteria are also responsible for the conversion of cholesterol to coprosterol in the gut [177–179]. While the former is slowly reabsorbed, the latter is considered to be completely non-absorbable. If cerebrosides are administered in the diets of rats, this conversion of cholesterol to coprosterol is accelerated. If sulfasuxidine is given, this conversion is inhibited. Modern concepts of the conversion of cholesterol to coprosterol are described by Rosenfeld et al. [180]. Although the mode of action of cerebrosides has not been investigated in human beings, Jones et al. [181] have found that serum cholesterol levels can be lowered in man by oral administration of brain cerebrosides.

Thus it is apparent that the intestinal flora may affect serum cholesterol levels in at least two ways, (1) by converting cholesterol to a non-reabsorbable compound, coprosterol, and (2) by degrading bile acids to products which are preferentially excreted in the feces, thus indirectly accelerating the conversion of cholesterol to bile acids. Bersohn et al. [182] have postulated that the intake of cellulose fiber may cause alterations in the bacterial flora as well as increased excretion of fecal fats. In animal husbandry it is well known that the indigestible portion of the feed markedly influences the digestibility of fat, protein and carbohydrate nutrients [183]. Lin et al. [184] have shown in rats that pectin and protopectin markedly affect the excretion of dietary fat but not of endogenous cholesterol. Whether such “bulk” agents have an effect on only intestinal motility and on food-stuff digestibility, or perhaps also on intestinal microorganisms, has not been defined.

We are impressed by the importance of defining (1) the types of intestinal bacteria which flourish in the gut under different feeding conditions and (2) their biochemical capabilities. It will be difficult enough to identify specific changes in flora caused by controlled alterations in dietary intakes in a given patient. It will be much more difficult, but nevertheless illuminating, to define what those bacteria need for their own nourishment, and in turn what they contribute to their host.

**Trace Substances**

*Magnesium.* In 1956 Malkiel-Shapiro et al. [185] reported that parenteral administration of magnesium sulfate produced clinical improvement in patients with ischemic heart disease, and reversion of abnormal serum lipoprotein patterns to normal values in many cases. This report was followed in 1957 by a study [186] of serum magnesium levels in Bantu and European South Africans, which purported to show signifi-
Nutritional Factors and Serum Lipid Levels—Ahrens

by Rosenman and Smith [793] indicated that significantly higher levels in the Bantu; a correlation in Europeans of serum magnesium and cholesterol levels was strongly negative. It was claimed that the higher the serum cholesterol level, the lower the serum magnesium. In view of the difficulties in making accurate analyses of serum magnesium, it is essential that these findings be independently verified. The clinical claims demand that a double-blind experiment be instituted.

Experimental studies of Vitale et al. [187–188] indicate that magnesium and lipid metabolism may be related, but it is not yet clear whether this relationship is direct or indirect. They have observed that in rats fed a diet containing 10 per cent protein and 24 mg. of magnesium per 100 gm. of diet magnesium deficiency develops only when cholesterol and cholic acid are included in the regimen. This was characterized by hyperexcitability, hyperemia of the ears, calcium deposition in the renal tubules, low serum magnesium levels and decreased oxidative phosphorylation of heart muscle mitochondria. All lesions were prevented by raising dietary magnesium four to eightfold. The lipid deposition in the aorta and heart valves caused by the cholesterol and cholic acid loads was greatly reduced when the dietary magnesium was increased, but the elevated serum cholesterol levels rose still further. Increasing the dietary protein intake to 20 per cent decreased the serum cholesterol levels, indicating that the 10 per cent protein intake had been limiting. Clearly, this is a complex phenomenon which deserves further exploration.

Other Metals. Schroeder [189] has presented an interesting postulate based on the abnormal occurrence in tissues of many trace metals (chromium, cadmium, nickel, aluminum, tin and lead). He suggested that these metals might interfere with numerous metal-activated enzyme systems, especially with those dependent on pyridoxine and recalled that this vitamin is a complex phenomenon which deserves further exploration.

REFERENCES

13. Progress in Chemistry of Fats and Other Lipids.
Nutritional Factors and Serum Lipid Levels—Ahrens


36. Snapper, I. Personal communication.


50. Watkin, D. M., Froeb, H. F., Hatch, F. T. and Gutman, A. B. Effects of diet in essential hyper-
Nutritional Factors and Serum Lipid Levels — Ahrens


Nutritional Factors and Serum Lipid Levels—Ahrens


107. See [56].


117. MENG, H. C. Preparation, utilization and importance of neutral fat emulsion in intravenous alimentation. Chap. 5 [77].


120. WADDELL, W. R. Personal communication.


131. WITTING, L. A., NISHIDA, T., JOHNSON, O. C. and

AMERICAN JOURNAL OF MEDICINE


169. 1213, 1957.


