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PLASMA VITAMIN A AND E IN THE STUDY OF LIPID AND LIPOPROTEIN METABOLISM IN CORONARY HEART DISEASE

BY

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INTRODUCTION

Patients with coronary heart disease frequently exhibit an abnormally high serum lipid content. This finding and the presence of an accelerated formation of atheroma in such endogenic defects of lipid metabolism as diabetes and essential hyperlipemia and hypercholesterolemia support the view that coronary heart disease is in essence an error of lipid metabolism. In the course of research on atherosclerosis, the metabolism of each of various serum lipids and lipoproteins has in turn been thought to be altered the most in coronary heart disease.

Purely exogenous lipids, such as carotenoids (Thomson 1934, Blankenhorn *et al.* 1956) and tocopherol (Mc Cormick and McCluer 1960), have been found in human atheromas, thus suggesting that the atheromatic lipids are derived from circulating lipids and are not synthetized in the arterial wall. According to the filtration theory of Page (1954), the primary process in atheroma formation is the lipid infiltration, the fibrous tissue present in atheromas being a secondary reaction to the fat imbibition. Moreton (1947, 1950) was the first to focus attention to the alimentary lipemia as a possible important factor in the deposition of lipid into the arterial wall.

In numerous investigations a prolonged and intensive postprandial lipemia has been found in patients with coronary heart disease. This has been thought to be due to an impaired fat removal from circulation, reflecting an abnormal lipoprotein metabolism. The reported harmful effects of lipemia, i.e., the acceleration of blood coagulation (Fullerton *et al.* 1953, O'Brien 1957), inhibition of fibrinolysis (Greig 1956), decrease of tissue oxygen tension (Joyner *et al.* 1960) and reduction of the coronary blood-flow (Regan *et al.* 1961) are also of great interest.

It was the object of the present investigation to study the behavior of purely exogenous lipids in the blood circulation of patients with coronary heart disease and of healthy persons. The fat-soluble vitamins A and E seemed to be well suited for this purpose, since they have been found to be present in human atheromas. Since these two vitamins are carried in a somewhat different manner by the serum lipoproteins (Krinsky *et al.* 1958, McCormick *et al.* 1960), it was considered probable that they would impart some information of the kinetics of lipoproteins.

REVIEW OF THE LITERATURE

SERUM CHOLESTEROL LEVEL IN CORONARY HEART DISEASE

The first reports of the presence of cholesterol in human atheroma are older than a hundred years. The great difference in the amount of cholesterol in atheromatic and intact aortas was observed much later (Windaus 1910). These basic observations as well as the production of atherosclerosis in rabbits by feeding a cholesterol-rich diet (Anitschkow 1913) focussed attention to cholesterol as an important factor in atherogenesis and led to a vast amount of research in this field.

Many surveys of this subject have been published. The following is only a brief summary of the outstanding studies and is far from complete.

The observations concerning the simultaneous occurrence of hypercholesterolemia and clinical atherosclerosis increased with time, and in 1925 Mjassnikow showed that, of the various clinical manifestations of atherosclerosis, hypercholesterolemia particularly was associated with coronary heart disease. In a review published in 1935 it was stated that serum cholesterol increased with age and with the extent of atherosclerosis, and that its determination was of clinical value (Hurxthal and Hunt 1935).

The important role of a disturbed cholesterol metabolism in the development of atherosclerosis then began to be generally accepted and was supported by many clinical observations (Hirsch and Weinhouse 1943). The findings of Mjassnikow were confirmed by Davis *et al.* (1937) in a systematical clinical study. The high incidence of coronary atherosclerosis in hypercholesterolemic xanthomatosis was stressed (Thannhauser and Magendantz 1938). It was emphasized that diabetic hyperlipidemia was often complicated by coronary heart disease (Rabinowitch 1935). Hypothyrotic patients frequently showed concomitant hypercholesterolemia and occlusive atheromatosis (Hurxthal and Hunt 1935). The hypercholesterolemia associated with coronary heart disease showed often a familial occurrence (Boas *et al.* 1948).

The first systematical clinical study by Davis *et al.*, cited above, revealed thus a significantly higher serum cholesterol content in patients with angina pectoris than in a somewhat younger control group. Similarly Morrison and co-workers (1948) found hypercholesterolemia in 68 per cent of 200 survi-

vors of myocardial infarction below the age of 60 years. The occurrence of hypercholesterolemia was less common (48 per cent) in the older group.

One of the best statistically analyzed studies is that of Gertler *et al.* (1950 a). Of 97 survivors of myocardial infarction, 24 had a cholesterol value below the mean control value, and only 2 were below control mean — 1 S.D. On the other hand, 15.4 per cent of the matched controls exceeded the mean coronary cholesterol, while only 2 control persons exceeded the mean +1 S.D. The significant difference observed was not age-dependent (Gertler *et al.* 1950 b). A similar significantly higher serum cholesterol mean content as well as a high phospholipid mean were found by Steiner *et al.* (1952) in 82 coronary patients, compared with 112 healthy individuals. The increase in serum lipid phosphorus was not proportional to the increase in serum cholesterol, and thus an elevation in the serum cholesterol/lipid phosphorus ratio became apparent. Supporting the view of Ahrens and Kunkel (1949) it was suggested that because of the shift in the ratio toward cholesterol, the amount of phospholipid may be insufficient to maintain all the cholesterol in solution. Further support to the significant role of a higher cholesterol mean level in coronary heart disease was brought by a statistically well analyzed study from England (Oliver and Boyd 1953). The 200 patients studied showed a significantly higher serum cholesterol level than the control persons through the entire age range from 30 to 70 years of age, with the exception of the sixth decade in women. The occurrence of true hypercholesterolemia was only found in a small coronary group below the age of 40 years. A significant elevation in the ratio of total cholesterol/phospholipid was seen also here, and was regarded as an important gauge of a disturbance in the lipid metabolism in atherosclerosis. In a Swedish series published by Björck *et al.* (1957) young coronary patients (40—49 years) showed a significantly higher serum cholesterol level than healthy males from the same population. In higher age groups the difference appeared to be smaller or even absent when compared with the control group of Keys *et al.* (1950). It was suggested that the young coronary patients may have derived from a somewhat different population than the older ones.

By using an arbitrary cholesterol value of 241 mg/100 ml, more than 80 per cent of the men in the fourth decade in control and coronary groups were separated correctly (Little *et al.* 1956). Like the latter, many other investigators have stressed the common occurrence of hypercholesterolemia in the younger coronary patients (Lawry *et al.* 1957, Björntorp and Malmcrona 1960, Scarborough *et al.* 1960, Albrink *et al.* 1961). On the other hand Carlson (1960 b) has postulated that the triglyceride metabolism was more disturbed in young coronary patients and that of cholesterol at a later age.

In the study of Lawry *et al.* (1957) the 75 per cent limit of healthy men of comparable age was used as discrimin-

ator, and 120 of 261 coronary patients exceeded this limit in serum cholesterol values. The serum cholesterol levels of 141 men with angina pectoris were not as high, while the values of female survivors were identical with those of male survivors. In contrast to the data from the Donner laboratory (Jones *et al.* 1951), Lawry and coworkers stressed that none of the lipid parameters studied (Sf 12—20 and 20—100 lipoproteins and cholesterol) showed any clear superiority in the prognostication of coronary heart disease. Mattingly *et al.* (1959), on the other hand, considered the serum cholesterol level more informative than the elaborate measurements of lipoproteins. In the study of Albrink *et al.* (1961) the cholesterol content of 260 mg/100 ml best separated 212 survivors of myocardial infarction from the healthy population. This was the concentration which in the Framingham study (Kannel *et al.* 1961) was the borderline for a marked increase of coronary heart disease. Only 5 per cent of young healthy men in the third decade exceeded this concentration. Half or more of the patients with coronary heart disease remained unidentified, however. The serum cholesterol level offered the best differentiation between normal subjects and those with the disease under the age of 50. The serum cholesterol levels of 23 women who had had myocardial infarction were entirely comparable with those of men with this disease.

To evaluate the significance of the serum cholesterol content in coronary heart disease, the National Advisory Heart Council organized a large-scale study in the United States in 1953. Four laboratories analyzed the cholesterol content of serum in 4,914 men. During a 2-year observation period, clinical manifestations of coronary heart disease developed in 82 men. The cholesterol measurements of all the laboratories combined showed a highly significant ability to place the subjects with definite events above the fiftieth percentile but not above the seventy-fifth percentile. Thus for the first time coronary heart disease was shown to be associated with an antecedent elevation of the serum cholesterol level (Gofman *et al.* 1956).

In 1948 the United States Public Health Service undertook a prospective study to investigate the incidence of coronary heart disease and factors related to its development. This study concerned 4,469 men 30 to 59 years of age in Framingham, Massachusetts. The serum cholesterol of all persons was determined. During a six-year follow-up 186 men developed coronary heart disease, representing an over-all incidence of 36.3 per thousand. A significantly higher mean cholesterol level was demonstrated among subjects who developed the disease than among the base population. The elevation was most marked in the men in the youngest age groups. It was concluded that a high cholesterol level increases the risk of development of coronary heart disease and that this risk in men 40 to 59 years of age is more than three-fold for individuals with serum cholesterol over 245 mg/100 ml than below 210 mg/100 ml (Kannel *et al.* 1961).

From the very beginning of research on cholesterol, the relationship between the serum cholesterol level and the extent of atherosclerosis found at autopsy has been a subject of investigation. The data available, however, are controversial. In an autopsy series of persons who had died of violence the serum cholesterol determined postmortem did not correlate with the aortic lipid content (Landé and Sperry 1936). In another study the cholesterol content of the aortic wall had a direct relationship to age but not to serum cholesterol (Faber 1946). The determination of cholesterol from postmortem blood samples was criticized by Morrison and Johnson (1950). They showed that the coronary arteries of patients who had died of acute coronary thrombosis contained, on an average, four times as much cholesterol as those of control patients. Similarly, hypercholesterolemia was found in most of the coronary patients, as compared to a normal average in the control group. The recent data of Paterson *et al.* (1960), again, showed that the severity of atherosclerosis was poorly correlated to the serum antemortem cholesterol level, except perhaps when it exceeded 300 mg/100 ml.

SERUM BASAL TRIGLYCERIDE LEVEL IN CORONARY HEART DISEASE

During the time that research of atherosclerosis has been carried on, different lipids or lipoprotein fractions have been thought to be the most atherogenic. The latest newcomer in the list of atherogenic lipids is neutral fat.

The alterations first observed in serum lipoproteins concerned the cholesterol-rich fraction Sf 10—20 (Gofman *et al.* 1950). Later, however, it appeared that the fraction Sf 12—400 of lower density showed a better correlation to clinical coronary heart disease (Gofman *et al.* 1954), suggesting an abnormally high serum triglyceride level (Havel and Carlson 1962). The presence of an abnormal metabolism of triglyceride was also supported by the observation of abnormalities in the alimentary hyperlipemia commonly seen in coronary patients, which will be described later. In addition, attention has been drawn to a higher triglyceride content of the human coronary arteries as compared to the aorta (Böttcher *et al.* 1959).

The study of Hauss and Böhle (1955) stressed for the first time the common occurrence of hypertriglyceridemia in coronary heart disease. In 21 survivors of myocardial infarction, the serum triglyceride content was estimated by subtracting the other lipids from the total lipid value. All patients exceeded the mean triglyceride level of age-matched controls, while the serum cholesterol values were in the upper limit of the control mean. The extensive studies of Schrade *et al.* (1959, 1960, 1961) supported this finding. Triglyceride was estimated by the sub-

traction method; however, similar data were obtained by absorption chromatography. Their largest series (published in 1960) consisted of 452 atherosclerotic patients, 321 of whom had either angina pectoris or a healed myocardial infarction. Hyperlipidemia occurred in 80 per cent of coronary patients. The serum triglyceride content was 44.2 per cent higher in the whole series than in 60 healthy persons of comparable age. The values for total cholesterol, total lipids and phospholipids were 16.1, 29.2 and 17.4 per cent, respectively. In a later publication (1961) they reported a 45 per cent increase of serum triglyceride with aging, calculated from two healthy male groups 18—42 and 46—71 years of age. Nevertheless, the significant difference remained also when the coronary patient group was compared with the older control group. As a »true» control group Antonis and Bersohn (1960) used 57 healthy Bantu negroes with a low incidence of coronary heart disease. The mean serum triglyceride +1 S.D. in the negroes was exceeded by 96 per cent of 23 European coronary patients in the same region, by 6 per cent of European male subjects below the age of 30 years, and by not less than 88 per cent of males over the age of 50 years. Since even the standard deviation increased with increasing age and serum triglyceride levels, the authors suggested the existence of two populations among older men, the one population consisting of the true healthy persons and the other of individuals with potent atherosclerosis. In a Swedish series studied by Carlson (1960 b) hypertriglyceridemia was characteristic of coronary patients below the age of 50 years, while the older patients showed hypercholesterolemia more often. Triglycerides were estimated by determining the glyceride glycerol. It was concluded that the coronary patients were composed of two different populations; the one with a primarily altered triglyceride metabolism and the other with a disturbed cholesterol metabolism.

Further light on this problem has been thrown by Albrink and co-workers (Albrink and Man 1959, Albrink *et al.* 1961, Albrink 1962). Triglycerides were calculated on the basis of the serum total esterified fatty acids. Contrary to the data of Carlson, hypertriglyceridemia was more common in patients over the age of 50 years (89 per cent) than in the younger patients, who showed a somewhat lower incidence (82 per cent). The younger patients showed a higher incidence of hypercholesterolemia, again in contrast to Carlson's data. The upper 95 per cent limit of healthy men of the age of 20—29 years was used as the normal value. This was exceeded, however, by 40 per cent of healthy men over 40 years of age. To solve the problem of the common occurrence of hypertriglyceridemia among older healthy persons, Albrink and co-workers (1962) showed that hypertriglyceridemia was often associated with a weight gain of over 4.5 kg after the age of 25 years and/or with a positive family history of atherosclerosis.

Recently Berkowitz and Croll

(1962), supporting the earlier findings, stated that hypertriglyceridemia and impaired radioactive fat tolerance were the most characteristic abnormalities in lipid metabolism in coronary heart disease. In 100 patients they observed hypercholesterolemia in 41 per cent and hypertriglyceridemia in 72 per cent, while 82 per cent exhibited an impaired fat tolerance.

DISTRIBUTION OF CHOLESTEROL AND TRIGLYCERIDE IN SERUM LIPOPROTEINS IN CORONARY HEART DISEASE

In the basic observation of Gofman and co-workers (1950) attention was focussed on the elevated lipoproteins as the most significant abnormality in the blood of patients with coronary heart disease. At first it was thought that the cholesterol-rich lipoprotein Sf 10—20 was altered the most. Later, however, the Donner group broadened the lipoprotein analyses to comprise almost the entire beta-lipoprotein spectrum Sf 0—12, 12—400 (Jones *et al.* 1951, Gofman *et al.* 1952, 1954). In order to weight the atherogenicity of each fraction, an atherogenic index was developed. It was found to correlate to clinical coronary heart disease better than any individual lipoprotein fraction. The atherogenic power of Sf 12—400 has been »weighted» at 1.75 times that of the Sf 0—12 fraction (Gofman *et al.* 1954).

In the prospective study organized by the National Advisory Heart Council in the United States it appeared that there existed a prior elevation of Sf 20—100 but not of Sf 12—20 lipoprotein in the men who developed coronary heart disease during the 2-year period of observation (Gofman *et al.* 1956). The Donner and Eastern laboratories disagreed, however, on the significance of these measurements, above all with respect to the predictive value of separating persons considered susceptible to coronary heart disease from the normal population. The existence of an elevated serum lipoprotein content in coronary patients has been confirmed by many other authors, but opinions differ concerning the superiority of the lipoprotein measurements as compared to the simple estimation of serum total cholesterol (Doyle *et al.* 1956, Little *et al.* 1956, Lawry *et al.* 1957, Mattingly *et al.* 1959, Page and Lewis 1959, Schlessinger *et al.* 1959).

Attempts have been made to clarify further the abnormal lipoprotein pattern in coronary heart disease by means of measurement of the lipid composition of the lipoproteins. Barr and co-workers (1951) were the first to show by lipoprotein-lipid analyses abnormalities in also high density lipoproteins. They showed that in the sera of survivors of myocardial infarction the alpha-lipoproteins separated by the Cohn method showed a significant decrease in the cholesterol content,

while the beta-lipoprotein cholesterol was elevated. This finding was confirmed by Pratt (1952) in an preliminary study using the ultracentrifugal technique.

The method of eletrophoretical separation for this purpose was introduced by Nikkilä (1952, 1953). The earlier observations were confirmed also in patients with a normal serum cholesterol content. If 70 per cent of the cholesterol bound to beta-lipoproteins was used as discriminator, this level was exceeded by one-fourth of healthy individuals over 40 years of age. On the other hand, one-eighth of the coronary group was below this limit, thus showing a normal distribution pattern. Similar data have been obtained by Jencks *et al.* (1956) in paper electrophoresis and by Carlson (1960b), who isolated the lipoproteins in a glass powder column. In the latter study the measurement of the lipoprotein-lipid distribution, however, separated the coronary patients from healthy population no better than did the serum cholesterol. Nor could Doyle and co-workers (1956) find any significant difference between healthy and coronary populations in the cholesterol or phospholipid content of the two major lipoproteins separated by Cohn fractionation. Serum cholesterol and phospholipids, however, were significantly higher in the coronary group. It was concluded that in a biologically homogeneous population universally susceptible to coronary atherosclerosis, the blood lipoprotein pattern is neither quantitatively nor qualitatively a satisfactory index of atherosclerosis.

Confirming the earlier observations of an abnormal distribution of cholesterol and phospholipids in lipoproteins, Schettler *et al.* (1957) focussed attention on the high neutral fat concentration in the $alpha_2$ region of starch electrophoresis in the sera of coronary patients.

A similar tendency to a decrease of $alpha_1$-lipoprotein lipid with a concomitant increase of beta-lipoprotein lipid has been shown by the lipid staining technique in coronary patients (Antonini *et al.* 1953, Kroetz and Fischer 1954, Fasoli *et al.* 1957). The existence of a large pre-beta-lipid band, roughly equated with Sf 20-100 lipoprotein, was regarded by Smith (1957) as the most significant alteration in the sera of coronary patients. This method was shown to have an excellent property of separating the two groups.

Numerous data are thus available concerning alterations in the cholesterol and phospholipid levels in the two major lipoprotein classes in the sera of coronary patients, and nearly all of them confirm one another. Much work has been done with lipoprotein measurements by the ultracentrifugal technique. However, the distribution of different lipids in the beta-lipoprotein subfractions in coronary heart disease is but poorly known. In one study, Havel and co-workers (1955) showed an elevation of the entire D. < 1.063 fraction in a few patients with atherosclerosis or with a disease predisposing to it. The increase in chol-

esterol and phospholipids was due to D 1.019—1.063, to D < 1.019, or to both fractions. Triglyceride estimations were not done. The Donner group observed no significant alterations in the lipid composition of Sf 0—12, 20—400 and HDL 2—3 lipoproteins (Lindgren and Gofman 1957).

FAT LOADING TESTS IN THE STUDY OF LIPID METABOLISM IN CORONARY HEART DISEASE

Postprandial lipemia is caused by the newly absorbed neutral fat, which is in the form of fairly large particles, often called chylomicra. The plasma lipid concentration curve obtained after ingestion of a lipid meal is a function of many simultaneously occurring biochemical processes: fat absorption and transport in the plasma, the plasma lipid pool already present, and the rate of removal of the absorbed fat from plasma. The effect of the quality and quantity of the ingested fat is equally of importance. Of all these processes, the mechanism of lipid transport and the disappearance of exogenic lipids from the plasma has been of particular interest in atherosclerosis research.

The first observations of the lactescence of the serum were made already in 18th century. Much later, however, it was shown that the postprandial lactescence was due to small fat particles, termed chylomicra, which were newly absorbed neutral fat (Gage and Fish 1924, Frazer and Stewart 1937, Elkes *et al.* 1939). At about the same time the first fat loading tests were performed in order to study the physiology of alimentary hyperlipemia (Nissen 1931, Page *et al.* 1930, Man and Gildea 1932, Wechsler 1932). In 1934 Chaikoff and co-workers studied also the lipemic response in a case of disseminated cutaneous xanthomata.

The observation of Moreton (1947, 1950) that newly absorbed fat in the postprandial plasma of healthy persons was in a state similar to that in the fasting plasma of hyperlipidemic patients led to a systematical study of postprandial hyperlipemia in atherosclerosis. Because atherosclerosis was common in such hyperlipidemic states as nephrosis, diabetes and essential xanthomatosis, Moreton stated that »the cumulative effect of many fatty meals over a lifetime, by producing these transient showers of large lipid particles in the plasma, may be the underlying cause of intimal lipid deposition in human atherosclerosis.»

After the observations of Moreton the study of postprandial hyperlipemia became one of the central problems in atherosclerosis research. It has been used to study the disturbed lipid metabolism often associated with coronary heart disease. Additionally, attempts have been made to use the fat loading tests for discovering the latent defects susceptible to coronary heart disease, which are not revealed by the analysis of fasting plasma.

Methods Used in the Study of Postprandial Lipemia

The method generally employed is to observe the plasma lipid concentration curve after the ingestion of a standardized fat meal. In recent years, parenteral administration has also come into use to eliminate intestinal absorption.

The large number of methods available for analyzing the lipemic plasma have been presented in detail in a recent review by Dole and Hamlin (1962). Only a few of them, however, have been used widely in the quantitation of the lipemic response in fat loading tests.

In order to study the size of the lipid particles in postprandial plasma, Moreton measured the scattering of light in a nephelometer. The counting of chylomicra or measurement of their size under the microscope was used earlier by many authors (Becker *et al.* 1950, Zinn and Griffith 1950, Grüner and Hilden 1953, Schettler and Jobst 1955). Because of the many sources of error, this method is no longer in use. The determination of newly absorbed neutral fat by measurement of the optical density in a photometer is one of the most common methods even today (Pomeranze and Beinfield 1951, White *et al.* 1951, Schwartz *et al.* 1952, Woldow *et al.* 1954, Barritt 1956, Eggstein and Schettler 1958, Mitchell and Bronte-Stewart 1959, Bouchier and Bronte-Stewart 1961, Brown *et al.* 1961). The direct chemical determination of triglyceride has been little used (Nikkilä and Konttinen 1962), but the estimation of plasma total fatty acids has been since earlier time in common use (Chaikoff *et al.* 1934, Hirsch and Carbonaro 1950, Pomeranze and Beinfield 1951, Eggstein and Schettler 1958, Brown 1961, Brown *et al.* 1961, Kingsbury *et al.* 1962).

The increase in the concentration of light lipoproteins (Sf 20-100) after an acute fat load has been known since the observations of Gofman *et al.* (1952). As a measure of lipoprotein concentration in fat loading tests, Woldow and co-workers (1954) used the thymol turbidity test, and the changes in ultracentrifugal lipoprotein fractions during alimentary hyperlipemia were studied by Goldner *et al.* (1954) and Horlick (1957). The electrophoretical mobility of lipemic plasma was studied by Swahn (1953), Kunkel and Trautman (1956) and Jobst and Schettler (1956). The electrophoretical changes during the fat loading test were also studied in hyperlipidemic and atherosclerotic patients (Kuo *et al.* 1956). The lipid distribution in ultracentrifugal lipoprotein fractions after an acute fat load was investigated by Havel (1957) and the kinetics of different lipoproteins after the administration of I^{131}-labeled fat was the subject of an extensive study in various hyperlipidemic states by Kruger *et al.* (1960).

The postprandial lipemia depends on the nature of the ingested fat. Intensive lipemia followed the ingestion of fats containing long-chain fatty acids independent of the iodine value, while the lipemic response to medium-chain fatty acids was poor (Eggstein and Schettler 1958, 1959). Various oils

studied by Kingsbury *et al.* (1960) all caused triglyceridemia of at least 60 mg/100 ml, but there were differences in the response of the concentrations of serum cholesterol and phospholipids and in their ratio. Emulsification of the lipid in the study of Brown *et al.* (1961) gave an earlier peak in the plasma lipid concentration curve.

The lipemia following the fat load depends also on the dietary habits preceding the test. The tissue distribution of the C^{14}-labeled chylomicra varied according to the nutritional status in laboratory animals used by Bragdon and Gordon (1958). The lipemic response to the fat load has been shown to diminish after restriction of the dietary fat (Pomeranze *et al.* 1954) and after corn oil supplements to the diet (Bronte-Stewart and Blackburn 1958). In one study, however, the alimentary hyperlipemia was reported to have been similar in three populations of different race and with quite different dietary habits (Bouchier and Bronte-Stewart 1961).

In addition to the natural butter fats, the test substances also in use are vitamin A, I^{131}-labeled triolein and C^{14}-labeled tripalmitin. Cholesterol loading tests have also been performed (Wang 1952), but because of the poor lipemic response it seems not to be of great value in these tests.

Oral administration of I^{131}-labeled triolein was introduced by Thannhauser and Stanley (1949) and has been widely used since in the study of lipid metabolism in coronary heart disease (Likoff *et al.* 1958, Hall *et al.* 1959, Seller *et al.* 1959, Berkowitz 1960, Kruger *et al.* 1960, Metz *et al.* 1960, Berkowitz *et al.* 1961, Brown 1961, Brown *et al.* 1961, Edelman *et al.* 1961, George *et al.* 1961, Berkowitz and Croll 1962, Levine and Cohen 1962, Malamos *et al.* 1962). As pointed out by Thannhauser and Stanley, the method has many advantages compared to the conventional fat loading. With this method it is possible to follow the disappearance of the labeled fat for a longer time. It was also thought that simultaneous determination of the specific activities of the free iodine and the protein-bound iodine gave some information on the lipid metabolism.

Although opinions differ concerning the similarity of the transport mechanism of neutral fat and vitamin A, as will be presented in the following section, vitamin A has been used as an indicator of neutral fat by some investigators in the study of lipid metabolism (Martt and Connor 1956, Beaumont *et al.* 1958, Beaumont and Beaumont 1960 a).

To eliminate the influence of the absorption component on the plasma concentration, and for better information on the plasma lipid disappearance rate, intravenous administration has been widely used since the experiments of Becker *et al.* (1950). They administered lipemic plasma intravenously in a study of the influence of age on lipid disappearance from the plasma. The method used the most, however, is the administration of I^{131}-labeled triolein emulsion (Berkowitz *et al.* 1961, Feinberg *et al.* 1961, Balodimos *et al.* 1962, Mayfield *et al.* 1962). The clearance

rates of I^{131}-labeled triolein and C^{14}-labeled tripalmitin have been shown to be similar (Balodimos *et al.* 1962). Many investigators (Berkowitz *et al.* 1961, Bouchier and Bronte-Stewart 1961, Mashford and Nestel 1961) have also used an artificial oil emulsion (Lipomul).

Influence of Age on Postprandial Lipemia

Some controversy exists concerning the effect of age on postprandial lipemia. One of the earliest observations is that of Wechsler (1932), who stated that the curve of plasma total lipids was flat in young persons and ascending in persons of middle age, and that a descending curve was obtained in association with arteriosclerosis. The difference in the chylomicron count seen in the two age groups after oral fat loading disappeared when lipemic plasma was given intravenously (Becker *et al.* 1950). The lipemia measured by determining the plasma total lipids lasted longer among older individuals in the study of Herzstein *et al.* (1953). Grüner and Hilden (1953), on the contrary, observed a higher chylomicron count during postprandial lipemia in young individuals. However, in studies by Barritt (1956) and Bouchier and Bronte-Stewart (1961), age did not at all influence the lipemic response. A higher peak level of the plasma total fatty acids and of the chylomicron count in the older group than in the younger one was observed by Schettler and Jobst (1955) after fat loading. Similarly, a faster plasma clearance rate of lipids in the younger group as compared with the older one was found in the study of Brown *et al.* (1961). The disappearance rate of intravenously administered I^{131}-labeled triolein correlated intimately with age in the study of Mayfield *et al.* (1962). The disappearance rate became slower with the increase of age independently of the clinical manifestations of atherosclerosis. They concluded that the slower lipid clearance rate observed by many authors in atherosclerotic patients reflects the age difference rather than a metabolic defect.

Meager data are available on the influence of age on alimentary lipemia in coronary heart disease. The expected abnormality in the I^{131} curve in diabetic and coronary patients over the age of 60 years was not found by Sandberg *et al.* (1960) and Edelman *et al.* (1961). In the study of Mayfield *et al.* (1962), however, the age correlation in coronary patients continued throughout the entire age span up to the age of 70 years, as was stated above. On the other hand, Barritt (1956) and Seller *et al.* (1959) observed among coronary patients no correlation between age and lipemia.

Effect of Physical Activity on Postprandial Lipemia

Methodologically the effect of physical activity is of some importance, since in many experiments the control group consists of subjects doing their normal daily work, while the patient

group is at bed rest. On the other hand, many authors have stated that coronary heart disease occurs less frequently in the physically active population (Morris *et al.* 1953).

During alimentary lipemia the peak level was somewhat lower and appeared earlier in ambulatory subjects than during bed rest (Nissen 1931, White *et al.* 1951). A similar result was obtained in coronary patients in a study by McDonald and Fullerton (1958). Accordingly, one coronary patient with an abnormal postprandial lipemia at bed rest responded normally to a similar fat load in ambulatory conditions (Hall *et al.* 1959). Barritt (1956), however, observed no difference in the lipemic response in hospitalized and ambulatory coronary patients.

Physical exercise did not influence the intensitivity of the lipemia in the study of Billimoria *et al.* (1959), but the peak level appeared earlier. In a similar study of Cohen and Goldberg (1960) the lipemic response after physical exercise decreased in 15 of 22 medical students. There was an increase in 4 cases and no change occurred in 3 cases. They stated that this phenomenon was due to an increased clearing effect and not to a decrease in the fat absorption. The effect of heavy exercise on alimentary lipemia in military recruits was studied by Nikkilä and Konttinen (1962), who observed a significantly smaller rise in the triglyceride level in exercising than in resting subjects, whereas the increase in the concentration of free fatty acids was greater in the former group. Very interesting was the observation that the effect of exercise was much lower in individuals with a high fasting serum triglyceride value.

Results of Fat Loading Tests in Coronary Heart Disease

The basic observation of Moreton was, as stated, that following a fatty meal the physical state of the lipid particles appearing in the plasma is qualitatively the same as that in sustained hyperlipidemic conditions »that are known to predispose to the relatively rapid and severe development of atherosclerosis». One of the oldest fat loading studies performed in survivors of myocardial infarction is that of Zinn and Griffith (1950), who demonstrated a significantly greater ratio of chylomicra (large fat particles) to lipomicra (any fat particles) in the fasting plasma of atherosclerotic patients than of healthy subjects. This difference could not, however, be found during the period of peak absorption following a fatty test meal. In the study of Schwartz *et al.* (1952) the coronary patients showed no difference in the optical density of fasting plasma as compared to the control group, but the increase of optical density was significantly higher in the former group after a standard fat meal. Similarly, Woldow and co-workers (1954) observed a higher degree and duration of alimentary lipemia and lipoproteinemia in coronary patients than in control persons, as estimated by the plasma optical density and the thymol turbidity test. After Block *et al.* (1951) had demonstrated a much lesser clear-

ing effect of heparin during alimentary lipemia in atherosclerotic patients than in normals, Woldow and co-workers also tested the heparin clearing effect. One of ten coronary patients showed no clearing effect, while the others cleared normally. In the studies of Pomeranze and co-workers (Pomeranze and Beinfield 1951, Pomeranze *et al.* 1954) there was in the two of three groups studied an abnormally high and prolonged lipemia after fat loading, as shown by the plasma optical density and total fatty acid content. The two abnormally responding groups consisted of elderly patients with clinical manifestations of atherosclerosis and healthy subjects with an abnormal fasting serum lipid pattern. The third group consisted of healthy persons with normal fasting lipid values. Similar results were obtained by Camelin *et al.* (1954) in atherosclerotic patients and by Schettler and Jobst (1955) in coronary patients. The coronary patients in the study of Barritt (1956) showed a significantly higher optical density of fasting plasma. The optical density was also higher after a fat load, but the difference was significant only at 5 and 7 hours, as compared to age-matched male control group. As stated above, a corn oil supplement in the diet normalized the abnormal lipemic response obtained in coronary patients (Bronte-Stewart and Blackburn 1958). In another experiment the team of Bronte-Stewart (Mitchell and Bronte-Stewart 1959) showed that the abnormally high optical density of plasma seen in coronary patients after fat loading was not due to a defect in the clearing factor. They studied the effect of heparin in cross-matched tests: CHD lipid — CHD clearing factor, CHD lipid — control clearing factor, CHD clearing factor — control lipid, and control lipid — control clearing factor. No difference was obtained, however.

The first systematical study of alimentary hyperlipemia in coronary heart disease using I^{131}-labeled triolein was performed by Likoff and co-workers (1958). They divided the healthy individuals and coronary patients according to the serum cholesterol content into normocholesterolemic and hypercholesterolemic subgroups. The maximum activity was observed in the normocholesterolemic control group before 6 hours had elapsed but in a great part of hypercholesterolemic groups first after 6 hours had elapsed from ingestion of the labeled fat. The mean peak level was also significantly higher in the hypercholesterolemic groups. When the normal value was defined as the mean value of the controls + 1 S.D., it was exceeded by 80 per cent of the coronary groups irrespective of the serum cholesterol value. The hypercholesterolemic group without manifestations of clinical atherosclerosis responded abnormally in 90 per cent. A good separation of the coronary group from the healthy population was obtained also by Seller *et al.* (1959) by determining the peak and 24-hour specific activities after a test dose of I^{131}-labeled triolein. They observed an abnormal response in every patient who had had myocardial infarction even though many of these showed a normal fasting lipid or lipo-

protein pattern. On the other hand, only two subjects in the control group responded abnormally. The ratio of peak protein-bound activity to peak supernatant activity was also significantly higher in patients who had an abnormal radioactivity curve. In another study the I^{131}-triolein clearance time (time required for the serum radioactivity to fall to half its peak value) was estimated in 40 patients with coronary heart disease (Hall *et al.* 1959). When this value was compared with the serum cholesterol content it appeared that both parameters were abnormal in 25 cases, the clearance rate in 10 cases only, and the serum cholesterol in 4 cases only.

However, no significant difference was obtained in the plasma radioactivity curves after the administration of I^{131}-labeled triolein to coronary and age-matched control groups (Metz *et al.* 1960). They studied also the behavior of labeled fat in a group of Bantu negroes representing a »true» control group having a rare occurrence of coronary heart disease. The result was unexpected because the plasma activity in coronary patients was significantly higher at 6, 8 and 12 hours, whereas the Bantus showed a higher plasma activity in 36 hours. They suggested that this was due to a liver dysfunction common among negroes or to an earlier feed-back phenomenon resulting from a faster clearance rate of the negroes.

Brown *et al.* (1961) examined the postprandial lipemia of 31 survivors of myocardial infarction and two control groups (age-matched subjects and young students) by determining the optical density, the total fatty acids and the I^{131}-activity pattern. There was a close relationship between the three measurements; however, the peak level of radioactivity appeared a little later than the peak level of optical density or of total fatty acids. It appeared that the measurement of lipid-bound radioactivity and the absolute increase of serum total fatty acids 9 hours after the test dose gave the best separation between the coronary group and the age-matched control group. The separation was more effective than by analysis of the fasting plasma lipids; 13 per cent of coronary patients showed, however, a normal response. In a recent study of Berkowitz and Croll (1962) the I^{131}-tolerance was compared with the fasting lipid content of plasma in 100 patients with coronary heart disease. The incidence of hypercholesterolemia was 41 per cent, 72 per cent showed hypertriglyceridemia, while 82 had an abnormal I^{131}-tolerance. An excellent correlation existed between the fasting triglyceride level and the radioactivity curve. In recent studies of small series, the abnormality to clear labeled fat in coronary heart disease has been confirmed by two investigator teams (Levine and Cohen 1962, Malamos *et al.* 1962).

An analysis of the kinetics of I^{131}-labeled fat in coronary heart disease has been presented by George *et al.* (1961). They suggested that the disappearance of ingested labeled fat was composed of a rapid phase during which 95 per cent of the ingested fat is

cleared and of a slower component accounting for 5 per cent representing either retained or recirculating fat. The abnormality in the fat removal in coronary heart disease was present in the latter phase.

Disappearance Rate of Intravenously Administered Fat in Coronary Heart Disease

Contrary to the oral fat loading test, fat emulsions given intravenously appear to clear with an equal efficacy in patients with coronary heart disease and in healthy persons. The oral administration of an artificial fat emulsion (Lipomul) induced in coronary patients a more prolonged and intensive lipemia than in controls when measured by the plasma optical density (Bouchier and Bronte-Stewart 1961). When the fat emulsion was given intravenously the lipid disappearance rate was identical in the two groups. Similar results were obtained by Mashford and Nestel (1961). The clearance rate of intravenously administered I^{131}-triolein emulsion was also the same in patients with coronary heart disease and healthy individuals (Feinberg *et al.* 1961). When patients with an abnormal oral I^{131}-fat curve were tested by the intravenous method, it appeared that abnormality was shown by 25 per cent only. The results were the same with orally and intravenously administered fat emulsions (Lipomul) (Berkowitz *et al.* 1961). The plasma protein-bound radioactivity curves obtained after intravenous administration of I^{131}-triolein and C^{14}-tripalmitin were similar in patients with coronary heart disease, patients with diabetes and healthy persons (Balodimos *et al.* 1962). This was confirmed in a recent rapport of Mayfield *et al.* (1962).

Fat Tolerance Studies in Hyperlipidemic States

A large amount of data are available on alimentary lipemia in hyperlipidemic states. The results of studies of diabetic patients vary considerably, probably according to the serum lipid values, diabetic complications and severity of the disease (Hirsch and Carbonaro 1950, Camelin *et al.* 1954, Beaumont *et al.* 1958, Beaumont and Beaumont 1960 a, Sandberg *et al.* 1960, Balodimos *et al.* 1962). The similarity in coronary and diabetic patients of the results of tests with I^{131}-labeled fat has been emphasized by Sandberg *et al.* (1960).

In spite of some controversy, most authors agree that the alimentary lipemia tends to be normal in pure hypercholesterolemia (Chaikoff *et al.* 1934, Thannhauser and Stanley 1949, Kuo *et al.* 1956, Beaumont *et al.* 1958, Likoff *et al.* 1958, Kruger *et al.* 1960). In an excellent study of lipoprotein kinetics in various hyperlipidemis states, Kruger and co-workers, using labeled triolein, showed that in all the subjects the labeled lipid appeared almost exclusively in the chylomicra and the Sf 10—400 lipoprotein fraction. They showed also that in hypercholesterolemic patients with an elevated Sf 3—9 lipoprotein fraction the labeled lipid peaked

early and was essentially cleared from the blood within 24 hours, as in control cases. Patients with hyperlipemia characterized by an elevated Sf 10—400 lipoprotein fraction exhibited a delayed peaking of the labeled lipid, which was still considerably elevated after 24 hours. A similar gross abnormality in the lipid curves of patients with hyperlipemia has been observed by many other authors (Thannhauser and Stanley 1949, Martt and Connor 1956, Beaumont *et al.* 1958, Hall *et al.* 1959, Beaumont and Beaumont 1960 a, Brown 1961, Meng 1961, Sigler and Rubini 1961).

PLASMA VITAMIN A AND ITS RELATION TO VITAMIN A METABOLISM

Vitamin A has been used since the 1930's as an indicator in the study of fat absorption owing to its fat solubility and relative ease of determination (Chesney and McCoord 1934). The vitamin A test is probably the most widely used test in the study of malabsorption. However, it has rarely been used in the study of fat metabolism in cases of hyperlipidemia.

Intestinal Absorption of Vitamin A

The absorption of vitamin A across the human intestine is an active process that requires energy. The energy is produced by oxidative phosphorylation (Loran *et al.* 1961).

In nature, vitamin A occurs in an esterified form. The first stage of vitamin A absorption appears to be a fairly complete hydrolysis in the intestinal lumen or on the surface of the epithelial cell (Loran *et al.* 1961, Mahadevan and Ganguly 1961).

It has been shown in animal experiments that at the stage of absorption the mesenterial lymph fluid and the intestinal wall contain, in addition to vitamin A in the form of free alcohol, vitamin A esterified only with long-chain fatty acids (Mahadevan *et al.* 1959). Re-esterification of vitamin A with palmitic acid has also been observed in the human intestine in *in vitro* experiments (Loran *et al.* 1961). According to the generally accepted opinion today, the transport of vitamin A from the intestine onward occurs esterified with long-chain fatty acids.

The absorption through the thoracic duct is well established. Drummond and his co-workers (1935) demonstrated in a patient with chylothorax that vitamin A is quite completely absorbed through the lymphatic system. They also observed that vitamin A administered as free alcohol was found in the esterified form in the chyle. A parallel rise in the vitamin A concentrations of serum and chyle after a dose of 500,000 units of vitamin A has been observed in another patient with chylothorax (Beaumont and Beaumont 1960 b). The absorption of vitamin A

through the thoracic duct has also been demonstrated in experimental animals (Eden and Sellers 1949). They also observed that the vitamin A content of portal and systemic blood was equal and that after the administration of vitamin A the increase in the portal blood was slightly lower.

After a single dose of vitamin A the concentration in blood rises, depending chiefly on the ester fraction regardless of the form in which the vitamin is administered. This has been shown by many investigators in human subjects (Hoch 1946, Popper *et al.* 1948, Week and Sevigne 1950, Dost and Rind 1957, Krinsky *at al.* 1958) and in laboratory animals (Ganguly and Krinsky 1953). A flat increase of the alcohol fraction has also been observed by some investigators (Hoch and Hoch 1946, Ganguly and Krinsky 1953).

The peak in the serum concentration after a single dose of vitamin A is reached in about 3 to 6 hours, and the level of the peak depends on the dosage. However, Dost and Rind (1957) reported that the height of the peak is not determined by the size of the dose alone, the vitamin disappearance rate being equally important. Free alcohol causes a more intensive and more rapid rise than the ester (Week and Sevigne 1950).

Vitamin A is absorbed much better in aqueous dispersion than in oily medium (Barnes *et al.* 1950, Sobel 1952, Moore 1957). In rats most of the absorption takes place in the upper jejunum with aqueous medium and in the lower jejunum with oily medium (Sobel 1952). Evidently bile is necessary in the absorption of vitamin A (Moore 1957).

Plasma Vitamin A Level

Despite the fact, presented above, that a single dose of vitamin A is followed by a rise only in the ester fraction of the serum, c. 80—90 per cent of vitamin A in postabsorptive plasma is free alcohol (Hoch and Hoch 1946, Popper *et al.* 1948, Week and Sevigne 1950, Ganguly and Krinsky 1953, Dost and Rind 1957).

The regulation of the plasma vitamin A alcohol content is poorly understood. It is quite evident that vitamin A is absorbed esterified and transported to the liver, which contains about 90 per cent of the body stores. The circulating free alcohol is independent of the liver stores, however (Glover *et al.* 1947, Ganguly and Krinsky 1953, High and Wilson 1956). Liver is not capable of hydrolyzing the long-chain fatty acid esters of vitamin A, and this probably occurs in the extrahepatic tissues (Ganguly 1960).

Much data are available in the literature on the plasma content of vitamin A in healthy individuals. There are considerable variations in the different reports, however. In his monograph, Moore (1957) calculated the average of reported concentrations, which in 1,040 subjects was 131 I.U. per 100 ml of plasma, being within the limits of 91—201 I.U. per 100 ml plasma. The averages in some of the largest materials were as follows:

Investigator	*Number of Subjects*	*Mean of Plasma Vitamin A I.U./100 ml.*	*Range*
Leitner et al. (1960 a):			
women	526	142	54—259 (95 %)
men	742	174	70—305 (95 %)
Vetter (1958)	220	225	175—275
Saksela (1940)	214	191	105—315
Abels *et al.* (1941)	124	160	
Pitkänen (1944):			
women	72	201	87—374

There is good agreement concerning the influence of sex on the plasma vitamin A content. The level of plasma vitamin A in male subjects usually exceeds that for the female by a significant amount (Kimble 1939, Abels *et al.* 1941, Week and Sevigne 1950, Leitner *et al.* 1960 a).

Data on the influence of age on the vitamin A content, on the other hand, are conflicting. One of the early studies of the subject indicated a considerable decline with age (Schneider and Widmann 1935). No clear correlation to age was seen in the series of Saksela (1940) consisting of subjects 12—50 years of age. Vetter (1958) reached the same conclusion. However, in the largest published series (Leitner *et al.* 1960 a) the mean plasma vitamin A levels increased with age up to the sixth decade in males and the seventh decade in females, at which time both sexes reached the same level.

The vitamin A levels are higher during the summer and spring seasons than in the late fall (Saksela 1940, Vetter 1958, Leitner *et al.* 1960 a), whereas no 24-hour fluctuations have been found (Lindqvist 1938, Kimble 1939).

Transport of Vitamin A in the Blood

The importance of a preliminary saponification of the plasma, apparently to remove vitamin A from some complex, was shown by Lindqvist (1938). A globulin complex was suggested by Pett and LePage (1940) because of the property of plasma vitamin A to precipitate with alcohol. After freezing of the plasma at —25°C, the amount extractable with ether rose significantly in the study of Dzialoszynski *et al.* (1945). They thought that the binding protein was albumin. In 1950 Oncley and co-workers found that beta-carotene, the provitamin A, was carried in plasma by beta-lipoproteins. In ultracentrifugal studies Hack (1956) observed, however, that vitamin A and carotenoids were concentrated in different protein layers.

A number of experiments with man and animals have brought out that the vitamin A esters and alcohol are carried by different proteins (Ganguly *et al.* 1952, Garbers 1958, Krinsky *et al.* 1958, Krishnamurthy *et al.* 1958, Garbers *et al.* 1960).

Krinsky *et al.* (1958), in their studies of the transport of vitamin A in plasma after a single dose of the vitamin, ob-

served no rise in the alcohol fraction. According to the results of ultracentrifugation, most of the free alcohol was in fraction D > 1.063 and a small amount in fraction Sf 3—9; the latter finding, however, they considered to be due to contamination. Immunologic experiments and ethanol fractionation suggested that the protein binding the vitamin A alcohol was not albumin. In experiments with rats, using C^{14}-labeled vitamin A, Garbers and co-workers (Garbers 1958 and Garbers *et al.* 1960) showed that vitamin A alcohol was bound to $alpha_1$-globulin and not to lipoproteins.

Evidently the esterified vitamin A is carried in plasma by lipoproteins, chiefly by low density lipoproteins. In a careful study, Krinsky *et al.* (1958) showed that after a single dose of vitamin A the absorbed vitamin ester was carried primarily in the Sf 10—100 lipoproteins. The chylomicra contained only a small part of the absorbed esters, suggesting a transport mechanism differing from that of neutral fat. There was also a small amount of vitamin A esters in the Sf 3—9 lipoprotein fraction.

The French investigators (Beaumont and Beaumont 1960 a) criticized this transport mechanism, however. They showed that vitamin A esters were primarily carried by chylomicra and that the newly absorbed vitamin appeared in beta-lipoproteins 6 hours after the ingestion of vitamin.

Very interesting is the observation by Schrieck and Kunkel (1956) of the influence of heparin on the different vitamin A fractions after vitamin A loading. There was a considerable fall in the total plasma vitamin A, with a rise in the free alcohol and a marked decrease in the ester fraction. Electrophoretic and ultracentrifugal analyses of the plasma indicated that heparin caused a partial shift of esterified vitamin from low density $alpha_2$-lipoproteins to beta-lipoproteins. Free vitamin alcohol appeared in the $alpha_1$-albumin fraction.

Storage of Vitamin A in the Liver

The vitamin A is distributed throughout the body. However, the liver contains 90 per cent of the body stores, the average concentration being about 250 I.U. per gm of liver tissue (Moore 1957). The liver is able to store a considerable amount of the vitamin esters but only a small portion of free alcohol (Ganguly 1960). In laboratory animals the liver vitamin A was esterified with palmitate only, regardless of the form in which the vitamin was fed (Mahadevan and Ganguly 1961). They thought that this was due to a selective binding property of the liproproteins. After the feeding of vitamin A, the liver of laboratory animals continued to store the ester fraction for a long time, but the alcohol fraction reached its maximum in 3 to 5 hours and did not increase thereafter (Ganguly and Krinsky 1953). It has been suggested that the esters are phagocytized directly by the Kupffer cells from the circulation. The free alcohol first underwent hydrolysis in the plasma and was then stored in parenchymal cells (Glover and Morton 1948). To confirm this, Krishna-

murthy and Ganguly (1956) showed that the blockage of the reticulo-endothelial system in laboratory animals led to a decrease in the liver ester fraction after vitamin A feeding. The blocking did not influence the liver alcohol fraction, however. In another study the blockage led to a marked slowing down of the plasma disappearance rate of vitamin A (Brown *et al.* 1952).

Metabolism of Vitamin A in Hyperlipidemic States

The exact role of vitamin A in lipid metabolism has not yet been established. The study of Lindqvist (1938) showed a significant parallellism in the serum cholesterol and vitamin A levels in response to iodine treatment in hyperthyroid patients. He suggested also that the simultaneous occurrence of hypercholesterolemia and hypervitaminosis A in hypothyrosis had a common cause, probably in a pathologic affinity to serum. In the reports of Wendt (1935) and Josephs (1942) a transient elevation of serum total cholesterol after vitamin A feeding was found in laboratory animals and human subjects. Similarly, during treatment with vitamin A and vitamin E of 3 weeks' duration Vannotti and Gervasoni (1957) observed in atherosclerotic patients and healthy subjects an increase of plasma total lipids and cholesterol. In another experiment, vitamin A showed an antisclerotic effect on aortic atheromas in hens; no change, however, was observed in the serum cholesterol levels (Weitzel 1957). Contrary to earlier results Kinley and Krause (1959) reported that treatment with 100,000 units of vitamin A daily resulted in a decrease of 20 to 175 mg/100 ml of the serum cholesterol in hypercholesterolemic survivors of myocardial infarction. Vitamin A had no influence on the normocholesterolemic subjects.

The importance of the function of the reticulo-endothelial system has been pointed out by Brown and coworkers (1952) as the common link in the metabolism of cholesterol and vitamin A. They observed an elevation of serum cholesterol and vitamin A levels in guinea-pigs after blockage of the reticulo-endothelial system with Thorotrast.

The presence of *hypercarotenemia* in various hyperlipidemias has been observed by many investigators (Rabinowitz *et al.* 1930, Wendt 1935, Ralli *et al.* 1936, Mandelbaum *et al.* 1942, Kimble *et al.* 1946, Cohen 1958, Blankenhorn 1960). The mechanism of hypercarotenemia is in many instances obscure. It has been suggested that the conversion of beta-carotene to vitamin A is impaired in diabetes and hypothyroidism (Ralli *et al.* 1936, Cohen 1958). In a large study of 116 diabetic patients (Kimble *et al.* 1946) the simultaneous occurrence of high blood carotene and low vitamin A levels was, however, unusual.

It also has long been known, that carotenoid pigments are responsible for the yellow color of early atheromas (Thomson 1934). Very interesting was the observation of Blankenhorn *et al.*

(1956) that human atherosclerotic lesions contained carotenoids in direct proportion to the severity of the atherosclerosis. He could also demonstrate that carotene feeding augmented the carotene content of xanthomas in a patient (Blankenhorn 1960).

Hypervitaminosis A has been a common finding in many cases of nephrosis (Saksela 1940, Popper *et al.* 1948, Kagan *et al.* 1950, Cohen 1958). The elevation depends on the ester fraction (Popper *et al.* 1948). Popper *et al.* suggested also that the elevation was due to increased solubility in the serum. The vitamin A metabolism in the nephrotic syndrome of children has been studied by Kagan *et al.* (1950), who observed the highest peak level in these children after the administration of vitamin A. The slow plasma disappearance rate also observed was thought to be due to failure by the body to store the plasma vitamin. They noted also an increase in the plasma total lipid level after the ingestion of vitamin A and connected this lipid mobilization to the vitamin retention in the plasma. The hypervitaminosis A in hyperlipidemic states was explained by Krinsky *et al.* (1958) as a block within the reticulo-endothelial system in the normal metabolism of the Sf 10—100 lipoproteins.

There is some controversy concerning the occurrence of hypervitaminosis A in hypothyroid states. As was stated above, it has been suggested to be a defect in the conversion of carotene to vitamin A (Cohen 1958). According to his study the vitamin A blood levels were chiefly within normal limits. High levels of vitamin A, however, have been reported by many investigators (Wendt 1935, Lindqvist 1938, Saksela 1940). A slow clearance rate after vitamin A loading was seen by Beaumont *et al.* (1958).

Among diabetic patients the plasma vitamin A values reported in the literature vary from low to high levels (Wendt 1935, Lindqvist 1938, Saksela 1940, Murril *et al.* 1941, Kimble *et al.* 1946, Beaumont *et al.* 1958, Cohen 1958). In the study of Wendt (1935), hypervitaminosis in diabetics was often accompanied by hypercholesterolemia. Insulin treatment did not alter the vitamin level. No correlation, however, between the serum total lipids and the carotene or vitamin A level occurred in the study of Kimble *et al.* (1946).

An abnormally high plasma concentration curve and delayed disappearance of vitamin A was observed by Martt and Connor (1956) in a case of idiopathic hyperlipemia associated with coronary atherosclerosis. The vitamin A metabolism in coronary heart disease with or without high serum lipid levels has been studied extensively by the French investigators (Beaumont *et al.* 1958, Beaumont and Ardaillou 1959, Beaumont and Lenègre 1959, Beaumont and Beaumont 1960 a, 1961). There were 23 abnormally high responses to vitamin A loading in 54 patients with angina pectoris. Of these 1 patient had myxedema, 8 essential hyperlipemia, and in 4 patients the lipid pattern was normal, while the others had moderately increased lipid values. A striking observation was that all the patients with

primary hypercholesterolemia responded normally. The high concentration of vitamin A in the chylomicron fraction was characteristic of the hyperlipemia group. There was still a marked retention of this fraction 24 hours after the loading. The investigators concluded that the common occurrence of high vitamin A concentration curves reflects a faulty chylomicron metabolism in coronary heart disease.

METABOLISM OF ALPHA-TOCOPHEROL

Alpha-tocopherol, which comprises most of the tocopherol pool of the human body (Quaife *et al.* 1949, Dju *et al.* 1958) is a natural vitamin E. Although vitamin E is regarded as a typical vitamin, the deficiency of which produces characteristic deficiency symptoms in experimental animals, its function is not known. Some investigators consider tocopherol to be a nonspecific anti-oxidant, since it is a readily oxidizing substance and since some of the deficiency symptoms disappear after the administration of synthetic anti-oxidants. On the other hand, a large group of investigators support the opinion that tocopherol plays a fully specific part in certain biochemical reactions, as do other vitamins (Schwartz 1961). In human pathophysiology the significance of vitamin E is slight. In induced vitamin E deficiency in humans, however, an increased peroxide hemolysis has been observed (Horwitt 1960).

Intestinal Absorption of Tocopherol

After a single dose of tocopherol the serum tocopherol concentration rises slowly and reaches a peak value at 6 to 12 hours, according to different authors (Quaife and Harris 1944, Popper *et al.* 1949, Klatskin and Krehl 1950, Week *et al.* 1952, Pomeranze and Lucarello 1953, Beckman 1955, McCormick *et al.* 1960).

According to the studies of Week and his co-wokers (1952) the shape of the serum concentration curve depends upon the form in which the tocopherol is administered. Free tocopherol produces a more rapid rise and a higher peak than the ester form, but in both cases the increase in concentration is due to free tocopherol. Pomeranze and Lucarello (1953) observed that the simultaneous ingestion of fat increases the absorption of tocopherol, and that a fat deficit diet preceding tocopherol loading decreases absorption. McCormick *et al.* (1960) obtained a two-peaked concentration curve if the test subject had a meal during the loading test.

Attention has been paid in several connections to the similarity of tocopherol and fat absorptions. Darby and co-workers (1946) studied the absorption of tocopherol in patients with sprue and observed that both the fasting value and the absorption curve

were low. Low tocopherol values have also been seen in other conditions of deficient absorption (Darby *et al.* 1949). Popper and his group (1949) reported poor tocopherol absorption in acute hepatitis and hepatic cirrhosis. In detailed studies of tocopherol absorption in liver disease, Klatskin and coworkers (1950, 1952 a, 1952 b) obtained a lower curve in hepatic cirrhosis than in control persons, but the surface area of the concentration curve was of the same size. The tocopherol concentration in the feces of cirrhotic patients was lower, however, and the administration of tocopherol in aqueous dispersion did not improve the absorption. They therefore suggested that the low plasma tocopherol values seen in cirrhotic patients were not a result of poor absorption.

The presence of tocopherol in feces has been observed in absorption studies (Cuthbertson *et al.* 1940, Hines and Mattil 1943, Hickman *et al.* 1944, Harris 1950, Klatskin and Molander 1952 b, Rosenkranz *et al.* 1951, 1953). In laboratory animals a part of parenterally given C^{14}-labeled tocopherol succinate was excreted in feces (Simon *et al.* 1956 a). This confirmed the earlier observations that there is an enterohepatic circulation of tocopherol, as in the case of cholesterol. Popper and his group (1949) had observed earlier that in patients with a biliary fistula the tocopherol concentration of the bile was of the same order of magnitude as that of the serum. A similar observation was reported by Klatskin and Molander (1952 a) in patients with hepatic cirrhosis, who stated, however, that the administration of tocopherol did not raise the bile tocopherol level.

Plasma Tocopherol Level

Since the early 1940's, much data has been published on the tocopherol content of the serum. Alpha-tocopherol makes up about 80 to 90 per cent of the total plasma tocopherol content (Quaife *et al.* 1949) and about one-third is in quinone form (Scudi and Buhs 1942, McCormick *et al. 1960)*. The greater proportion of plasma tocopherol is free alcohol and only about 10 per cent is esterified (Rindi and Perri 1957). Furthermore, the tocopherol is entirely bound to the lipoproteins (Lewis *et al.* 1954, McCormick *et al.* 1960).

Many surveys are available of the plasma tocopherol level (Rauramo 1946, Beckman 1955, Feldheim 1957, Harris *et al.* 1961). The largest series that have been published are the following:

	Number of Subjects	*Mean of Serum Alpha-Tocopherol mg/L*	*sd*
Leitner *et al.* (1960 b)	583	10.5	± 2.3
Harris *et al.* (1961)	197	10.5	± 3.2
Chieffi and Kirk (1951)	188	9.8	± 3.0
Engel (1949)	122	7.7	± 3.5
Kramer (1955): women	116	9.9	± 2.5

The mean serum tocopherol value of all the cases published in the literature is 10.1 ± 2.4 mg/L, according to Harris *et al.* (1961), with a range from 0.8 to 20 mg/L, according to Feldheim (1957), showing thus a considerable variation.

According to the large survey of the literature prepared by Beckman (1955), the serum tocopherol content is lower in children than in adults. A positive age trend is also present in adults (Darby *et al.* 1949, Lemley *et al.* 1949, Chieffi and Kirk 1951, Leitner *et al.* 1960 b).

In the study of Rauramo (1946), women had a significantly higher serum tocopherol content than men, as also was the case in the study of Chieffi and Kirk (1951). No difference in this respect was present in Leitner's series when calculated from his total series. However, the different age groups showed significant differences in both directions.

Transport of Tocopherol in the Blood

Very little is known concerning the fate of tocopherol after its absorption from the intestine into the blood circulation. Sternberg and Pascoe-Dawson (1959), in studying the metabolism of tocopherol in experimental animals with C^{14}-labeled alpha-tocopherol succinate found an equal radioactivity in the different lipoproteins in both the portal venous blood and the aortic blood. The absorption would thus take place by way of the thoracic duct.

Tocopherol evidently is completely bound to the lipoproteins (Lewis *et al.* 1954, Sternberg and Pascoe-Dawson 1959, McCormick *et al.* 1960). On the other hand, Ames and Risley (1949) were able to produce a tocopherol-protein complex in *in vitro* experiments, and Voth and Miller (1958) demonstrated the existence of a fairly strong affinity between tocopherol and bovine albumin. On the basis of these studies it was suggested that tocopherol is bound to all proteins also under *in vivo* conditions. Evidence speaking against this opinion, however, are the studies of Sternberg and Pascoe-Dawson (1959) using labeled tocopherol, in which no radioactivity was found in the albumin fraction.

Data on the binding of tocopherol to the various lipoprotein fractions are contradictory. According to Lewis *et al.*, tocopherol was bound under fasting conditions mainly to the high density lipoproteins (c. 54 per cent), while the beta-lipoproteins contained only 20 per cent of the plasma tocopherol. In the study of McCormick *et al.*, however, most of the plasma tocopherol was in the Sf 3—9 fraction. After the ingestion of tocopherol the serum concentration curve was two-peaked, in similarity to the curve for the chylomicron and Sf 10—400 fractions. The first peak was reached about 3—4 hours and the second peak about 12 hours after the intake of tocopherol. The tocopherol contents of fractions Sf 3—9 and of the high density lipoproteins increased at a slow rate and reached maximum in about 8—10 hours. On the basis of these results the investigators concluded that te tocopherol was converted from the lighter lipo-

proteins into Sf 3—9 and high density lipoproteins.

In experiments with rats, using labeled tocopherol, the radioactivity was distributed in paper electrophoresis as follows: Chylomicron fraction, 27.5 per cent; alpha$_1$- and beta-fractions, 58.4 per cent, and the remainder in the alpha$_2$-fraction (Sternberg and Pascoe-Dawson 1959).

The plasma disappearance rate of tocopherol is slow. According to Beckman (1955) the plasma tocopherol content at 24 hours after tocopherol loading was higher than the fasting value if the dose exceeded 200 mg. In rats the plasma half-life of C^{14}-labeled alpha-tocopherol was 60 hours (Sternberg and Pascoe-Dawson 1959).

Tissue Storage of Tocopherol

Most of the tocopherol is stored in tissues in the alpha form, although the latter comprises only one-half of the total vitamin E intake (Quaife *et al.* 1949, Dju *et al.* 1958). Accordingly, alpha-tocopherol has been found to be absorbed from the intestine better than the next most common gamma-tocopherol (Quaife *et al.* 1949). In the opinion of Dju, tocopherol is incorporated into the cells as a natural part of the exogenous fat in connection with the normal lipid metabolism of the cells.

Tocopherol is distributed throughout the body, the largest deposits being in adipose tissue. However, the highest concentrations per gram of fat are found in the plasma, hypophysis, adrenals and gonads (2.0, 1.2, 1.0 and 0.7 mg, respectively, of tocopherol per gram of fat). Considerably lower concentrations are found in the liver, heart and skeletal musculature, in which it is about 0.3 mg per gram of fat. The tocopherol content of the tissues increases with age and attains the maximum at the age of 20—30 years, after which it gradually declines until in old age it reverts to the childhood level (Dju *et al.* 1958).

Chemical Transformations of Alpha-Tocopherol in the Metabolic Process

Tocopherol is evidently excreted in the bile into the intestine. It probably is not excreted unchanged in the urine, even if a reducing substance has been encountered in the urine after large doses of tocopherol and has been interpreted to be tocopherol or its quinone (Cuthbertson *et al.*, 1940, Rosenkranz *et al.*, 1953). According to Simon *et al.* (1956 a) and Sternberg and Pascoe-Dawson (1959), however, tocopherol metabolites in the form of glucuronides are probably excreted in the urine.

It is the prevailing opinion that the first stage of tocopherol metabolism is the oxidation to tocopherol quinone, followed by reduction to hydroquinone (Scudi and Buhs 1942, Rosenkranz *et al.* 1953, Simon *et al.* 1956 a, Diplock *et al.* 1960, McCormick *et al.* 1960). Complete unanimity has not been reached on this point, however, since these metabolites have not been encountered by some investigators in the tissues of

experimental animals (Hines and Mattil 1943, Pollard and Bieri 1959, Alaupović *et al.* 1961).

Simon and his co-workers (1956 b) isolated from the urine two metabolites and identified them as 2-(3-hydroxy-3-methyl-5-carboxypentyl)-3, 5, 6, -trimethyl benzoquinone and its gamma-lactone. They also advanced the theory that tocopherol is first oxidized into quinone, reduced to hydroquinone and conjugated with glucuronic acid. The last methyl group in the side chain is then oxidized to a carboxyl group and conjugated with CoeA. By means of beta-oxidation the side chain is shortened to contain six carbon atoms, after which lactonization and excretion in the urine occur. Alaupović *et al.* (1961) were unable to isolate these metabolites from the liver of experimental animals, but they presented instead three new metabolites, the chemical structure of which is still unclear. From the mitochondria of the rabbit liver Martius and Costelli (1957) isolated, in addition to unchanged alpha-tocopherol, a metabolite which they regarded as the active form of tocopherol: the trimethyl phythyl benzoquinone.

Relation of Tocopherol to Cholesterol and Other Lipids

Numerous animal experiments have revealed a rise in the muscle and serum cholesterol concentrations in vitamin E deficiency. Oppenheimer *et al.* 1958) further observed a decrease in the plasma alpha-lipoprotein cholesterol but an increase in the beta-lipoprotein cholesterol, giving the net result of an increased total cholesterol level in the plasma. Shull *et al.* (1958) and Alfin-Slater (1960), again, were able to inhibit with synthetic antioxidants the development of muscular dystrophy in vitamin E deficiency, but obtained no effect on the cholesterol metabolism. In the light of these results they suggested, therefore, that tocopherol has a quite specific role in the cholesterol metabolism.

The interrelationship of cholesterol and tocopherol has also been studied in humans. Darby and his co-workers (1949), in examining the plasma tocopherol levels in various pathological conditions, observed that in diseases with associated hypercholesterolemia there frequently were elevated plasma tocopherol values. Such conditions were, for example, xanthomatosis, diabetes and hypercarotenemia. Further they pointed out that hypertocopherolemia, as well as hypercholesterolemia, is often present during pregnancy and in cardiovascular diseases, whereas in conditions with low serum cholesterol values the tocopherol also was decreased. After demonstrating that this was not a methodological error, they concluded that the explanation was »an increased lipid-carrying power of the serum». Attention was also paid by Popper and his group (1949) to the parallel behavior of cholesterol and tocopherol in diseases of the liver and the biliary tract. In diseases in which the excretion of cholesterol in the bile was impaired and retention into the

blood circulation occurred there was hypertocopherolemia. However, contrary to the case with cholesterol, the tocopherol concentration in the bile did not rise above that in the serum. Klatskin and Krehl (1950), on the other hand, observed no correlation between the serum cholesterol and tocopherol in patients with hepatic cirrhosis.

The relationship between tocopherol and cholesterol in diabetes has been studied by Bensley and his co-workers (1950) and Vanzetti and his group (1956). The first mentioned observed a significant positive correlation between the plasma level of these two lipids and suggested that the plasma tocopherol content is directly dependent on the serum cholesterol level. On the other hand, no relationship was seen between the blood sugar and the serum tocopherol. Vanzetti *et al.* studied the interrelationship between cholesterol, total lipids and tocopherol in healthy aged persons and patients with arteriosclerosis (mean age 72 years) and in diabetics. No significant difference in the plasma tocopherol level was seen between the arteriosclerosis and control groups, whereas the mean tocopherol level in the diabetics was higher than in the healthy subjects and showed a good correlation to the cholesterol values.

In the study of Postel (1956) a positive correlation between the serum tocopherol and cholesterol levels in thyroid disorders was observed. He concluded that the thyroid hormone had a similar effect on the tocopherol and cholesterol metabolisms.

Tocopherol and Atherogenesis

The high peroxide number of the lipids in the wall of the atherosclerotic artery (Glavind *et al.* 1952) has given rise to a suspicion that tocopherol may be effective in preventing the formation of atheroma. The atherosclerotic aorta, however, contains considerably more tocopherol than the normal aorta (Vannotti and Gervasoni 1957). It was stated by McCormick and McCluer (1960) that the amount of tocopherol present in the atheromatous aorta is sufficient to prevent the peroxidation of lipids. Weitzel (1957), in an extensive study of the antisclerotic effect of the fat-soluble vitamins, observed that vitamin E had only a low action in this respect.

Conflicting results have also been obtained in studies of the effect of large vitamin E doses on the serum lipids. Bronte-Stewart *et al.* (1956) and Beveridge *et al.* (1957) stated that the lowering action of unsaturated fatty acids on serum cholesterol was not due to their high tocopherol content. Greenblatt (1957) administered massive doses of vitamin E (40 gm of tocopherol per day) to 6 test subjects and observed the greatest decrease of serum cholesterol in persons with hypercholesterolemia, the drop being in some cases as much as 100 mg/100 ml. After the administration of 100 mg of alpha-tocopherol acetate during 12 days to healthy subjects, Gray and Loh (1958) recorded a significant increase in the serum cholesterol and phospholipid levels. Hammerl and Pichler (1960) effected a decrease in

the serum cholesterol level in atherosclerotic patients by combined treatment with vitamins A, E and K. In his monograph Pezold (1961) concluded that the results of animal and clinical experiments have been so contradictory that tocopherol deficiency or its administration in pharmacological doses apparently has no effect on atherogenesis.

OBJECT OF THE PRESENT INVESTIGATION

The present investigation is an attempt to throw further light on lipid metabolism in coronary heart disease.

In order to eliminate disturbances in the endogenous synthesis of lipids and thus facilitate the interpretation of the results, vitamins A and E, representing purely exogenous lipids, were chosen as the subjects of investigation. This seemed to be useful also for the reason that only few data are available of the serum tocopherol content or of the lipoprotein kinetics of the two vitamins in states of altered lipid metabolism. The serum cholesterol and triglyceride levels were also analyzed in order to classify the lipid disorder of each subject. In addition, the interrelationships of all these lipids may be of some interest.

The investigation is divided into five parts:

1) Determination of the serum cholesterol, triglyceride and vitamin E and A levels in coronary and control subjects;
2) Interrelationship of the serum cholesterol, triglyceride, vitamin E and A levels in coronary and control subjects;
3) Influence of acute myocardial infarction on the serum cholesterol, triglyceride and vitamin E levels;
4) Vitamin E and A loading tests in coronary and control subjects;
5) Lipoprotein kinetics of vitamins E and A in coronary and control subjects.

The investigation was begun with the vitamin A studies. However, vitamin A exhibited some disadvantages. The method for determining vitamin A in the plasma and particularly in the lipoprotein fractions was not fully adequate and, furthermore, side effects — nausea and headache — frequently occurred during the vitamin A loading tests. The vitamin A studies were therefore discontinued before the series of experiments was completed.

Some of the present data have been previously published as preliminary reports (Nikkilä and Pelkonen 1961, 1962 a, 1962 b, 1963).

MATERIAL

The series studied consisted of one group of 124 survivors of myocardial infarction and of two control groups, i.e., 322 blood donors and 101 healthy persons.

Survivors of myocardial infarction

This group included 110 male and 14 female patients between the ages of 30 to 65 years. All the patients in this group were admitted to the First or the Third Department of Medicine, University of Helsinki, in 1960—1962 because of symptoms of acute myocardial infarction or angina pectoris. All had electrocardiographic changes unequivocally indicating an old or recent myocardial infarction.

Patients with diseases known to affect the lipid metabolism, such as diabetes mellitus and thyroid disorders, as well as patients with other severe diseases were excluded. Heart failure, which may influence the fat absorption (Mäkelä *et al.* 1960), was also regarded as an excluding factor.

During the acute stage of illness the patients were on a light caloric diet (1000 Cal., including 50 gm of fat per day) and thereafter on an ordinary hospital diet (1500 Cal., including 90 mg fat per day). The majority of the patients (100 of 124) were treated with oral anticoagulants.

The lipid analyses were made after a minimum of three weeks had passed from the acute stage of the illness. In the majority of cases they were done during the fifth week. The patients were then already mobilized from the bed.

In the text to follow this group is briefly termed »coronary».

Blood donors

This group included 176 male and 146 female blood donors of the Blood Bank of the Finnish Red Cross. The age range was 20 to 65 years. No medical examination was performed with the exception of the routine hemoglobin determination. Donors who had taken any vitamin preparations within one week were excluded. The samples were taken without regard to the state of absorption and were analyzed even though the plasma was lactescent.

Healthy subjects

The test subjects in this group comprised 88 males and 13 females in the age range of 20 to 58 years.

The persons were either patients under medical observation in the First Department of Medicine, University of Helsinki, or medical students working in the hospital.

The patients included in this group were admitted to the hospital for a cardiologic examination because of congenital heart disease without marked hemodynamic changes. The medical examination revealed no organic disorders in some patients and they were thus included in this group. Values of 350 mg/100 ml for serum cholesterol and of 500 mg/100 ml for serum triglyceride were regarded as criteria of hypercholesterolemic and hyperlipemic diseases. Persons who exceeded these lipid concentrations were excluded from this group. The medical students underwent no detailed medical examination but they were subjectively healthy.

The medical students continued their customary dietary habits and the patients received the usual hospital diet (1500 Cal., including 90 gm fat per day).

In the following text this group is briefly termed »healthy».

METHODS

All samples, with the exception of those for plasma tocopherol and serum cholesterol analyses from the blood donors, were taken in the morning after an overnight fasting of 12 hours. In order to standardize the postabsorptive state, all the patients had one plate of porridge in the preceding evening, 12 hours before the blood tap. Drinking of water was allowed during the fasting.

The plasma or serum was separated immediately after the blood was drawn, and was stored at +4°C. The analyses were made within one week after the blood tap. The vitamin A determinations, however, were done within two days.

All analyses were performed in duplicate. It the estimations of duplicates differed by more than 10 per cent, the samples were reanalyzed.

Serum Total Cholesterol

The serum total cholesterol was determined by the method of Pearson *et al.* (1953). When this method was compared with the method of Abell *et al.* (1952) it appeared that the latter gave cholesterol concentrations approximately 10 per cent lower irrespective of the cholesterol concentration (Nikkilä and Pelkonen 1963).

Serum Triglyceride

The serum triglyceride was determined by measurement of the glycerol component of the triglyceride molecule after saponification. The method used in this study was a combined modification of two methods (van Handel and Zilversmit 1957, Carlson and Wadström 1959). The extraction and purification of triglyceride was performed according to van Handel and Zilversmit with a mixture of chloroform and a zeolite (Doucil, W. A. Taylor Company), since this procedure was simpler than the chromatographic method of Carlson and Wadström. To avoid the common occurrence of opalescence in the final step of the van Handel and Zilversmit procedure, the extraction of fatty acids with petroleum ether after saponification was included as in the method of Carlson and Wadström.

Plasma vitamin A

The plasma vitamin A determinations were made from heparinized

plasma, with the exception of the lipoprotein analyses, where Na-EDTA plasma was used. The different plasmas gave the same results when compared. A modification of the spectrophotometric method was used (Bessey *et al.* 1946).

After saponification with a mixture of absolute alcohol — potassium hydroxide, the vitamin was extracted with n-heptane. The extraction showed a recovery of 70—80 per cent even at the high plasma concentrations obtained during the vitamin A loading tests. The heptane extract was divided into two parts, one of which was irradiated in ultraviolet light to destroy the vitamin A. The difference in the optical densities of these two parts measured at wave length 328 mμ in the spectrophotometer (Beckman DU) thus showed the actual amount of vitamin A in the extract. However, the optical densities of the irradiated extracts were so fixed at the level of 0.002—0.008 that the irradiation was discontinued. In making the calculations a value of 0.005, corresponding to the mean optical density of the irradiated extracts, was subtracted from the reading. All the samples were stored in complete darkness and direct daylight was avoided during the analysis procedure.

Plasma Tocopherol

The plasma tocopherol was determined after extraction with xylene by means of the dipyridyl-ferric chloride color reaction (Rindi 1957). Heparinized plasma was used; the lipoprotein tocopherol, however, was estimated from the Na-EDTA plasma. Analyses of the different plasmas as well as of the serum showed no differences in the tocopherol concentration. The addition of cholesterol in increasing amounts (1.6—3.2—4.8 mg) into the extracts of tocopherol analyses did not alter the optical density readings at wave lengths 520 mμ.

Isolation of the Plasma Lipoproteins

The lipoprotein particles were fractionated according to their hydrated densities by flotation in a Spinco Model L preparative ultracentrifuge. Two runs were needed to obtain the following five particle classes: D. $<$ 1.006, D. $>$ 1.006, D. 1.006—1.019, D. 1.019—1.063, and D. $>$ 1.063 (Havel *et al.* 1955).

In the primary separation 5 ml Na-EDTA plasma was layered with a syringe and needle under an equal volume of 0.85 per cent sodium chloride solution in three lusteroid tubes with a volume of 13.5 ml. After a run in a rotor 40 for 30 minutes at a speed of 21,000 r.p.m. (28,360 x G) two layers were obtained. The upper turbid layer contained thus D. $<$ 1.006 particles and the lower, clear layer D. $>$ 1.006 particles. From one of the tubes the two layers were separated for further chemical analysis. From the other two tubes the lower parts were removed by suction and placed in two empty tubes. To obtain the desired densities (D. 1.019 and 1.063), equal volumes of 5.1 per cent sodium chloride solution

(D. 1.032) was added to the one tube and 18.7 per cent sodium chloride solution (D. 1.120) to the other tube. The tubes were then run in the ultracentrifuge for 18 hours at a speed of 40.000 r.p.m. (105,400 x G). Three layers were obtained. The turbid top layers contained the D. 1.006—1.019 and D. 1.006—1.063 particles, the water-clear, colorless middle layers were free of lipoproteins, and the yellow bottom layers consisted of D. > 1.019 and D. > 1.063 particles. Then 2 ml of the surface layers D. < 1.019 and D. < 1.063 and of the bottom layer D. > 1.063 were again suctioned and distilled water was added to make the initial plasma volume of 5 ml. The fraction D. 1.019—1.063 was calculated by subtracting the D. 1.006—1.019 fraction from the D. 1.006—1.063 fraction.

The concentration of vitamin A or tocopherol in lipoprotein fractions was determined. The method of vitamin A determination, however, was not sensitive enough for lipoprotein analyses of fasting plasma. The recovery of vitamin A in the lipoprotein analyses showed a great variability in the range of 40 to 150 per cent. The samples where the recovery was less than 50 per cent or more than 125 per cent were discarded.

The recovery of tocopherol in the lipoprotein analyses was, on the average, as high as 90 per cent in the primary separation and 80 per cent in the second separation. Here the samples were discarded if the recovery was less than 60 per cent or more than 125 per cent.

Vitamin A Loading Test

The vitamin A and E tests were identical in principle. At 8 a.m., after overnight fasting of 12 hours' duration, the vitamin was given with 100 ml of cream containing 40 per cent fat. The total amounts of fat were 45.9 gm in the vitamin A loading tests and 49.2 gm in the vitamin E loading tests. The subjects continued to fast until the 6-hour blood sample was taken, after which they had the usual lunch. The 10-hour sample was taken after dinner. On the next morning the last, or 24-hour, sample was tapped, again preceded by overnight fasting.

During fasting, the ingestion of small amounts of water and smoking were allowed. Movement was limited to ambulation in the ward. The only ambulatory subjects included in this study were the medical students who underwent the vitamin A loading test.

To reach sufficiently high plasma concentrations for lipoprotein analyses rather large amounts of vitamin A was used. Thus 1.5 million I.U. of vitamin A palmitate in 5 ml of soyabean oil (A-Vitol forte, Orion) were given.

For the complete test, seven samples (at 0, 2, 3, 4, 6, 10, and 24 hours) were taken for the vitamin A determinations. Only the fasting and 24-hour samples were studied in the medical students, however. The vitamin A content of lipoproteins was analyzed in the 4, 6, 10, and 24-hour samples.

In two subjects the vitamin A loading test was done twice, and the results give some information of the reproducibility of the test (table I).

TABLE I. Plasma vitamin A level in two subjects during two successive vitamin A loading tests

Subjects	*Date*	*Fasting*	*2*	*3*	*4*	*6*	*10*	*24 hrs.*
Case 1 (healthy)	March 13, 1961	111	687	718	3957	3276	1319	421 I.U./100 ml.
	March 27, 1961	128	887	2600	4356	4163	—	457 »
Case 2 (coronary)	Oct. 24, 1960	241	477	1022	2762	4000	3313	— »
	Nov. 10, 1960	267	326	—	3107	5375	5003	— »

The above data show that in spite of a marked difference in the rate of absorption in the two tests with each of the subjects, the reproducibility of the test increased toward the end of the curve.

Tocopherol Loading Test

Two grams of alpha-tocopherol acetate in 10 ml arachidis oil (Evitol, Orion) was given with 100 ml of cream (40 per cent fat). No side effects were observed during this test.

The blood samples were taken in the complete test 4, 6, 10, 24 hours after ingestion of vitamin E. Because of the limited laboratory capacity, only the 0, 4, and 24-hour or the 0 and 24-hour samples were taken from some subjects. The complete lipoprotein analyses were made from the 0, 4, 6, 10, and 24-hour samples.

The ordinary hospital meal had no influence on the plasma tocopherol content studied in 5 subjects. The loading test without tocopherol supplement was also performed in 5 subjects and no changes in the plasma tocopherol content were observed.

In order to study further the reproducibility, the tocopherol test was performed twice in one patient with hyperlipemia who showed a very abnormal response, and the following results were obtained (table II).

Here again the best reproducibility was obtained at 24 hours.

In both the loading tests the serum triglyceride levels as well as the triglyceride content of the lipoproteins were determined, but will be published elsewhere.

Statistical Procedures

The mean values presented are the arithmetic means calculated from the individual observations and are compared by means of the »Student's» *t*-test.

TABLE II. Plasma tocopherol level in a patient with hyperlipemia during two successive tocopherol loading tests

Patient with hyperlipemia	*Fasting*	*4*	*6*	*10*	*24 hrs.*
Sept. 15, 1961	38.9	58.4	118.2	231.9	108.5 mg/L
June 12, 1962	45.1	79.1	83.6	123.5	110.1 mg/L

In the correlation studies, linear regression analyses were made and the correlation coefficient of Pearson was used, with the exception of the vitamin A studies, in which Spearman's rank order correlation coefficient (r_S) was used. The significance of the correlations was tested by the test variable:

$$t = \sqrt{n-2}\ \frac{r}{\sqrt{1-r^2}}$$

For the statistical calculations employed the reader is referred to the monography of Walker and Lev (1953).

RESULTS

Since all the control series are not comparable with the coronary series with respect to age, they were divided into age groups below and over the age of 35 years. This age limit was chosen because, according to autopsy data, the incidence of atherosclerosis increases sharply after the 35th year. Thus, the younger age group best represents a population free from active atherosclerosis. The coronary group was also divided into two age groups below and over 50 years, because the various degenerative processes associated with aging become more apparent after this age. The younger coronary group is therefore best suited for the study of a possible metabolic defect.

SERUM TOTAL CHOLESTEROL LEVEL

Blood Donors and Healthy Subjects

The serum cholesterol content was determined in 312 blood donors (mean age 41 years), 175 of whom were males (mean age 39 years) and 137 were females (mean age 44 years), and in 101 healthy subjects (mean age 31 years). The healthy group consisted of 85 men (mean age 32 years) and 16 women (mean age 26 years). The data are given in tables 1 and 2 as mean values with corresponding standard deviations, grouped according to the age of the subjects. Figure 1 depicts the influence of age. In addition, the cumulative frequency distribution curves of serum cholesterol in the blood donors are presented in fig. 2.

The range of the serum cholesterol content in the male blood donors was from 145 to 438 mg/100 ml and in the female blood donors from 182 to 408 mg/100 ml. By definition »true» hypercholesterolemic persons did not occur in the healthy group. The 350 mg/100 ml limit for »true» hypercholesterolemia was exceeded by 10 blood donors (3 per cent), 7 of whom were females and only one was younger than 35 years.

Comparison of healthy subjects and blood donors revealed a higher mean cholesterol content in blood donors. This, however, was not unexpected since hypercholesterolemic subjects were excluded from the healthy group.

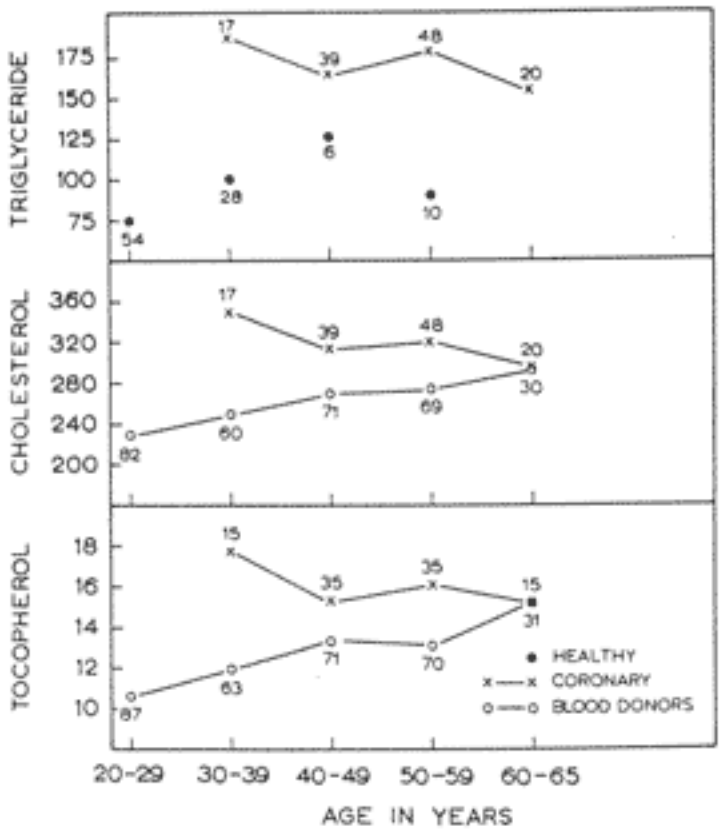

Fig. 1. Serum triglyceride (mg/100 ml), total cholesterol (mg/100 ml) and tocopherol (mg/L) levels in blood donors, healthy persons and in survivors of myocardial infarction grouped according to age. Figures refer to number of subjects.

On the other hand, the age distribution was different. Two-thirds of the healthy subjects were under 35 years of age, while three-fifths of the blood donors were in the older group. Accordingly the mean values in the two younger groups were almost identical.

A definite age trend was found (table 1 and fig. 1). The younger blood donors (age below 35 years) showed a significantly lower ($p < 0.001$) mean cholesterol content than the older ones (table 2, fig. 2). The mean cholesterol content in blood donors increased with each decade throughout the entire age range (table 1, fig. 1). The increase from the fifth to the sixth decade was, however, only 2.5 mg/100 ml. This was due to the different age-cholesterol relationship between the two sexes. The men showed an increase up to the fifth decade, after which the cholesterol values began to decrease. Unfortunately the oldest male group (60—65 years) was too small to allow definite conclusions. On the other hand, the female cholesterol values continued to increase up to seventh decade.

Women showed a slightly higher mean cholesterol content than men, and the difference remained when the blood donor material was divided into the two age groups seen in table 2. This no longer was the case when the five decades were compared (table 1). Here the men of middle age showed higher mean value. The differences, however, were hardly significant.

TABLE 1. Serum total cholesterol level (mg/100 ml) in 175 male and 137 female blood donors and in 124 survivors of myocardial infarction, grouped according to age

Subjects	20—29 years			30—39 years			40—49 years			50—59 years			60—65 years		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Blood donors															
males	56	226.2	50.4	34	251.1	41.2	38	275.6	52.0	41	268.8	48.0	6	256.2	24.4
females ..	26	236.2	31.7	26	249.8	45.1	33	265.9	52.4	28	280.3	46.3	24	300.5	47.2
total	82	229.3	45.4	60	250.6	42.6	71	271.0	50.6	69	273.5	47.3	30	291.7	46.8
Coronary	—	—	—	17	348.2	125.9	39	312.7	68.9	48	319.0	91.9	20	296.4	60.1

TABLE 2. Serum total cholesterol level (mg/100 ml) in blood donors and healthy persons, in age groups below and over 35 years

Subjects	20—35 years			36—65 years			Total		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Blood donors									
males	85	234.6	49.0	90	269.9	47.4	175	252.8	51.5
females	45	241.6	40.6	92	278.3	48.5	137	266.2	49.0
total	130	237.0	46.6	182	274.1	48.0	312	258.7	50.8
Healthy	67	233.6	33.9	34	244.5	40.6	101	237.3	36.5

Survivors of Myocardial Infarction

The serum cholesterol content was determined in 114 male (mean age 50 years) and 10 female survivors (mean age 51 years) of myocardial infarction. The mean age of the whole coronary group was 50 years. The data are given in tables 1 and 3 as mean values with standard deviations, grouped according to the age of the patient. Fig. 1 shows the influence of age, and in fig. 2 the cumulative frequency distribution of the serum cholesterol values is presented.

The range of cholesterol values in men was from 195 to 810 mg/100 ml and in women from 207 to 502 mg/100 ml. The female patients showed a considerably higher mean content than the male patients (table 3), but the female group was too small for statistical treatment. The frequency of »true» hypercholesterolemia, when the lower limit was defined as 350 mg/100 ml, was about 25 per cent; two-thirds of these 31 patients were under 50 years of age. The younger patients, i.e., those under age 50, showed a higher mean content than the older patients (table 3), but the difference was not significant. When the patients were divided according to age in decades (table 1, fig. 1), the age dependence became more apparent. Definite decrease occurred from the fourth to the fifth and from the sixth to the seventh decade.

The coronary patients showed a significantly higher ($p < 0.001$) mean serum content of cholesterol than the blood donors. The age distribution as well as the age-cholesterol relationship were different, as seen in fig. 1. However, the mean levels in coronary patients were significantly higher than those of blood donors in each decade

TABLE 3. Serum total cholesterol level (mg/100 ml) in survivors of myocardial infarction, in age groups below and over 50 years

Subjects	30—50 years			51—65 years			Total		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Coronary									
males	60	326.6	106.5	54	300.9	57.4	114	314.4	87.4
females	4	—	—	6	—	—	10	351.1	74.6
total	64	330.6	106.3	60	303.2	56.6	124	317.4	86.8

($p < 0.01$) up to the seventh decade, when they were nearly identical. The female blood donors showed in seventh decade even a higher mean content than the coronary patients.

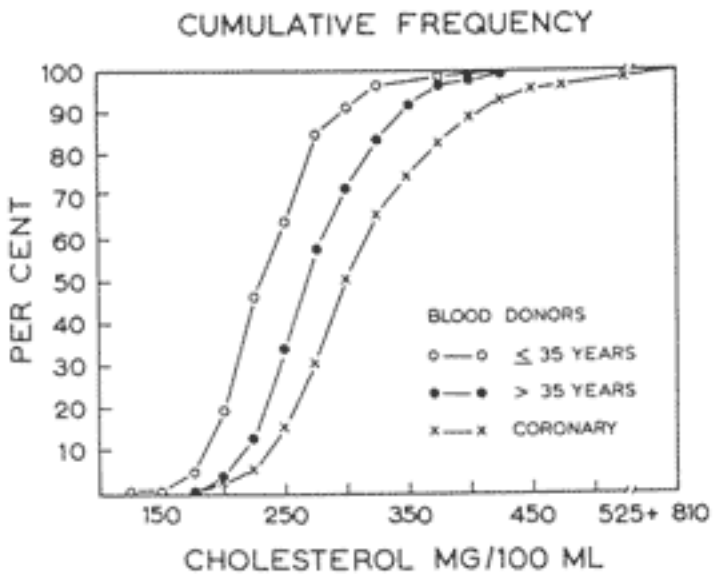

Fig. 2. Cumulative frequency distribution of serum total cholesterol in 130 blood donors below and 182 blood donors over the age of 35, and in 124 survivors of myocardial infarction.

In order to study the effectiveness of the various lipid parameters in separating the normal population from the diseased, the 90 per cent upper limit of the younger blood donors (age below 35 years) was used as discriminator. As is seen in the cumulative frequency distribution curves (fig. 2), this limit for cholesterol was 290 mg/100 ml.

Of the healthy subjects, 4 persons in both age groups (table 2) exceeded this limit, making 6 and 12 per cent. On the other hand, 58 older blood donors (age over 35 years) (31 per cent) were above this limit (fig. 2). Of the coronary patients, 60 per cent showed higher values. This percentage was 70 per cent for the younger patients (age below 50 years) and only 50 per cent for the older patients.

SERUM TRIGLYCERIDE LEVEL

Healthy Subjects

The serum triglyceride was determined in 84 healthy men (mean age 32 years) and in 14 healthy women (mean age 27 years). The mean age of the total healthy group was 31 years.

The data are given in tables 4 and 5 as mean values with standard deviations, grouped according to the age of the subjects. Fig. 1 shows the influence of age, and the cumulative frequency distribution of the serum triglyceride values is seen in fig. 3.

TABLE 4. Serum triglyceride level (mg/100 ml) in 98 healthy persons and 124 survivors of myocardial infarction, grouped according to age

Subjects	20—29 years			30—39 years			40—49 years			50—59 years			60—65 years		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Healthy	54	75.0	34.8	28	101.9	91.0	6	129.7	56.1	10	90.4	32.4	—	—	—
Coronary ..	—	—	—	17	187.8	84.8	39	165.4	75.4	48	177.9	90.8	20	154.3	69.1

TABLE 5. Serum triglyceride level (mg/100 ml) in healthy subjects, in age groups below and over 35 years

Subjects	20—35 years N.	Mean	S.D.	36—65 years N.	Mean	S.D.	Total N.	Mean	S.D.
Healthy									
males	56	79.0	33.4	28	107.1	39.9	84	88.4	37.9
females	11	—	—	3	—	—	14	83.1	36.8
total	67	79.1	33.9	31	105.8	39.1	98	87.6	37.4

In men the triglyceride levels ranged from 32 to 188 mg/100 ml and in women from 41 to 152 mg/100 ml. The small group of females thus had an almost identical mean content as the males (table 5). Because of the unequal age distribution, as seen in fig. 1, it is difficult to evaluate the influence of age on the serum triglyceride content. However, the older subjects over the age of 35 years (table 5) showed a significantly higher mean content than the young persons ($p < 0.01$).

Survivors of Myocardial Infarction

The serum triglyceride content was determined in 114 male (mean age 50 years) and in 10 female (mean age 51 years) survivors of myocardial infarction. The mean age of the total group was 50 years.

The data are presented in tables 4 and 6 as mean values with corresponding standard deviations, grouped according to the age of the patients. The relation to age is shown in fig. 1, and the cumulative frequency distribution of serum triglyceride is seen in fig. 3.

The range of serum triglyceride values was from 63 to 453 mg/100 ml in men and from 78 to 322 mg/100 ml in women. The mean level in the younger patients (age below 50) was higher than that in the older patients, but the difference was not significant (table 6). When the patients were grouped by age decades (fig. 1 and table 4) the mean values of the groups showed a tendency to decrease with increasing age. The small group of female patients had a higher mean serum triglyceride content than the men (table 6).

The coronary patients had, on an average, significantly more serum triglyceride (tables 4, 5 and 6) than the healthy subjects ($p < 0.001$). How-

TABLE 6. Serum triglyceride level (mg/100 ml) in survivors of myocardial infarction, in age groups below and over 50 years

Subjects	30—50 years N.	Mean	S.D.	51—65 years N.	Mean	S.D.	Total N.	Mean	S.D.
Coronary									
males	60	182.0	83.6	54	158.0	76.1	114	170.6	79.1
females	4	—	—	6	—	—	10	181.6	98.0
total	64	180.3	83.6	60	162.1	79.5	124	171.5	81.8

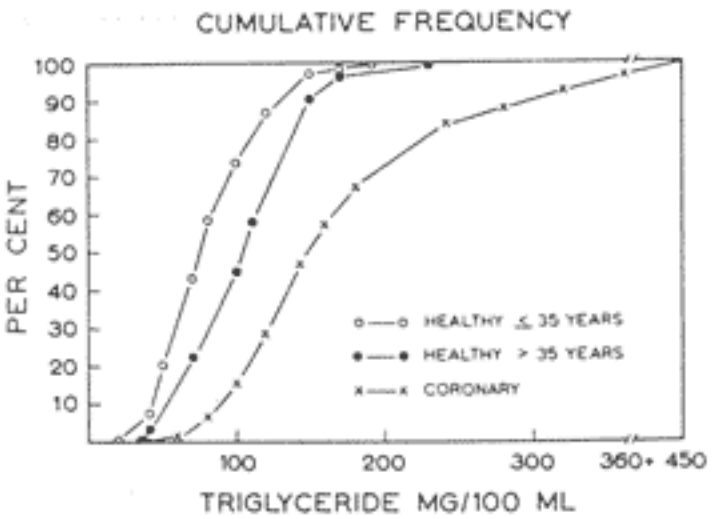

Fig. 3. Cumulative frequency distribution of serum fasting triglyceride in 67 healthy persons below and 31 healthy persons over the age of 35, and in 124 survivors of myocardial infarction.

ever, as is seen in table 4, the age distribution was quite different. When the various age groups were compared the difference was nevertheless highly significant ($p < 0.001$) in the fourth and sixth decades. On the other hand, no significant difference was seen in the fifth decade, but this age group consisted of 6 healthy persons only.

The 90 per cent upper limit for the young healthy persons revealed a serum triglyceride content of 125 mg/100 ml (fig. 3). Thirty per cent of the older healthy persons (age over 35 years) exceeded this limit and the same percentage of the younger coronary group (age below 50 years) was below the limit, while 65 per cent of the older coronary patients exceeded it. The percentage of the whole coronary group was 68 per cent.

PLASMA TOCOPHEROL LEVEL

Blood Donors and Healthy Subjects

The plasma tocopherol content was determined in 176 male (mean age 39 years) and 146 female (mean age 43 years) blood donors, and in 62 healthy men (mean age 33 years) and 10 healthy women (mean age 26 years). The mean age of the total blood donor group was 41 years and that of the total healthy group 32 years.

The data are given in tables 7 and 8 and in the figure 1 as mean values with standard deviations. Fig. 4 depicts the cumulative frequency distribution of the plasma tocopherol values in the blood donor series.

TABLE 7. Plasma tocopherol level (mg/L) in 176 male and 146 female blood donors and in 100 survivors of myocardial infarction, grouped according to age

Subjects	20—29 years			30—39 years			40—49 years			50—59 years			60—65 years		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Blood donors															
males	56	10.1	2.7	34	11.7	3.0	38	12.9	3.7	42	12.9	3.1	6	12.6	2.8
females ..	31	11.5	2.0	29	12.1	2.7	33	13.9	3.4	28	13.5	3.6	25	15.7	3.4
total	87	10.6	2.5	63	11.9	2.9	71	13.3	3.6	70	13.1	3.5	31	15.1	3.5
Coronary	—	—	—	15	17.7	3.9	35	15.2	3.4	35	16.1	3.6	15	15.1	2.9

TABLE 8. Plasma tocopherol level (mg/L) in blood donors and healthy persons, in age groups below and over 35 years

Subjects	20—35 years			36—65 years			Total		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Blood donors									
males	85	10.7	3.0	91	12.8	3.3	176	11.8	3.3
females	53	11.6	2.5	93	14.2	3.6	146	13.3	3.5
total	138	11.0	2.8	184	13.5	3.5	322	12.4	3.5
Healthy	48	10.5	2.1	24	11.1	2.5	72	10.7	2.3

The plasma tocopherol values of the male blood donors ranged from 6.1 to 23.3 mg/L, of the female blood donors from 6.9 to 23.6 mg/L, of the healthy males from 6.6 to 16.5 mg/L, and of the healthy women from 6.8 to 15.6 mg/L. The healthy subjects had, on an average, a lower plasma tocopherol content than the blood donors, but the age distribution was different. Thus, no difference existed in the younger groups (age below 35 years), as is seen in table 8. The age dependence was strikingly similar to that of serum cholesterol in fig. 1. No increase occurred, however, in the female blood donors from the fifth to the sixth decade, thus differing from the cholesterol-age relation. The men showed a decrease after the fifth or sixth decade also here (table 7). Independent of the age, the females had slightly higher mean values than the men (tables 7 and 8).

Survivors of Myocardial Infarction

The plasma tocopherol content was determined in 91 male (mean age 49 years) and 9 female (mean age 50 years) survivors of myocardial infarction, the mean age of the total group being 49 years.

The data are presented as mean values with standard deviations in tables 7 and 9 and in fig. 1. The cumulative frequency distribution of the plasma tocopherol values is given in fig. 4.

The range of plasma tocopherol values was from 8.2 to 26.2 mg/L in males and from 8.9 to 20.4 mg/L in females. The women had, on an average, a slightly higher plasma tocopherol level than the men, but the number of women was too small for statistical treatment (table 9). The young patients showed a higher mean

TABLE 9. Plasma tocopherol level (mg/L) in survivors of myocardial infarction, in age groups below and over 50 years

Subjects	30—50 years			51—65 years			Total		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Coronary									
males	52	16.2	3.9	39	15.3	2.8	91	15.8	3.5
females	4	—	—	5	—	—	9	16.8	3.8
total	56	16.3	3.9	44	15.4	2.9	100	15.9	3.1

content than the old patients (age over 50 years) but the difference was not significant (table 9). Fig. 1 shows the similar age relations of the plasma tocopherol and the serum cholesterol values in the coronary population, revealing a definite tendency of plasma tocopherol content to decrease with age.

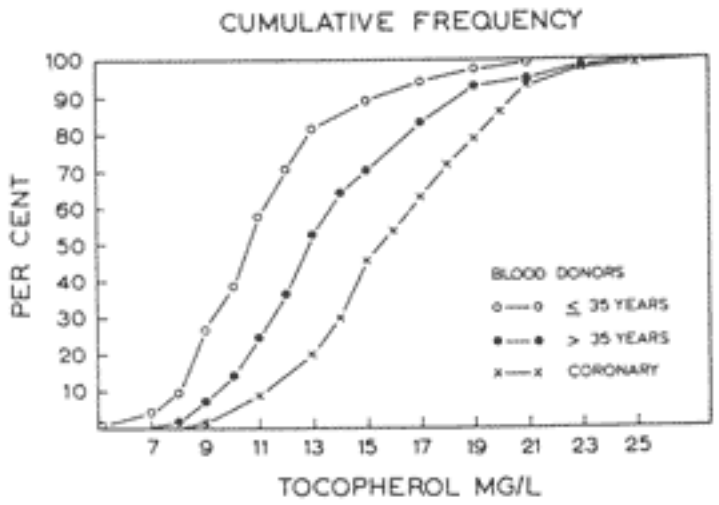

Fig. 4. Cumulative frequency distribution of plasma tocopherol in 138 blood donors below and 184 blood donors over the age of 35, and in 100 survivors of myocardial infarction.

On an average the coronary patients had more plasma tocopherol ($p < 0.001$) than the blood donors (tables 7, 8 and 9). The difference was also statistically significant in all but the oldest decade (table 7 and fig. 1), where the mean contents were identical ($p < 0.001$ in fourth and sixth decades, $p < 0.01$ in the fifth decade).

The 90 per cent upper limit for the younger blood donors (age below 35 years) was 15 mg/L, as is seen in the cumulative frequency distribution curve (fig. 4). Only one of the younger (age below 35 years) and 2 of the older healthy group exceeded this limit, but not less than 30 per cent of the older blood donors had higher values. On the other hand, 53 per cent of the coronary patients were above this limit, i.e., 55 per cent of the younger (age below 50 years) and 50 per cent of the older patients.

INTERRELATIONSHIPS OF SERUM TOTAL CHOLESTEROL, TRIGLYCERIDE AND TOCOPHEROL LEVELS

The correlation between the serum levels of the three lipids was studied by linear regression analysis and is shown in scattergrams in figs. 5—10. The regression equations and the correlation coefficients are presented in the legends to the figures.

A significant correlation ($p < 0.001$) was found to be present between the serum cholesterol and plasma tocopherol levels both in the coronary group and in the combined control group consisting of the healthy subjects and the blood donors. The regressions showed a somewhat greater increase of the serum cholesterol content with increasing tocopherol levels in the coronary group than in the control group (figs. 5 and 6).

On the other hand, a significant correlation ($p < 0.001$) between the serum triglyceride and plasma tocopherol levels was present only in the coronary group (figs. 7 and 8).

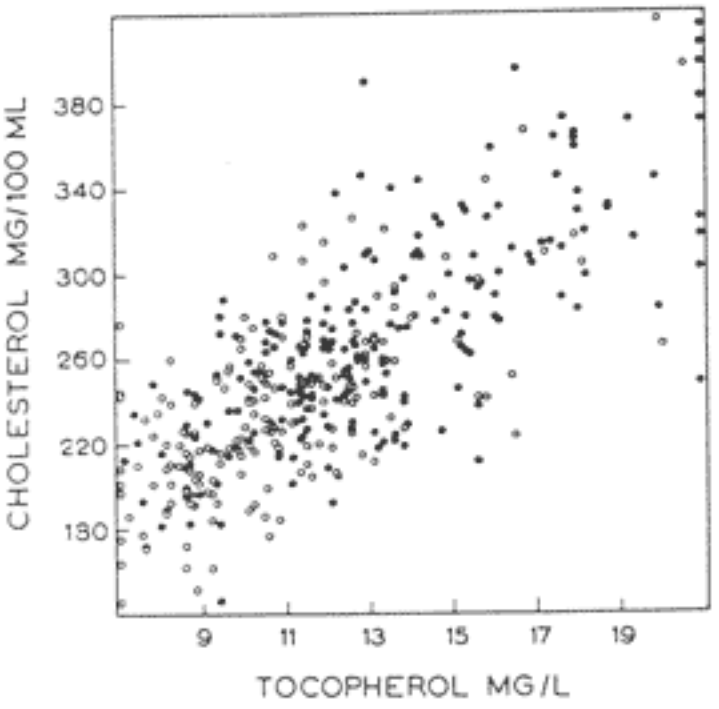

Fig. 5. Relationship of serum cholesterol and plasma tocopherol levels in 312 blood donors and 68 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation: $y = 122.4 + 10.94\,x \pm 32.6$ ($p < 0.001$), $r = 0.75$ ($p < 0.001$).

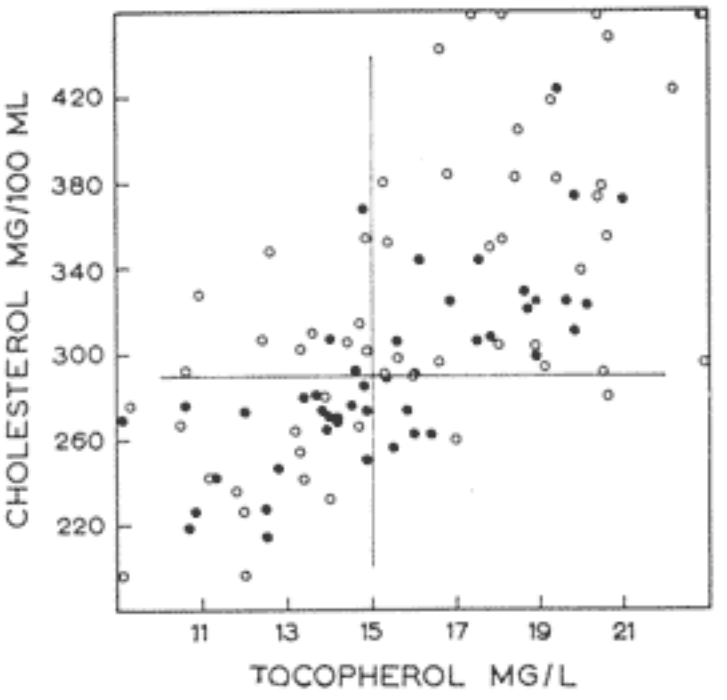

Fig. 6. Relationship of serum cholesterol and plasma tocopherol levels in 100 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 35. Lines = upper normal limits. Regression equation: $y = 54.0 + 16.65\,x \pm 68.3$ ($p < 0.001$), $r = 0.65$ ($p < 0.001$).

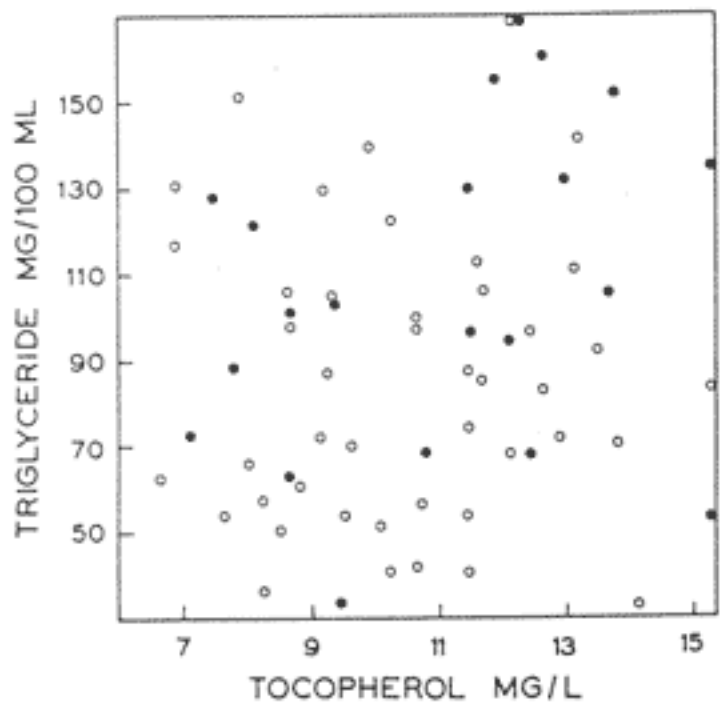

Fig. 7. Relationship of serum triglyceride and plasma tocopherol levels in 68 healthy persons. Open dots = age below 35, black dots = age over 35.

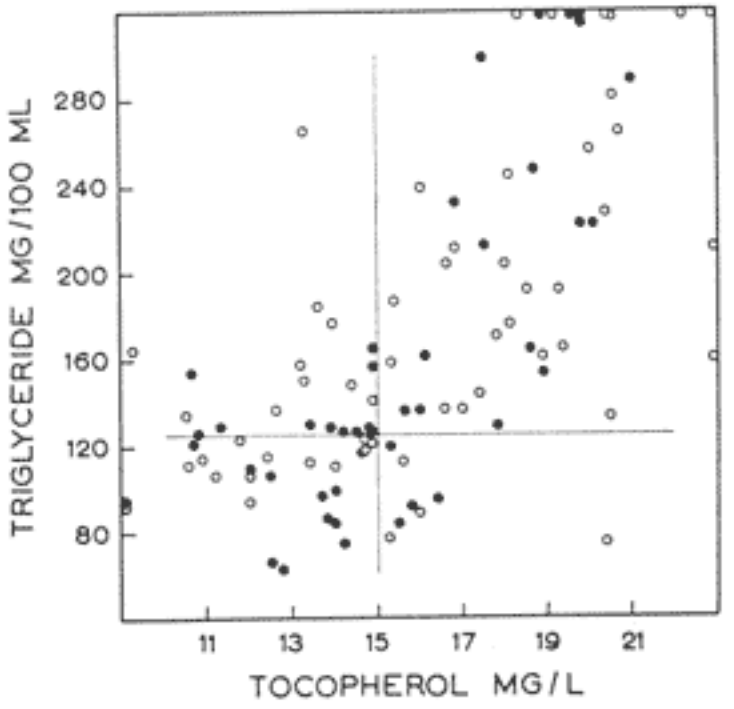

Fig. 8. Relationship of serum triglyceride and plasma tocopherol levels in 100 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation: $y = -70.5 + 15.25\,x \pm 66.3$ ($p < 0.001$), $r = 0.63$ ($p < 0.001$).

The regression analyses revealed a significant correlation between the serum triglyceride and cholesterol levels (figs. 9 and 10) in the coronary group ($p < 0.001$) and in the healthy group ($p < 0.05$). The regression line in both groups had a parallel course, but in the coronary group it had a higher level, indicating a higher triglyceride content at the same cholesterol content.

The serum cholesterol, triglyceride and tocopherol contents were determined in 100 coronary patients. All the values were abnormally high in 44 cent and normal in 17 per cent. At least two abnormal lipid values were found in 56 per cent of the patients, while 27 per cent exhibited only one abnormal value. If only one lipid parameter was pathologic, this was most commonly the serum triglyceride (in 14 per cent of patients) and least commonly the plasma tocopherol (in 5 per cent).

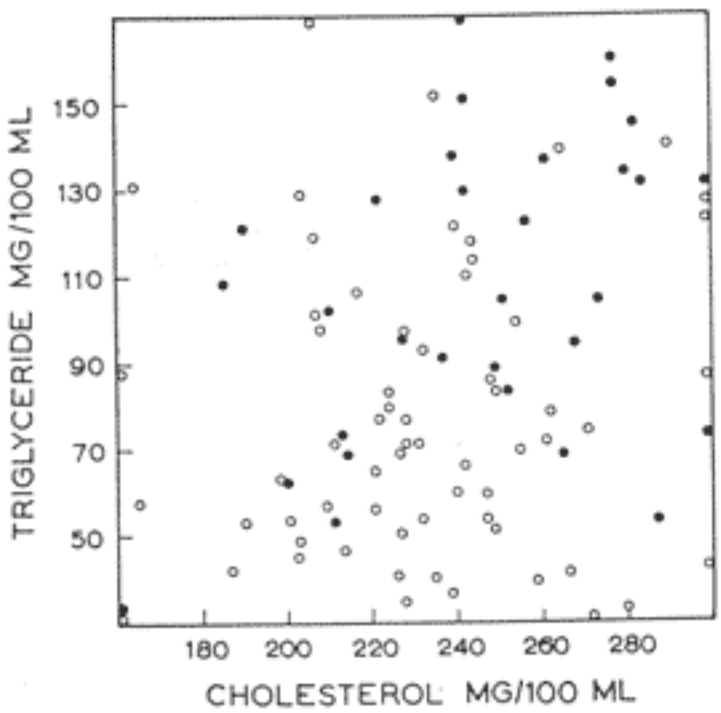

Fig. 9. Relationship of serum triglyceride and cholesterol levels in 94 healthy persons. Open dots = age below 35 years, black dots = age over 35. Regression equation: $y = 28.8 + 0.2507\ x \pm 37.9$ ($p < 0.05$), $r = 0.23$ ($p < 0.05$).

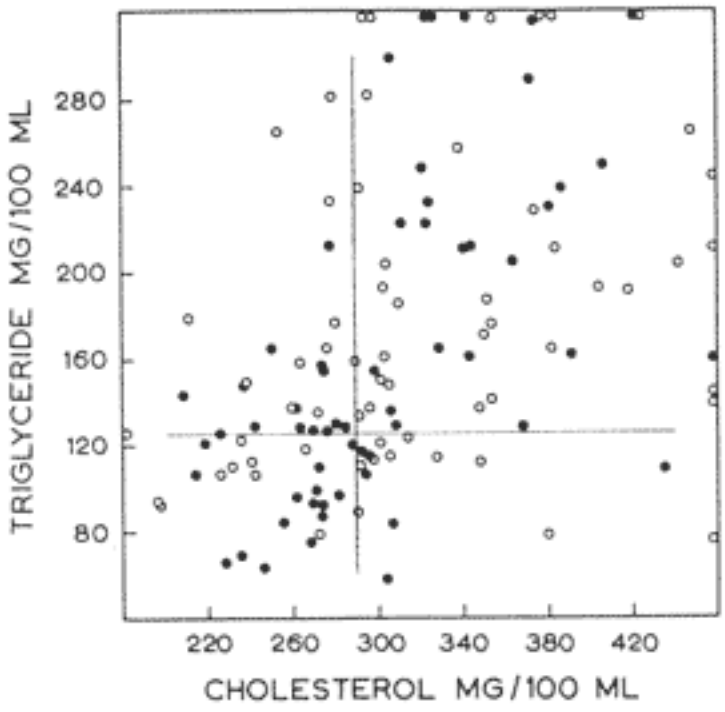

Fig. 10. Relationship of serum triglyceride and cholesterol levels in 124 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation: $y = 76.6 + 0.2991\ x \pm 77.9$ ($p < 0.001$), $r = 0.32$ ($p < 0.001$).

INFLUENCE OF ACUTE MYOCARDIAL INFARCTION ON SERUM CHOLESTEROL, TRIGLYCERIDE AND TOCOPHEROL LEVELS

Numerous reports have been published of the changes occurring in the plasma lipids, lipoproteins and electrophoretic pattern after acute myocardial infarction (Welin 1948, Kroetz and Fischer 1954, Haus and Böhle 1955, Björck *et al.* 1957, Smith 1957, Dodds and Mills 1959, Page and Lewis 1959, Pomerantz 1962). However, the data are somewhat controversial.

In order to study the influence of acute myocardial infarction on the plasma tocopherol, serum triglyceride and serum cholesterol levels, these values were followed in 9 patients until five months at least had elapsed from the acute attack. The first sample was taken within 24 hours after the patient's admission to this hospital. All the patients showed definite electrocardiographic changes due to the acute myocardial infarction and an increased serum activity of G—O-transaminase and lactic acid dehydrogenase at that time. The following three samples were taken at intervals of one week, and the last sample under ambulatory conditions when 5 to 11 months had elapsed from the acute infarction.

On admission an oral anticoagulant therapy was instituted in all cases, but none of the patients was given heparin. All but one patient were receiving anticoagulant therapy at the time the last sample was taken. No marked changes occurred in the dietary habits during the time of observation.

The mean tocopherol, triglyceride and cholesterol levels at each of the five dates are presented in fig. 11.

A significant fall ($p < 0.01$) occurred in the mean cholesterol and tocopherol levels during the first week. This was apparent in all the patients. On the other hand, no systematic change occurred in the triglyceride level. In one-half of the patients the triglyceride level decreased, while the other half showed an increase.

Two weeks after admission the plasma tocopherol already reached the initial level, and under ambulatory conditions the plasma tocopherol and serum cholesterol showed higher, though not significantly higher, mean values than in the acute phase. The tocopherol mean at this time was the sum of an increase of greater magnitude in 5 patients than the decrease that occurred in 4 patients. On the other hand, all but one patient showed a higher cholesterol content during ambulation than on admission. The similar behavior of these two lipids after the infarction was striking.

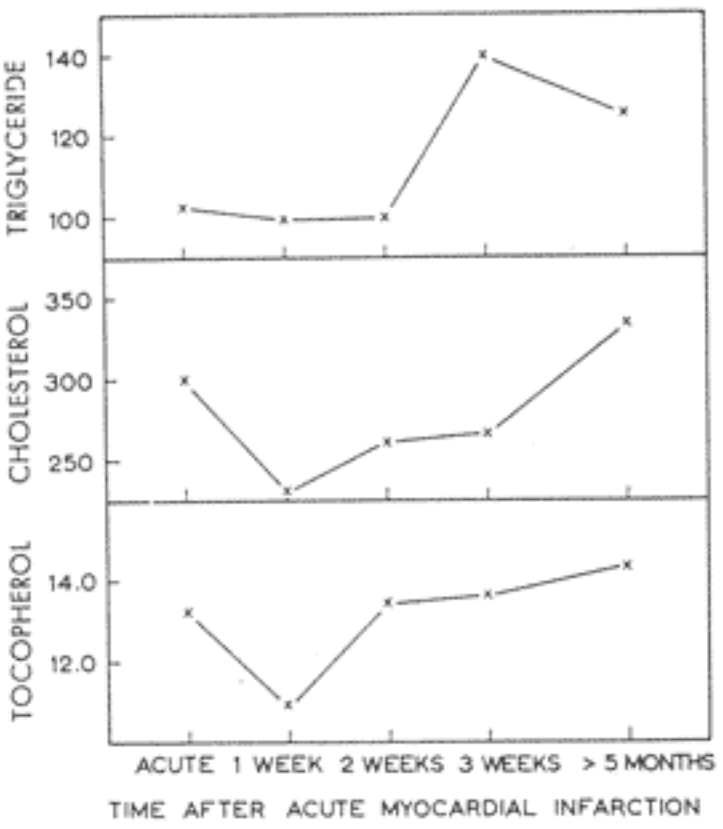

Fig. 11. Influence of acute myocardial infarction on serum cholesterol (mg/100 ml), triglyceride (mg/100 ml) and tocopherol (mg/L) levels in 9 patients.

The serum triglyceride level also was, on an average, higher at the last examination than on admission. This was due to a definite increase in 6 patients, while the other 3 patients showed a negligible decrease. The high mean level in the third week was chiefly due to an unexpectedly high value in one patient.

TOCOPHEROL LOADING TEST

Two grams of tocopherol acetate and 100 ml of cream (40 per cent fat) was given under hospital conditions to 64 survivors of myocardial infarction (mean age 48 years) and to 38 healthy subjects (mean age 35 years). Six female coronary patients and three healthy females were included in the two groups. The coronary and healthy groups were divided into two categories according to age. The age borderline for coronary patients was 50 years and for healthy persons 35 years. The mean ages for the two coronary groups were 43 and 57 years and for the two healthy groups 26 and 45 years.

Table 10 presents the mean values at each time, with standard deviations. Fig. 12 shows the individual values and fig. 13 the absolute increase from the basal level of plasma tocopherol after the ingestion of vitamin E.

The complete loading test concerned five samples (basal sample and at 4, 6, 10 and 24 hours) but as is seen in the table 10, all the samples were not examined at each time of sampling. This concerns particularly the 6- and 10-hour samples in the coronary group.

The basal levels of serum cholesterol and triglyceride were determined in all subjects. When the criteria for normal limits were those presented above, these measurements revealed in coronary patients who underwent the test a hypercholesterolemia incidence of 73 per cent, while 75 per cent showed hypertriglyceridemia, and 61 per cent hypertocopherolemia. Thus, hyperlipidemia was somewhat more common in these subjects than in the whole series in this study. With respect to lipid values the healthy subjects were comparable to the »base population» of this study with the exception of the older healthy subjects (age over 35 years), who showed hypertriglyceridemia in 39 per cent of subjects.

Fasting samples. — The coronary patients had a significantly higher mean tocopherol level ($p < 0.001$) than the healthy persons. The difference was highly significant also when the mean levels of the older healthy group and the younger coronary group were compared ($p < 0.001$).

4-hour samples. — The mean tocopherol level of the coronary group was still significantly higher than the mean level of the healthy subjects. However, there was considerable overlapping of the individual values in the two populations. The younger subjects in both groups showed higher mean values than the older subjects due to more rapid mean increases. On the average, the whole healthy group showed a slightly higher increase from the basal level, but statistically this was not significant.

6-hour samples. — The difference between the mean levels of the two groups was greater than in the 4-hour samples. The mean increase of the plasma tocopherol from the basal level was now greater in the coronary group than in healthy subjects, but it was not yet statistically significant. In both

groups the mean tocopherol content as well as the mean increase from the basal level still tended to remain higher in the younger subjects than in the older ones.

10-hour samples. — This value represented the peak level in all the subjects. Despite the significant difference in the mean values of coronary patients and healthy subjects, there was still marked overlapping of individual values in the two groups. The mean tocopherol level —1 S.D. in coronary patients was exceeded by 31 per cent of healthy subjects. On the other hand, 28 per cent of the coronary patients had values below the mean +1 S.D. of the healthy group. The older group of healthy subjects now showed a slightly higher mean content than the younger healthy group, while in the coronary group the two age categories had almost identical mean values. The mean increase from the basal level was now significantly higher in the coronary than in the healthy population ($p < 0.01$).

Both groups exhibited a significant correlation between the basal tocopherol level and the 10-hour peak level. The correlation coefficient in the coronary group was 0.63 ($p < 0.001$) and in the healthy group 0.41 ($p < 0.02$).

24-hour samples. — The difference of the mean levels in the two main groups was significant at 24 hours ($p < 0.001$). The difference between the older healthy group and the younger coronary group was also statistically highly significant ($p < 0.001$). Nevertheless, a considerable overlapping of the individual values of coronary and healthy subjects was present. Thus 27 per cent of the healthy subjects exceeded the mean level —1 S.D. of the coronary patients, and 24 per cent of the coronary patients were below the mean level +1 S.D. of the healthy subjects. The older group of healthy subjects had a higher mean level than the younger group due to a significantly greater increase from the basal level ($p < 0.05$). Among the coronary patients the situation was the reverse, the younger coronary subjects

TABLE 10. Plasma tocopherol level (mg/L) in healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat)

Subjects	Fasting			4 hours			6 hours			10 hours			24 hours		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Healthy															
age below 35	20	10.8	1.9	20	18.5	4.5	20	22.0	5.4	18	31.2	7.7	20	19.5	3.3
age over 35	18	11.2	2.6	17	16.6	5.2	16	21.1	7.6	17	34.0	10.3	18	22.6	5.2
total	38	11.0	2.3	37	17.6	4.8	36	21.6	6.4	35	32.6	9.0	38	21.0	4.5
Coronary															
age below 50	41	16.7	4.0	40	23.7	6.3	24	31.1	7.7	25	45.6	11.3	40	31.9	7.9
age over 50	23	16.4	3.0	22	20.7	4.4	7	27.1	6.4	7	46.2	12.3	23	28.5	6.2
total	64	16.6	3.6	62	22.7	5.9	31	30.2	7.5	32	45.8	11.3	63	30.7	7.5

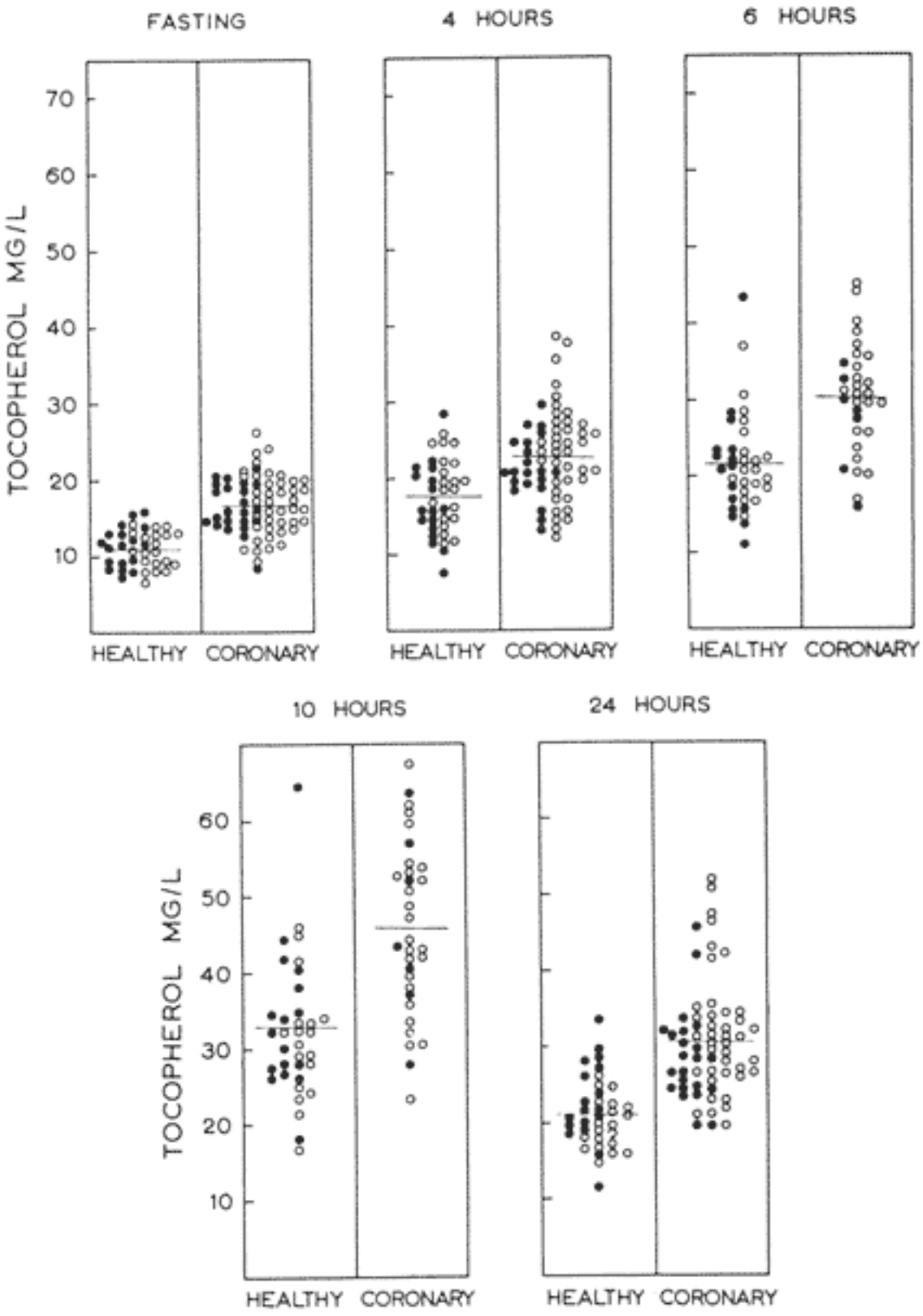

Fig. 12. Plasma tocopherol levels in healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat). Healthy: Open dots = age below 35, black dots = age over 35. Coronary: Open dots = age below 50, black dots = age over 50. Horizontal lines = mean tocopherol levels.

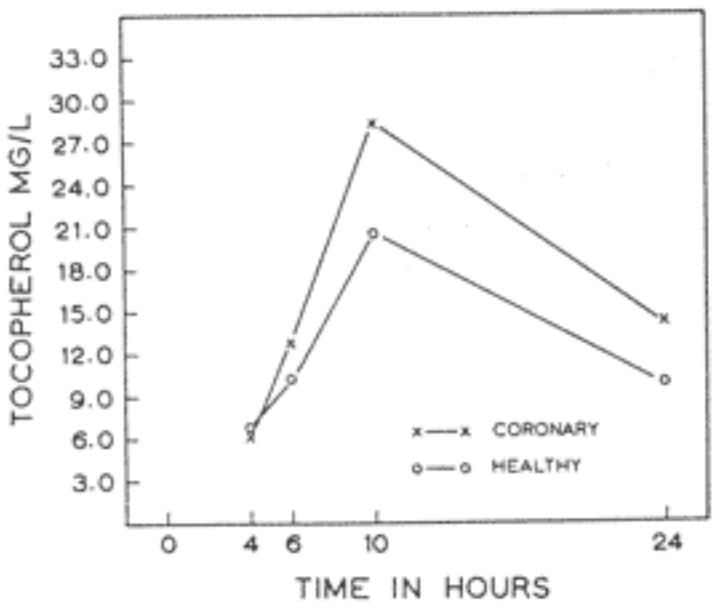

Fig. 13. Absolute increase of plasma tocopherol content from the basal level in 38 healthy persons and 63 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat).

having a higher mean level than the older patients and the increase from the basal level also being higher ($p < 0.05$). The mean increase from the basal level remained significantly higher in the coronary group than in the healthy group ($p < 0.02$).

The increase of plasma tocopherol content from the basal level was continuous up to the peak level in all the subjects with the exception of two who showed no increase in the interval from 4 to 6 hours, and one, who showed no increase from the basal level in the 4-hour sample. In all the subjects the 24-hour level was higher than the basal level but lower than the 10-hour level.

The plasma tocopherol concentration curve calculated on the basis of samples taken 4, 6, 10 and 24 hours after the ingestion of tocopherol was thus significantly higher in the coronary patients than in the somewhat younger healthy group. Considerable overlapping of individual values was present at each time, the least in the 24-hour samples. Because of a different age distribution of the two groups, the data are not readily comparable. The mean ages and age distributions, however, were identical in the older healthy group and in the younger coronary group. These groups are therefore the best suited for comparison. The results in these groups revealed a similar significant difference as the healthy and coronary groups as a whole.

An abnormal retention of tocopherol at 24 hours may reflect some defect in the lipid metabolism, particularly in the disappearance of exogenic lipids from the circulation. The relation of the level at 24 hours to the basal level of tocopherol and to those of triglyceride and cholesterol, the two important constituents of plasma lipids, was therefore studied by linear regression analysis. The relationship of these lipid parameters is demonstrated by plotting the individual values in scattergrams (figs. 14—19). The regression equations and correlation coefficients are presented in the legends to figs. 14—19. The analyses revealed a significant correlation between the 24-hour tocopherol level and the basal lipid level in both the healthy and the coronary groups, thus showing that no one of these parameters was an independent variable.

On the basis of this consideration it seemed to be useful to study the validity of the 24-hour tocopherol value in

separating the coronary population from the normal population, as compared to the validity of the basal level of plasma tocopherol, serum triglyceride and serum cholesterol. The 90 per cent upper limit of the young healthy subjects was used as a discriminator. In the 24-hour tocopherol determination this was 24.5 mg/L. Six persons in the older healthy group exceeded this limit, equivalent to 33 per cent. This percentage thus was almost the same as the incidence of hypertriglyceridemia. On the other hand, 83 per cent of the coronary patients exceeded this value, while the corresponding percentage of basal cholesterol, triglyceride and tocopherol were 73, 75 and 61 per cent. Accordingly, the metabolic abnormality was best revealed by the loading test.

Comparison of the values of all these lipid parameters in the coronary group showed that all the parameters were abnormally high in 51 per cent of cases and within normal limits in 3 cases only. The 24-hour tocopherol level only was pathologic in 2 cases, but it was never normal alone.

It has been suggested that oral anticoagulants impair the removal of circulating fat in postprandial lipemia (Mashford and Nestel 1961). In the present series all but 9 coronary patients received oral anticoagulants. However, only one of these 9 subjects had a normal 24-hour plasma tocopherol level.

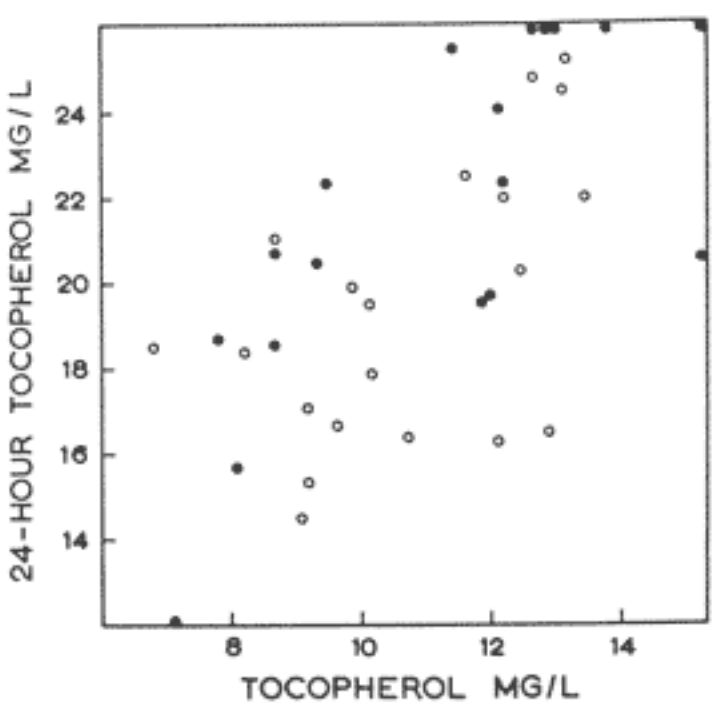

Fig. 14. Relationship of plasma 24-hour tocopherol and fasting tocopherol levels in 38 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation: $y = 6.95 + 1.275\ x \pm 3.49$ ($p < 0.001$), $r = 0.64$ ($p < 0.001$).

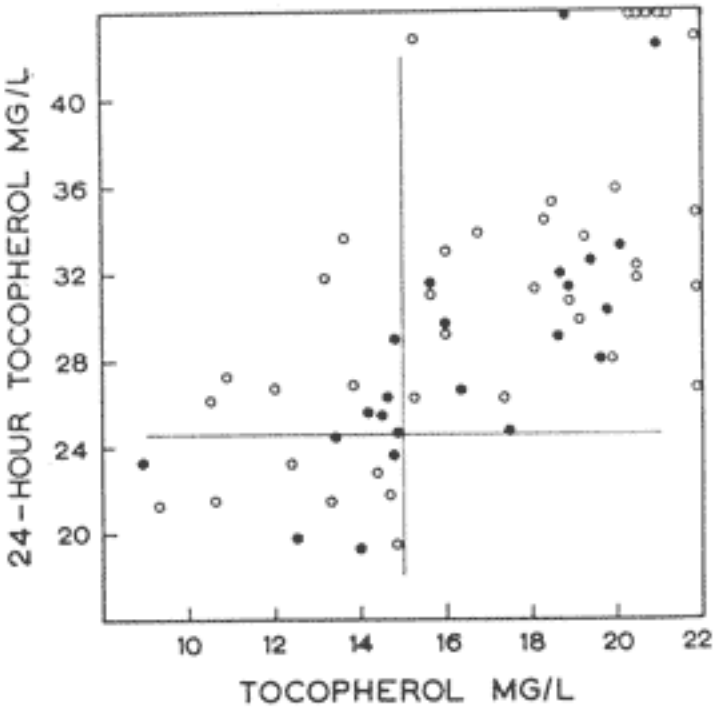

Fig. 15. Relationship of plasma 24-hour tocopherol and fasting tocopherol levels in 63 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation: $y = 7.15 + 1.420\ x \pm 5.46$ ($p < 0.001$), $r = 0.69$ ($p < 0.001$).

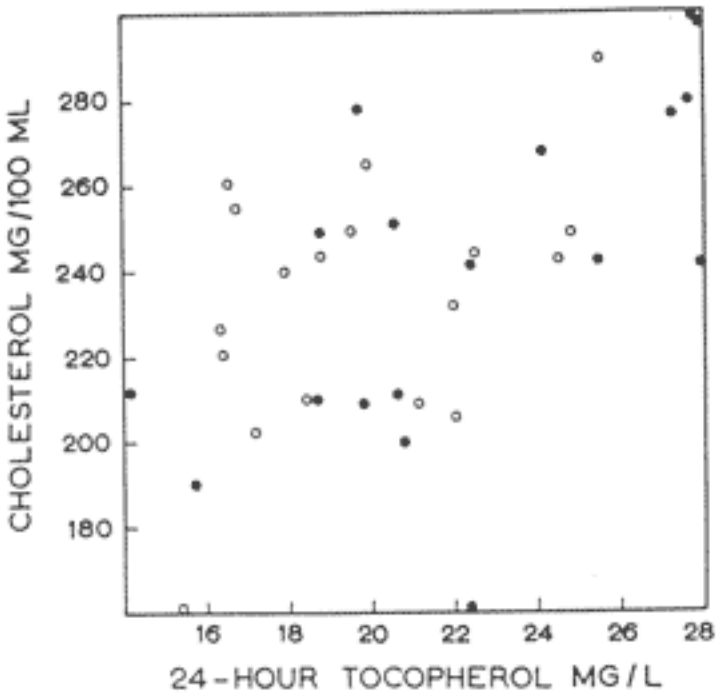

Fig. 16. Relationship of serum cholesterol and plasma 24-hour tocopherol levels in 36 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation: $y = 140.4 + 4.619\,x \pm 33.3$ ($p < 0.001$), $r = 0.54$ ($p < 0.001$).

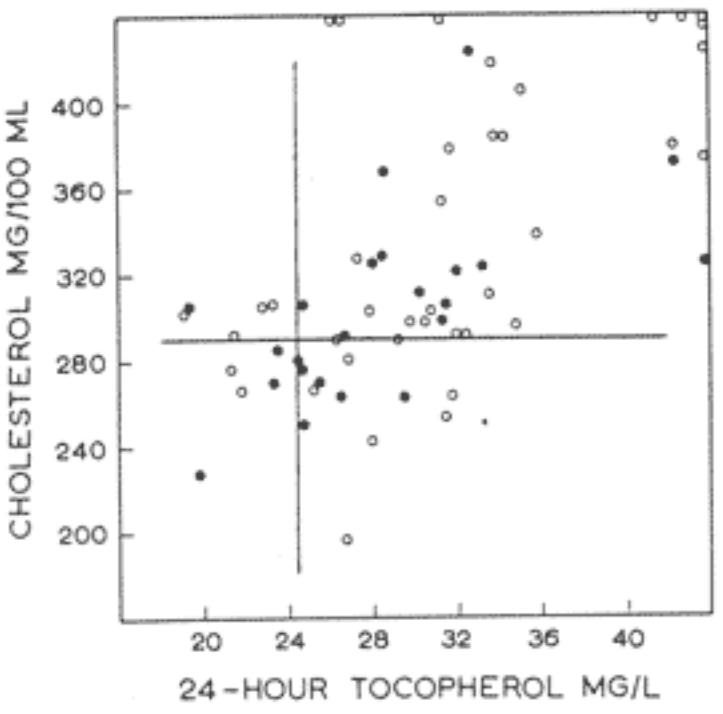

Fig. 17. Relationship of serum cholesterol and plasma 24-hour tocopherol levels in 63 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation: $y = 86.1 + 8.212\,x \pm 81.8$ ($p < 0.001$), $r = 0.60$ ($p < 0.001$).

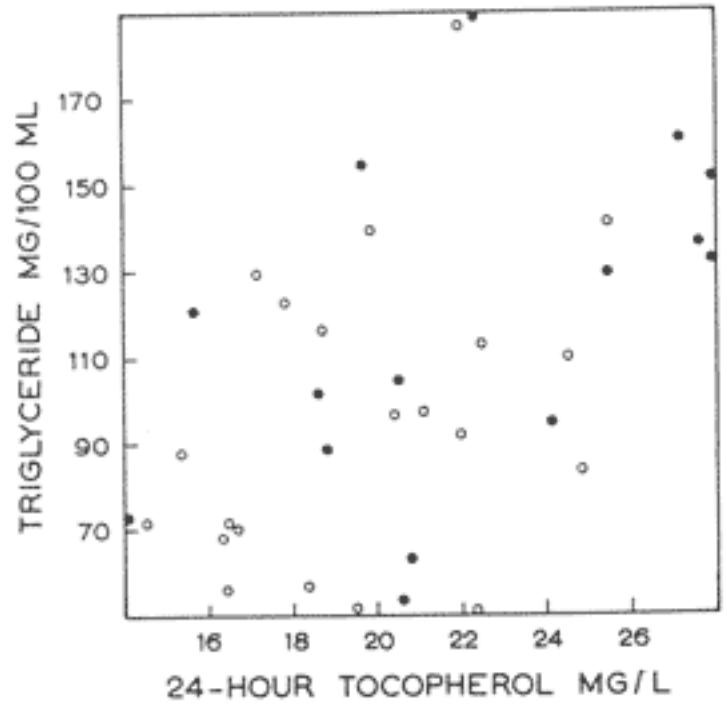

Fig. 18. Relationship of serum fasting triglyceride and plasma 24-hour tocopherol levels in 36 healthy persons. Open dots = age below 35, black dots = age over 35. Regression equation: $y = 19.5 + 4.149\,x \pm 36.9$ ($p < 0.01$), $r = 0.45$ ($p < 0.01$).

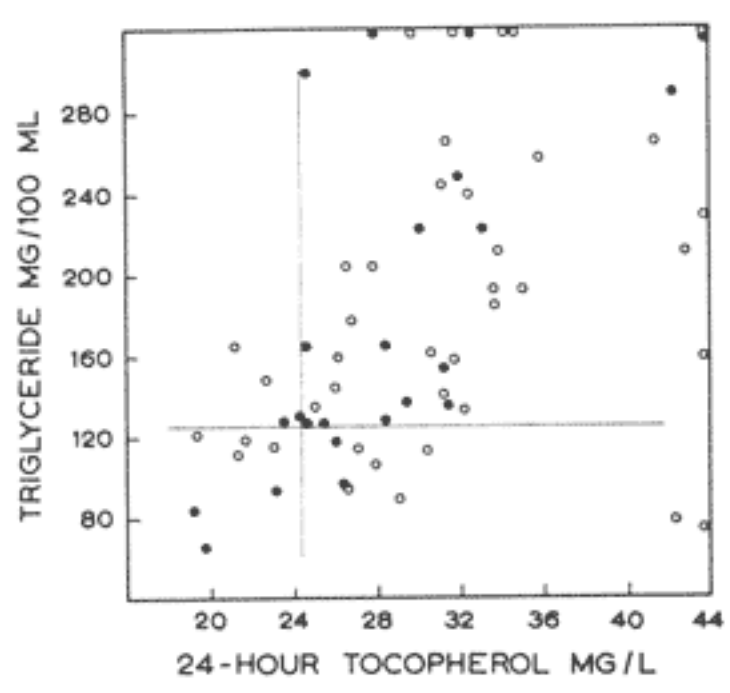

Fig. 19. Relationship of serum fasting triglyceride and plasma 24-hour tocopherol levels in 63 survivors of myocardial infarction. Open dots = age below 50, black dots = age over 50. Lines = upper normal limits. Regression equation: $y = 35.6 + 4.980\,x \pm 85.8$ ($p < 0.01$), $r = 0.40$ ($p < 0.01$).

TOCOPHEROL IN PLASMA LIPOPROTEINS DURING THE LOADING TEST

The lipoproteins were isolated from the plasma by two successive ultracentrifugal runs. In the primary separation fractions D. < 1.006 and D. > 1.006 were obtained. This separation was performed of fasting plasma samples and of samples taken 4, 6, 10 and 24 hours after the ingestion of 2 grams of tocopherol acetate and 100 ml of cream (40 per cent fat) by 17 survivors of myocardial infarction (mean age 47 years) and 25 healthy persons (mean age 33 years). The healthy group was divided according to age into two groups, the age borderline being 35 years. The mean ages of the younger healthy group was 27 years and of the older group 41 years. The subjects were men, with the exception of 3 women in the coronary group and 2 in the healthy group. The D. > 1.006 fraction of 8 coronary patients (mean age 47 years) and of 7 healthy persons (mean age 33 years) was separated further into D. 1.006—1.019, D. 1.019—1.063 and in D. > 1.063 fractions. This secondary separation was done of fasting plasma and of samples taken 4, 10 and 24 hours after the administration of tocopherol.

The results, expressed as mean values with standard deviations or ranges are shown in tables 11 and 12. The concentration curves for tocopherol in the fractions are presented in figs. 20 and 22 and the corresponding percentile

TABLE 11. Tocopherol content (mg/L) of D. < 1.006 and D. > 1.006 particles in sera of healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat). Data corrected to 100 per cent recovery of total plasma content.

Subjects	D. < 1.006 Fasting N.	Mean	S.D.	4 hours N.	Mean	S.D.	6 hours N.	Mean	S.D.	10 hours N.	Mean	S.D.	24 hours N.	Mean	S.D.
Healthy															
age below 35	15	1.9	1.3	10	6.0	4.1	12	5.0	2.9	12	7.0	4.7	14	2.9	1.6
age over 35	8	1.5	0.9	8	5.0	2.4	7	4.6	1.8	11	8.4	3.7	11	3.9	2.0
total	23	1.8	1.2	18	5.6	3.4	19	4.9	2.5	23	7.6	4.2	25	3.3	1.8
Coronary ..	15	2.1	1.6	14	6.2	4.0	13	7.0	6.1	17	10.3	4.3	17	5.3	2.1

Subjects	D. > 1.006 Fasting N.	Mean	S.D.	4 hours N.	Mean	S.D.	6 hours N.	Mean	S.D.	10 hours N.	Mean	S.D.	24 hours N.	Mean	S.D.
Healthy															
age below 35	15	8.9	2.1	10	11.5	2.2	12	15.0	2.5	12	22.8	3.9	14	16.3	2.7
age over 35	8	10.5	3.2	8	13.7	4.0	7	15.5	5.0	11	26.2	9.5	11	18.7	5.8
total	23	9.4	2.6	18	12.5	3.2	19	15.2	3.5	23	24.4	7.2	25	17.4	4.4
Coronary ..	15	15.9	3.1	14	19.1	4.8	13	25.2	6.8	17	37.0	8.9	17	29.3	7.9

distributions in figs. 21 and 23. As will be seen in the tables, all the samples were not analyzed each time.

Serum cholesterol and fasting triglyceride were determined in all these subjects. Of the coronary patients all but 2 had hypercholesterolemia and all but 4 had hypertriglyceridemia. Only 2 coronary patients showed a normal tocopherol »clearing» at 24 hours, while in 6 healthy persons the 24-hour tocopherol level was abnormally high.

Tocopherol Content of D. < 1.006 and D. > 1.006 Particles

Fasting Samples. — Most of plasma tocopherol was found in the D. > 1.006 particles. The mean ratio of D. > 1.006 tocopherol to the total plasma tocopherol, expressed as per cent, was higher in coronary patients than in healthy persons, but the difference was not significant. The higher mean content of plasma tocopherol in the coronary patients as compared to the healthy subjects (calculated from table 11) was wholly accounted for by fraction D. > 1.006, in which the tocopherol content was significantly higher in the coronary patients ($p < 0.001$). The ratio (D. > 1.006/total plasma tocopherol) tended to be higher in older healthy persons than in the younger ones, but the groups were small.

Samples Taken 4, 6, 10 and 24 Hours after the Ingestion of Tocopherol. — In the early phase up to 4 hours the tocopherol content increased in both fractions, the increase being more rapid in D. < 1.006 particles. *At 4 hours* the mean tocopherol content as well as the mean increase from the basal level in both particle classes were higher in the coronary than in the healthy

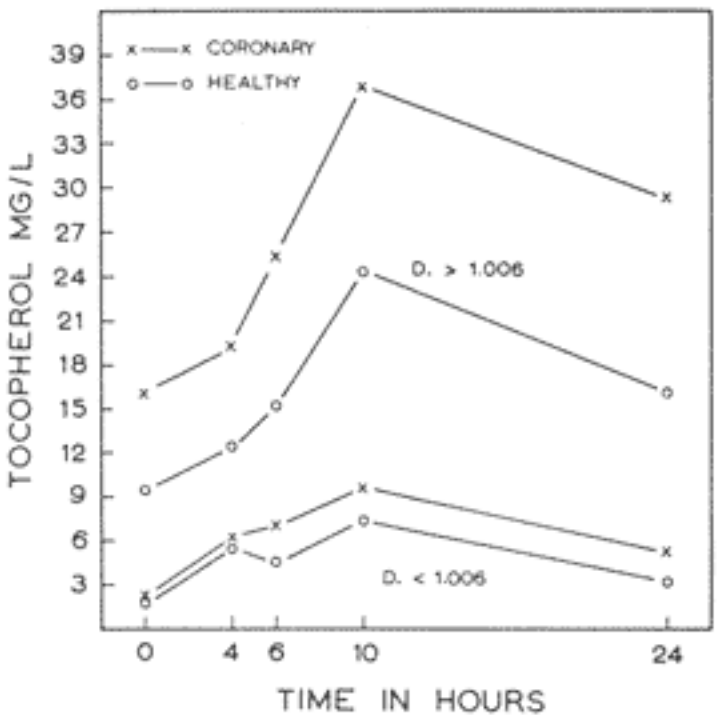

Fig. 20. Tocopherol content of D. < 1.006 and D. > 1.006 particles in sera of 25 healthy persons and 17 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat).

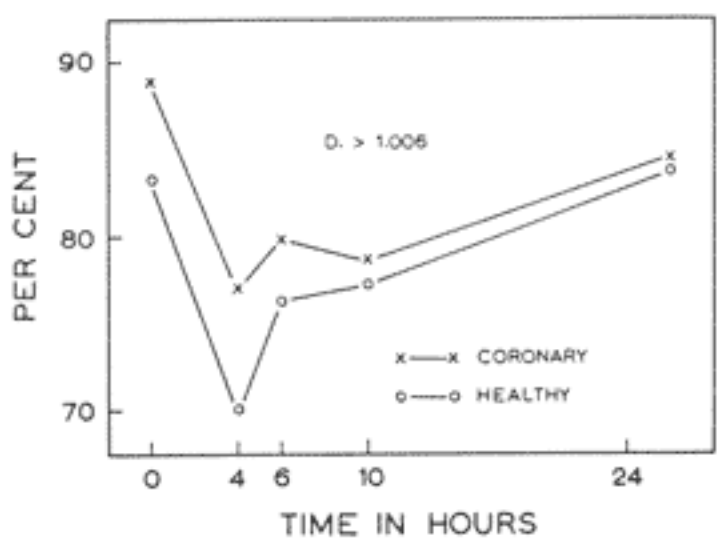

Fig. 21. Tocopherol content of D. > 1.006 particles as per cent of total plasma content in 25 healthy persons and 17 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat).

group. The younger healthy subjects showed at 4 hours a higher mean content of D. < 1.006 tocopherol and a lower content of D. > 1.006 tocopherol than the older ones.

After 4 hours the D. > 1.006 tocopherol showed a sharp and continuous increase in both main groups until the peak level was attained at 10 hours. The difference between the two main groups with respect to the tocopherol in this fraction increased with time until the peak level was reached, and thus was significant at each time. Similarly, the older healthy persons maintained, on the average, a higher content of D. > 1.006 tocopherol up to the peak level at 10 hours, as compared to the young healthy persons.

The peak level of tocopherol in the light particles (D. < 1.006) was also reached at 10 hours in both groups. The mean increase between 4 and 10 hours was, however, considerably smaller than that of tocopherol in D. > 1.006 particles. Moreover, the tocopherol content of the lighter particles decreased between 4 and 6 hours in the majority of healthy persons. During the same interval the increase in tocopherol in the D. < 1.006 particles was the least apparent also in coronary population. Again, at each time the coronary group showed a higher mean content of tocopherol in the lighter particles than the healthy persons. The higher mean content of tocopherol in these particles observed up to 6 hours in the young healthy persons as compared to the older healthy persons was exceeded by old healthy persons at the peak level.

After the peak level, D. > 1.006 tocopherol showed in both groups a somewhat more rapid rate of decrease than the lighter particles. The rates of decrease of tocopherol in both particles was almost identical in the coronary and the healthy groups. At 24 hours none of the fractions had as yet declined to the initial level, and the tocopherol content of both fractions was higher in the coronary group than in the healthy group (D. < 1.006 $p < 0.001$, D. > 1.006 $p < 0.01$). The rate of decrease of the particulate tocopherol was almost identical in both healthy age groups. The tocopherol content of both fractions at 24 hours was somewhat higher in the older healthy persons as compared to the younger group.

Tocopherol Content of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 Particles

Fasting Samples. — The mean tocopherol concentration in all subfractions with a density above 1.006 was higher in the coronary patients than in the healthy subjects. However, the difference in the high density particles (D. > 1.063) was insignificant. Particles D. 1.019—1.063 appeared to have the highest tocopherol content. In coronary patients, on the other hand, the tocopherol content of D. 1.006—1.019 was only slightly lower than that of D. 1.019—1.063. In both groups the high-density lipoproteins contained the least tocopherol.

Samples Taken 4, 10, and 24 Hours after the Ingestion of Tocopherol. —

During the first hours the tocopherol content appeared to increase in all the fractions, the most in the D. 1.006—1.019 fraction. *At 4 hours* the coronary group had a higher mean tocopherol content in D. 1.006—1.019 and D. 1.019—1.063 particles as compared to the healthy group, while the particles of highest density had an equal tocopherol content in the two groups due to a higher mean increase from the basal level in the healthy subjects. The peak level of tocopherol in the particle classes D. 1.006—1.019 and D. 1.019—1.063 was attained at 10 hours and was higher in coronary patients than in healthy subjects. From 4 to 10 hours the coronary group showed a higher mean increase of tocopherol in D. 1.006—1.019 particles as compared to that in D. 1.019—1.063 particles, while the situation was the opposite in healthy persons. The differences within the two groups were small, however.

After 10 hours D. 1.006—1.019 tocopherol in both groups showed the most rapid decrease, the rate being slightly greater in the coronary than in the healthy group. Only a slight decrease of the tocopherol content of D. 1.019—1.063 occurred between 10 and 24 hours in coronary patients, while the healthy persons showed a definite decrease. A difference between the two

TABLE 12. Tocopherol content (mg/L) of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 particles in sera of healthy persons and survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat). Data corrected to 100 per cent recovery of tocopherol content of D. > 1.006 fraction.

D. 1.006—1.019

Subjects	Fasting			4 hours			10 hours			24 hours		
	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range
Healthy	5	3.4	2.6— 5.6	7	5.0	4.2— 6.7	7	11.3	3.8—22.0	7	5.3	3.8— 9.6
Coronary	6	7.6	4.7—11.8	8	9.2	6.9—11.6	6	20.4	12.2—30.9	6	13.6	8.8—18.4

D. 1.019—1.063

Subjects	Fasting			4 hours			10 hours			24 hours		
	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range
Healthy	5	5.3	2.8— 7.0	7	6.2	2.4— 9.8	7	13.3	7.3—23.8	7	10.5	5.7—16.7
Coronary	6	8.1	6.2—12.8	8	8.5	1.0—11.9	6	18.5	10.8—33.6	6	17.6	9.6—31.6

D. > 1.063

Subjects	Fasting			4 hours			10 hours			24 hours		
	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range
Healthy	5	1.5	1.4— 2.8	7	2.4	0.8— 5.7	7	3.1	2.5— 5.0	7	3.1	1.8— 6.1
Coronary	6	1.8	1.5— 2.9	8	2.4	1.6— 4.6	6	3.4	0.5— 6.4	6	4.5	0.4— 9.9

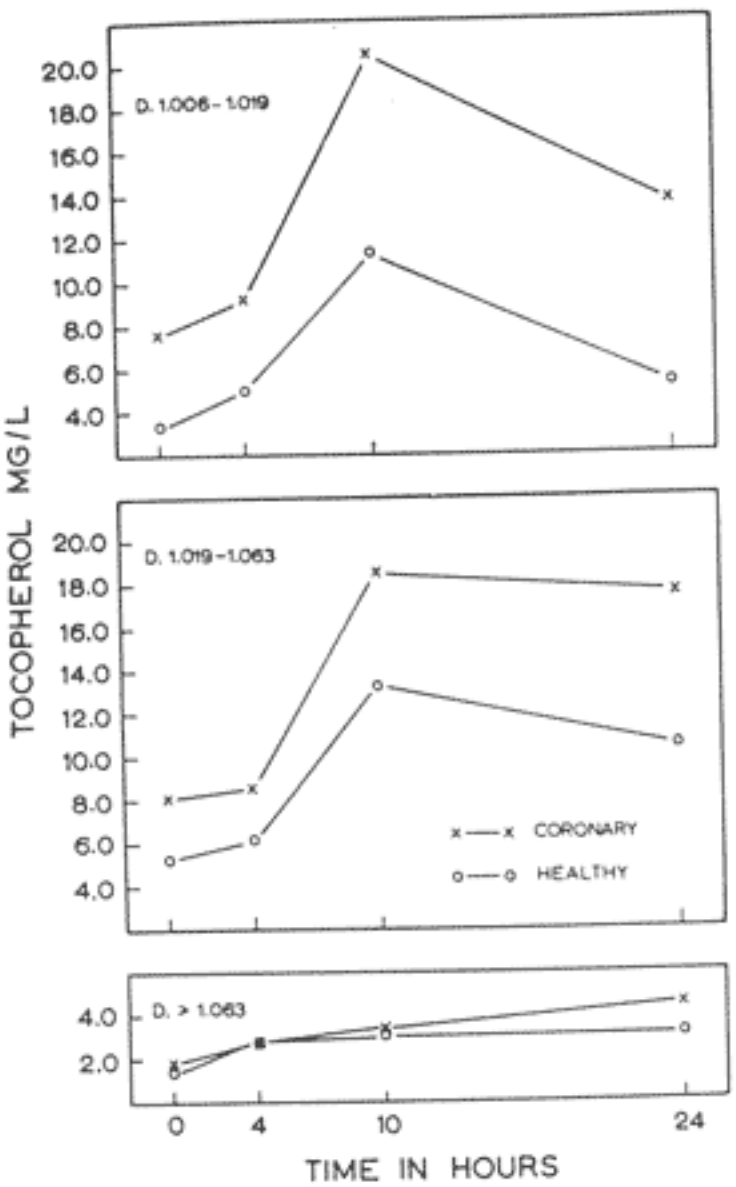

Fig. 22. Tocopherol content of D. 1.006—1.019, D. 1.019—1.063 and D. 1.063 particles in sera of 7 healthy persons and 8 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat).

groups was also obtained in this late phase with respect to the high-density lipoprotein-bound tocopherol. Coronary patients showed a slight increase, while the concentration in healthy subjects was unchanged. At 24 hours the content of tocopherol had not yet declined to the basal level in any of the particles. All the particles showed a higher mean content in coronary patients than in healthy subjects. At this time, fraction D. 1.019—1.063 in

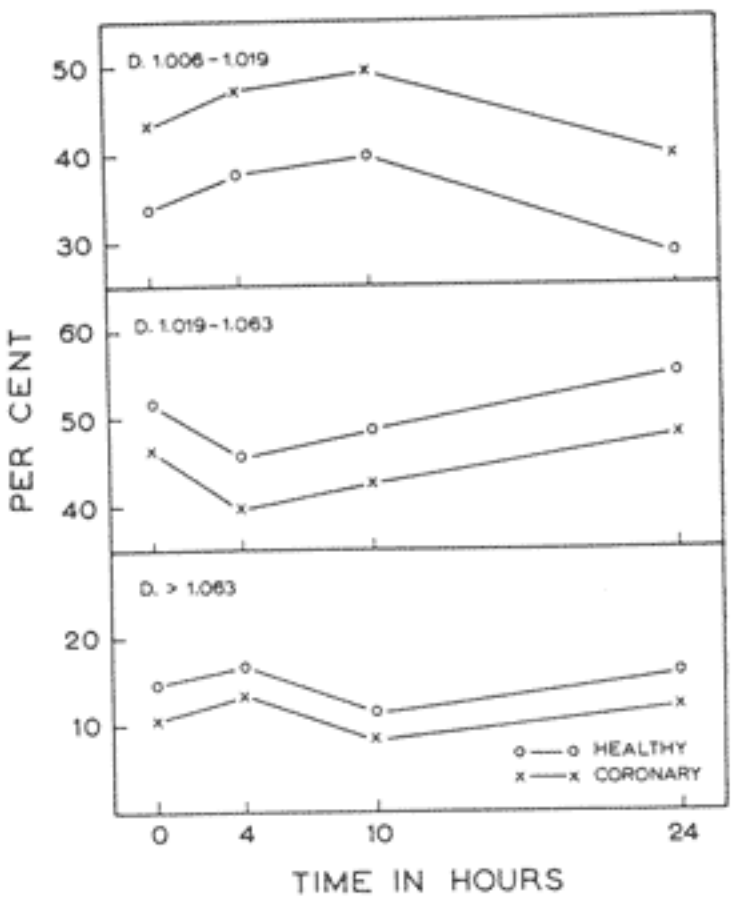

Fig. 23. Tocopherol content of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 particles as per cent of D. > 1.006 fraction in sera of 7 healthy persons and 8 survivors of myocardial infarction after ingestion of 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat).

both groups again had the highest tocopherol content, as was the case in healthy group at all times, while the coronary patients showed more tocopherol in the D. 1.006—1.019 particles than in D. 1.019—1.063 at 4 and 10 hours. The »percentile distribution curves» of the two groups ran fairly parallel from 0 to 24 hours, with small divergences in the late phase.

These few observations suggest that in the very early phase up to 4 hours most of the newly absorbed tocopherol was carried in the healthy group by the D. < 1.006 and D. 1.006—1.019 particles. Later, however, a large proportion of the newly absorbed toco-

pherol was found in the D. 1.019—1.063 particles, but an almost equal amount was present in the D. 1.006—1.019 particles. The high density lipoproteins were quantitatively less important. The disappearance rates of tocopherol from particles with a density of over 1.006 tended to decrease with increasing density.

In the coronary group, on the other hand, slightly more of the newly absorbed tocopherol was found, after the early phase was passed, in the D. 1.006—1.019 particles than in the D. 1.019—1.063 particles. The disappearance rates of tocopherol from D. 1.019—1.063 and D. > 1.063 particles appeared to be slower than in healthy group.

VITAMIN A LOADING TEST

The vitamin A loading test was performed in 34 survivors of myocardial infarction (mean age 51 years) and in 36 healthy persons (mean age 29 years). Fifteen subjects in the latter group were ambulant young medical students. The coronary group included 2 females and the healthy group 5 females. Both groups were divided according to age into two subgroups, the age borderline being 50 years in the coronary group and 35 years in the healthy group. The mean ages in the coronary age groups were 43 and 58 years, and in the healthy age groups 24 and 43 years, respectively.

The complete test comprised 7 blood samples drawn at 0, 2, 3, 4, 6, 10, and 24 hours, but all the samples were not analyzed in each test. The fasting and 24-hour samples were analyzed in the medical students only.

The results are given in table 13, expressed as mean plasma vitamin A concentrations with corresponding standard deviations. Fig. 24 presents the individual data in the fasting state and at 10 and 24 hours. The concentration curves for the two main groups are shown in fig. 25.

The serum cholesterol was determined in all but one subject and the serum triglyceride in 65 of all 70 subjects who underwent the vitamin A loading test. These measurements revealed among coronary patients hypercholesterolemia in 65 per cent and hypertriglyceridemia in 69 per cent. Only 4 persons in the healthy group showed hypercholesterolemia and 4 had hypertriglyceridemia.

Fasting Samples. — The older healthy persons showed a higher mean content of plasma vitamin A than the younger ones, but the difference was not significant. In the coronary group the mean values for both age groups were almost identical.

The mean vitamin A content was significantly higher ($p < 0.001$) in the coronary group than in the healthy group. Here, again, the age distribution was different in the two groups. However, the difference also was signifi-

cant when the older healthy group and the younger coronary group are compared ($p < 0.02$). Overlapping of the individual values in the two groups of subjects was considerable. Not less than 45 per cent of coronary patients fell below the mean content + 1 S.D. of the healthy subjects, and the same proportion of healthy subjects exceeded the mean value —1 S.D. of the coronary group. The 90 per cent upper limit of the young healthy subjects was 156 I.U./100 ml and was exceeded by 65 per cent of the coronary patients. No significant correlation was found between the plasma vitamin levels and serum triglyceride or cholesterol levels.

Samples at 2, 3, and 4 hours. — All the subjects showed a continuous rise of the plasma vitamin A content from 0 to 4 hours. At each time the young healthy subjects had a higher mean content than the older healthy persons, the difference being the most marked at 3 hours. In the coronary group, on the other hand, the younger patients had a higher mean content than the older patients only at 2 hours, the difference was negligible at 3 hours, while at 4 hours the older patient group had a higher mean level. Comparison of the two main groups revealed that the increase from the basal level to 4 hours was on an average higher in the healthy group than in the coronary group. In spite of their lower initial mean content, the healthy subjects showed markedly higher

TABLE 13. Plasma vitamin A level (I.U./100 ml) in healthy persons and survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat).

Subjects	Fasting N.	Fasting Mean	Fasting S.D.	2 hours N.	2 hours Mean	2 hours S.D.	3 hours N.	3 hours Mean	3 hours S.D.	4 hours N.	4 hours Mean	4 hours S.D.
Healthy												
age below 35	26	112	42	9	877	744	9	2064	1553	9	3123	2002
age over 35	10	131	45	8	869	577	8	1694	1041	9	3018	1114
total	36	117	43	17	873	650	17	1890	1310	18	3071	1572
Coronary												
age below 50	14	171	54	10	685	542	10	1476	1148	11	2382	1379
age over 50	20	165	45	19	510	433	19	1539	1400	18	2737	1844
total	34	167	48	29	570	471	29	1517	1298	29	2603	1666

Subjects	6 hours N.	6 hours Mean	6 hours S.D.	10 hours N.	10 hours Mean	10 hours S.D.	24 hours N.	24 hours Mean	24 hours S.D.
Healthy									
age below 35 ..	9	3378	2355	10	1564	949	25	265	130
age over 35 ..	9	2977	1399	10	1867	671	10	399	134
total	18	3177	1890	20	1716	864	35	303	143
Coronary									
age below 50 ..	13	4828	1621	15	5248	3589	13	1134	1087
age over 50 ..	19	4652	2059	18	3898	2271	16	739	377
total	32	4724	1867	33	4511	2973	29	917	789

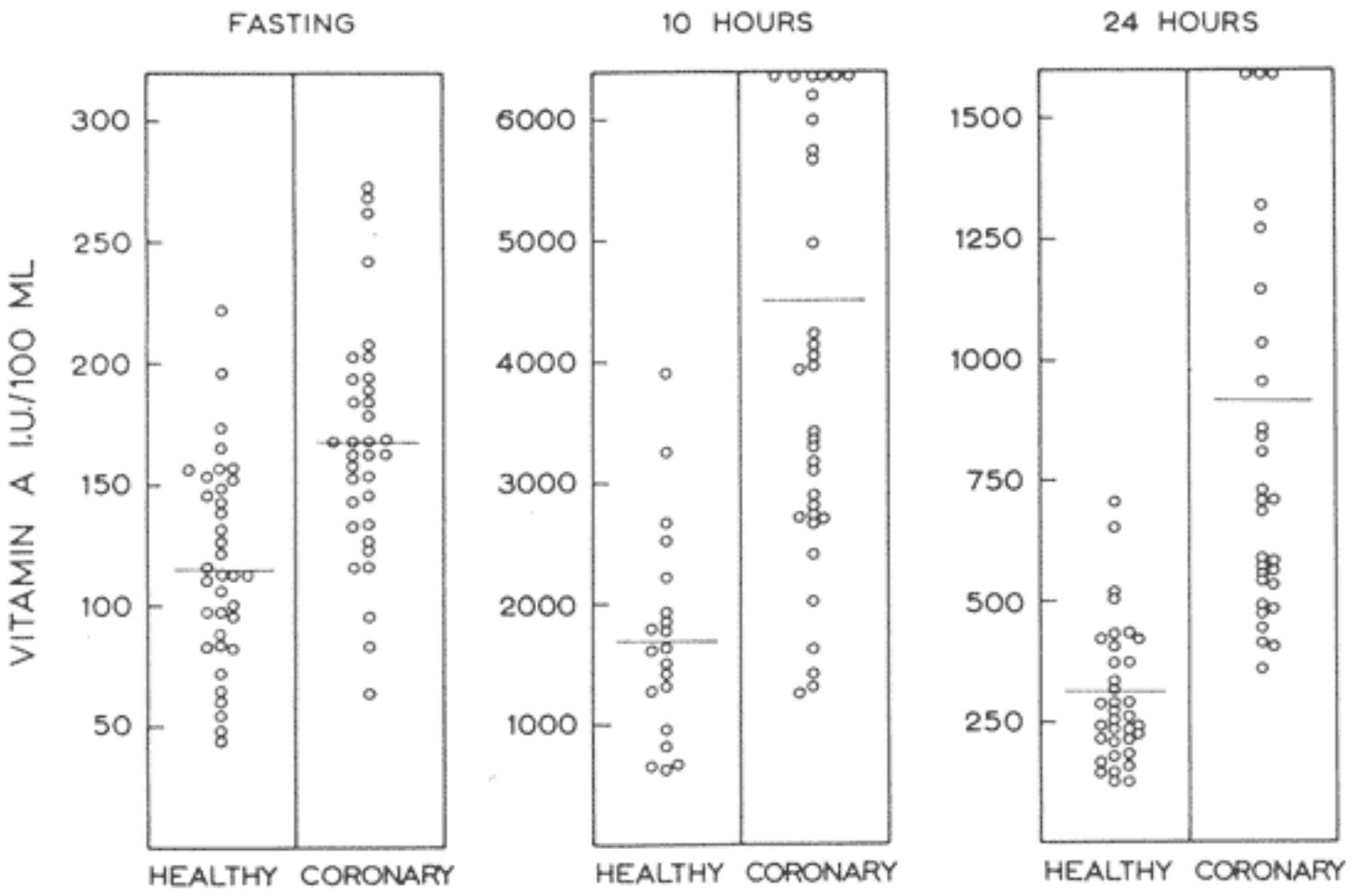

Fig. 24. Plasma vitamin A level in healthy persons and survivors of myocardial infarction at fasting stage and 10 hours and 24 hours after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat). Horizontal lines = mean vitamin A levels.

mean values at 4 hours. In 6 healthy subjects and in 2 coronary patients the 4-hour level was the peak level. A marked overlapping of the values between the two groups was present each time.

Samples at 6 Hours. — All but 2 healthy subjects reached the peak level in 6 hours. In the coronary group, on the other hand, 17 of the 28 patients (61 per cent) from whom a sufficient number of samples was examined to estimate the time of the peak level had it at 6 hours. However, the mean peak level in both groups occurred at 6 hours. Of the healthy subjects the younger category still showed a higher mean level. The young coronary patients also showed a slightly higher mean level than the older ones. At 6 hours the mean level of the coronary group was above that of the healthy group ($p < 0.01$), though overlapping of the individual values was great.

Samples at 10 Hours. — Only two subjects in the healthy group showed a »delayed peaking» at 10 hours. They both had a normal fasting serum lipid pattern, including the vitamin A level. »Delayed peaking» occurred in the coronary group in 39 per cent. Only one of these patients was normocholesterolemic, three had normal triglyceride values, and not less than 7 of these 11 patients had a normal fasting vitamin A content.

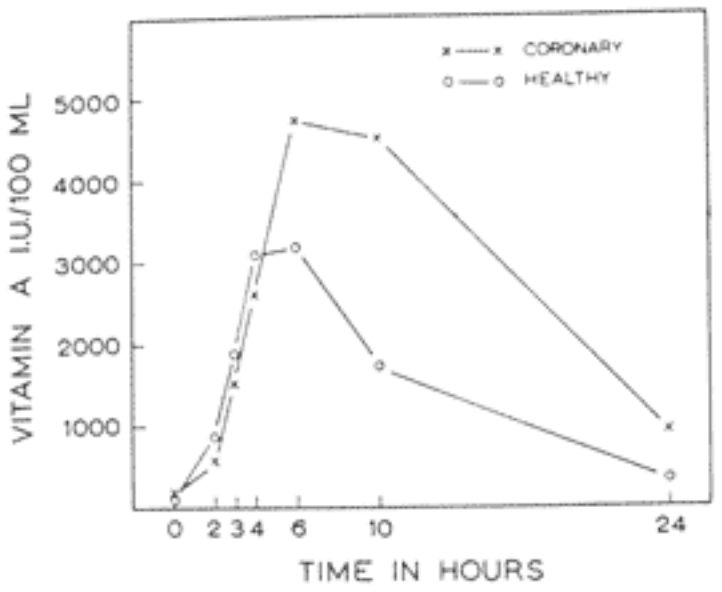

Fig. 25. Plasma vitamin A level in 36 healthy persons and 34 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat).

At 10 hours the older healthy persons showed, for the first time after the load, a higher mean level than the younger ones. The young coronary patients, on the contrary, had a markedly higher mean content than the old patients. The coronary group had a significantly higher mean level ($p < 0.001$) than the healthy group and overlapping was no longer as marked as earlier, as seen in the fig. 24.

Samples at 24 Hours. — None of the subjects had reached the basal vitamin A level at 24 hours, but in all subjects the values were lower than at 10 hours. The difference in the mean levels of the two age categories of the healthy group was marked. In none of the older healthy subjects was the circulating vitamin A content at 24 hours below the mean level of the younger healthy subjects. The medical students showed a lower mean content (221 I.U./100 ml) than the hospitalized young healthy group (329 I.U./100 ml). The younger coronary group, on contrary, had a markedly higher mean level than the older coronary group.

The plasma vitamin A retention at 24 hours was, on an average, higher in coronary patients than in healthy subjects ($p < 0.001$). A significant difference ($p < 0.02$) existed also between the older healthy and the younger coronary groups. As seen in fig. 24, the overlapping of individual values of the two main groups was, however, still present.

As was done in the tocopherol loading test, the 90 per cent upper limit of the vitamin A level at 24 hours in young healthy persons was calculated and was found to be 450 I.U./100 ml. This level was exceeded by 2 subjects in the older healthy group. A lower value at 24 hours was seen in only 3 patients in the older coronary group and in none of the younger patients. One of these normally »clearing» patients had a normal fasting lipid pattern, another showed only hypertriglyceridemia, and the third had hypervitaminosis A only.

When the fasting plasma vitamin A levels and the 24-hour vitamin A levels were compared in each group, the only significant correlation was found in the healthy group ($p < 0.01$), the correlation coefficient being 0.53 (fig. 26). Serum basal triglyceride levels were not in significant correlation with the plasma 24-hour vitamin A levels, but a significant correlation was found between serum basal cholesterol and the plasma 24-hour vitamin A levels

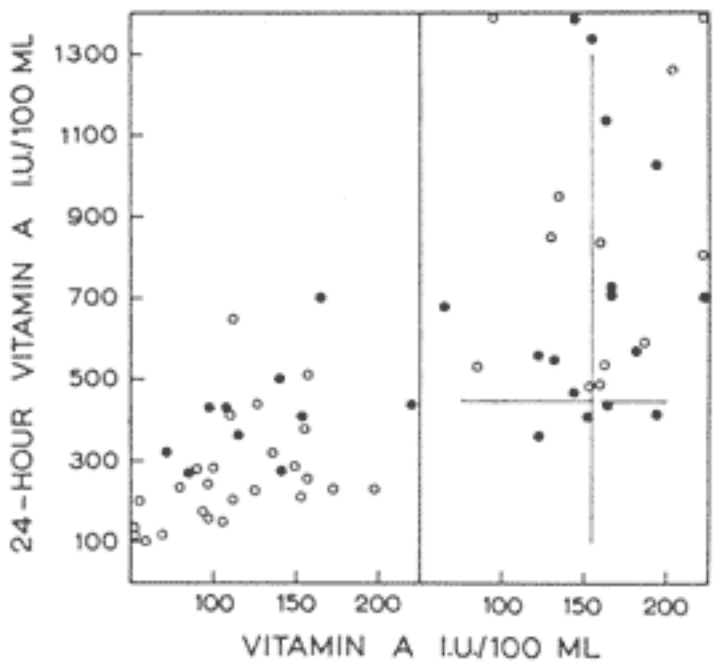

Fig. 26. Relationship of 24-hour plasma vitamin A and fasting vitamin A levels in (left) healthy persons and (right) 28 survivors of myocardial infarction. Healthy: Open dots = age below 35, black dots = age over 35. Coronary: Open dots = age below 50, black dots = age over 50. Lines = upper normal limits.

in the coronary group ($p < 0.001$) and in the healthy group ($p < 0.001$). The correlation coefficients were 0.63 and 0.55, respectively.

Thus the coronary and healthy groups handled the ingested vitamin A differently in many respects. It is, however, once again pointed out that these two groups were not comparable with respect to age. The older healthy group (age over 35 years) and the younger coronary group (age below 50 years) had, however, an equal mean age and are best suited for comparison.

The healthy subjects showed a faster initial rise in the plasma vitamin A level. At 6 hours, but not before, the coronary patients had a higher mean plasma vitamin A content than the controls and thereafter the difference between the two groups became more apparent. The occurrence of the peak level varied from 4 to 10 hours. Regardless of the time, the mean content at peak level in healthy subjects was 3466 I.U./100 ml (S.D. 1787), the mean of 3558 I.U./100 ml (S.D. 2403) in the younger healthy persons being higher than the 3385 I.U./100 ml (S.D. 1147) seen in the older healthy group. The coronary patients had a significantly higher ($p < 0.001$) plasma vitamin A peak content, 5899 I.U./100 ml (S.D. 2789), than the healthy group. The younger coronary patients had higher peak level, 6665 I.U./100 ml (S.D. 3189), than the older coronary patients, whose peak was 5324 I.U./100 ml (S.D. 2382).

On the whole, the healthy subjects reached the peak level earlier or, in other words, »delayed peaking» was more common in the coronary group. The peak level was attained at 10 hours by 11 of the 28 coronary patients but by only 2 of the 17 healthy subjects.

In 5 coronary subjects, all exhibiting a marked hypercholesterolemia and hypertriglyceridemia, both the vitamin A and the tocopherol loading tests were done. In both tests all the 5 subjects responded abnormally showing a high vitamin level at 24 hours. In addition, 4 of these subjects showed a »delayed peaking» in the vitamin A loading test.

Only 1 of the 8 patients who did not recieve oral anticoagulants had a normal 24-hour plasma vitamin A level, while the others showed an abnormally high vitamin A retention at 24 hours.

VITAMIN A IN PLASMA LIPOPROTEINS DURING THE LOADING TEST

Vitamin A was determined in five lipoprotein fractions which were isolated from plasma by two successive ultracentrifugal runs. In the primary separation, fractions D. < 1.006 and D. > 1.006 were obtained. This primary fractionation was performed from samples taken 4, 6, 10 and 24 hours after ingestion of the vitamin in 12 survivors of myocardial infarction (mean age 52 years) and in 9 healthy men (mean age 33 years) . One woman was included in the coronary group. The D. > 1.006 fraction of 9 coronary patients (mean age 54 years) and of the 9 healthy men were separated further and in this secondary fractionation the D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 fractions were obtained. The data are listed in tables 14 and 15 as mean values with standard deviations or ranges. Figs. 27 and 29 show the concentration curves for vitamin A in the different lipoproteins during the loading test. The percentile distribution of vitamin A in lipoproteins is presented in figs. 28 and 30. Because of technical difficulties the number of samples studied varied at each time, as seen in the tables.

The serum cholesterol and triglyceride were studied in all the subjects. These measurements revealed that all but four of the coronary patients had hypercholesterolemia. Two of these 4 had also a normal triglyceride value. In addition 2 other patients showed a normal triglyceride content. Only 2 patients in the coronary group showed normal »clearing» at 24 hours.

Vitamin A Content of D. < 1.006 and D. > 1.006 Particles

At 4 hours slightly more vitamin A (V.-A) was found in D. < 1.006 than in D. > 1.006 particles. The V.-A content of both particles was higher, on the

TABLE 14. Vitamin A content (I.U./100 ml) of D. < 1.006 and D. > 1.006 particles in sera of healthy persons and survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat). Data corrected to 100 per cent recovery of total plasma content.

	D. < 1.006											
Subjects	4 hours			6 hours			10 hours			24 hours		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Healthy	9	1385	895	8	1207	748	8	749	461	9	60	43
Coronary	12	1725	1032	12	3014	1417	10	2794	2412	11	287	279

	D. > 1.006											
Subjects	4 hours			6 hours			10 hours			24 hours		
	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.	N.	Mean	S.D.
Healthy	9	1029	536	8	1585	700	8	1127	351	9	439	460
Coronary	12	1212	915	12	2129	691	10	2706	1979	11	871	846

average, in coronary patients than in healthy subjects, but the distribution pattern was equal in both groups. Thus, contrary to the findings in the whole V.-A loading series, presented above, this small coronary group showed already at 4 hours a higher mean total content of V.-A than the healthy group.

The difference in the average total plasma vitamin A in the healthy and the coronary groups (calculated from table 14) became more apparent at the time interval from *4 to 6 hours*. This was chiefly due to vitamin transported in the light particles (D. < 1.006), which showed a sharp increase up to 6 hours in the coronary group, while in healthy persons the peak level was reached already at 4 hours. V.-A in the D. > 1.006 particles showed a definite increase from 4 to 6 hours in both groups, the rise being more marked in the coronary group. Accordingly, as seen in fig. 28, the percentile distribution of vitamin A showed a considerable change towards the D. > 1.006 particles in the healthy group.

The mean peak level of plasma total vitamin A occurred in the healthy group *at 6 hours* and in the coronary group 4 hours later. This »delayed peaking» is reflected nicely in the lipoprotein concentration curves (fig. 27). Thus, V.-A in fraction D. > 1.006 in the coronary group showed on an average a marked increase up to 10 hours, while in healthy persons V.-A in this fraction was then already continuously decreasing after the peak level had been attained at 6 hours. The vitamin in the light particles showed a decrease

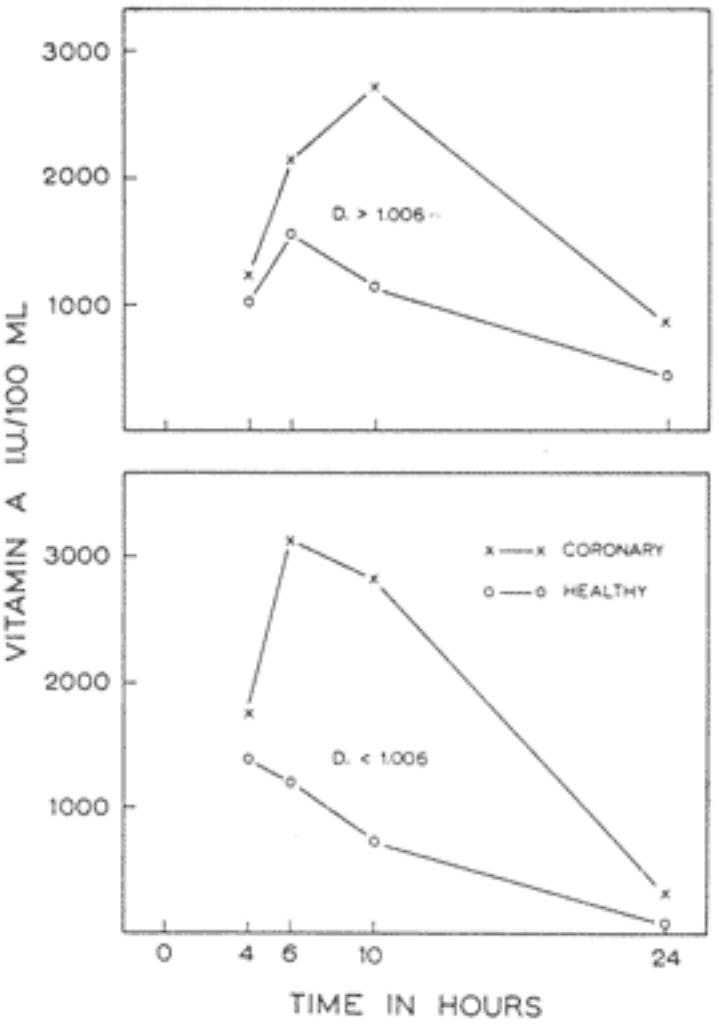

Fig. 27. Vitamin A content of D. < 1.006 and D. > 1.006 particles in 9 healthy persons and 12 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat).

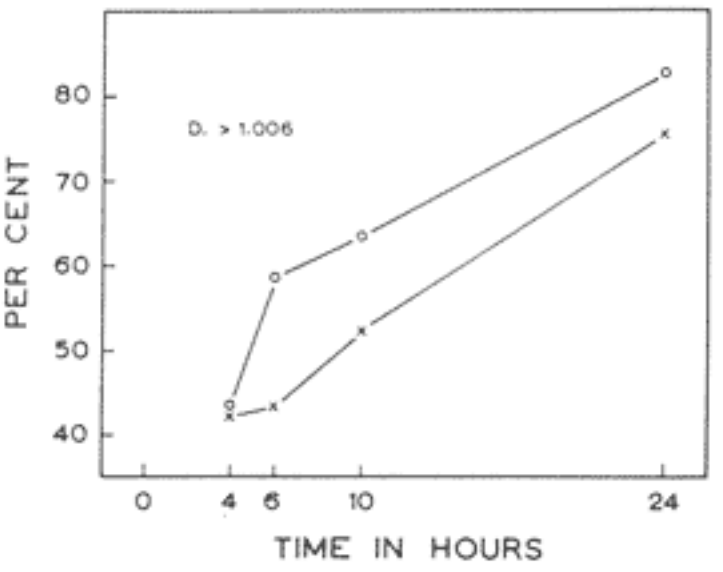

Fig. 28. Vitamin A content of D. > 1.006 particles as per cent of total plasma content in 9 healthy persons and 12 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat).
o = healthy, x = coronary.

in both groups after 6 hours, the disappearance rate being somewhat faster after 10 hours in the coronary group.

After 6 hours the percentile distribution of vitamin A in these two fractions (fig. 28) changed with time towards D. > 1.006 particles at an approximately equal rate, the ratio of V.-A in D. > 1.006 particles to V.-A in total plasma being somewhat lower in the coronary group than in the healthy group.

Vitamin A Content of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 Particles

These data, obtained from the secondary lipoprotein separation, do not cover all the samples that underwent the primary separation. Therefore the mean data and lipoprotein concentration curves obtained from the primary separation (D. < 1.006 and D. > 1.006) are not comparable to the data from this second separation.

From 4 to 6 hours the vitamin showed an increase in all the particles, with the exception of V.-A in D. > 1.063 particles in healthy subjects, who maintained the same content as at 4 hours. Most marked was the increase in the D. 1.006—1.019 fraction, where also the major difference between the healthy and coronary groups existed. The vitamin A content in D. 1.019—1.063 at 6 hours was nearly similar in

TABLE 15. Vitamin A content (I.U./100 ml) of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 particles in sera of healthy persons and survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat). Data corrected to 100 per cent recovery of tocopherol content of D. > 1.006 fraction.

D. 1.006—1.019

Subjects	4 hours			6 hours			10 hours			24 hours		
	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range
Healthy	6	474	128— 952	7	963	344—2082	8	699	175—1346	5	78	52—114
Coronary	6	716	179—1342	6	1673	931—2520	6	1515	563—3051	6	260	50—499

D. 1.019—1.063

Subjects	4 hours			6 hours			10 hours			24 hours		
	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range
Healthy	6	198	44— 408	7	359	127— 750	8	207	73— 360	5	57	36— 81
Coronary	6	222	11— 640	6	343	195— 491	6	270	124— 569	6	243	117—456

D. > 1.063

Subjects	4 hours			6 hours			10 hours			24 hours		
	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range	N.	Mean	Range
Healthy	6	295	172— 511	7	292	192— 489	8	191	73— 298	5	104	57—142
Coronary	6	199	126— 253	6	272	120— 445	6	262	119— 474	6	134	80—218

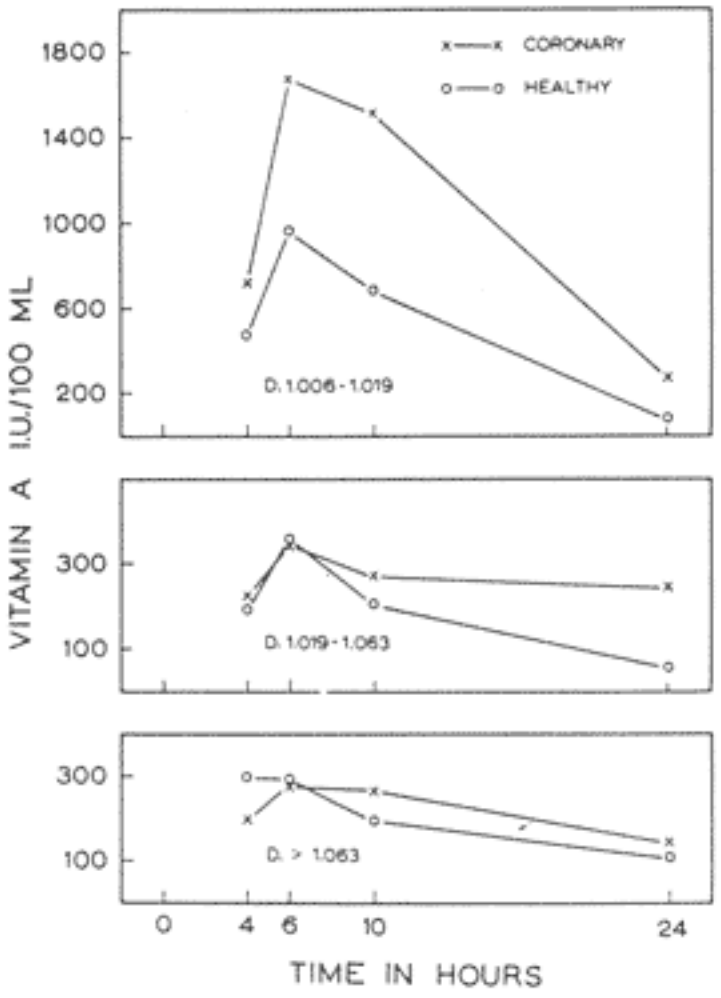

Fig. 29. Vitamin A content of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 particles in sera of 9 healthy persons and 9 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat).

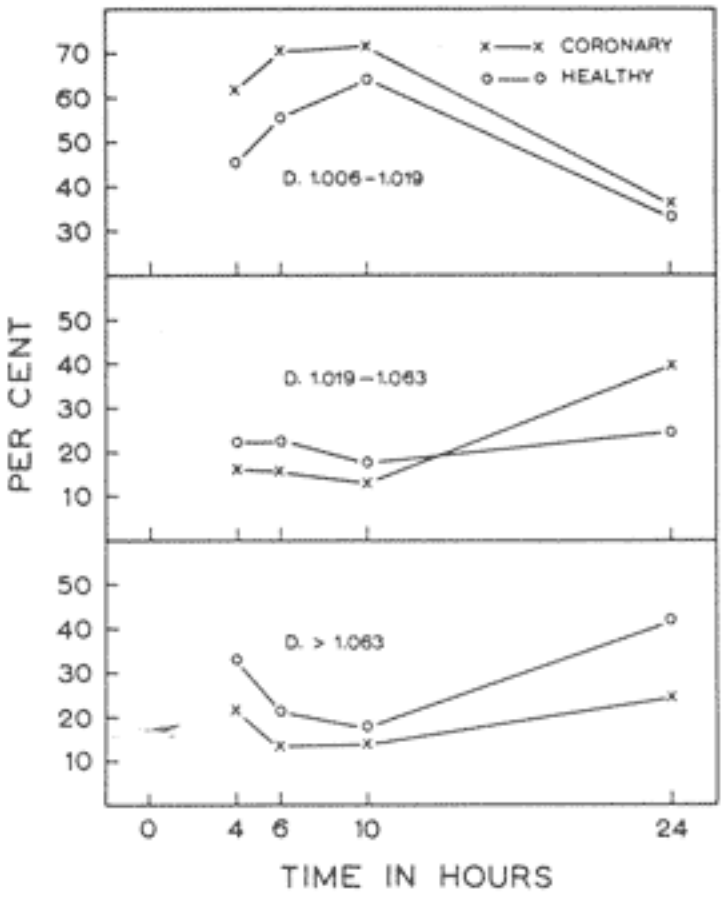

Fig. 30. Vitamin A content of D. 1.006—1.019, D. 1.019—1.063 and D. > 1.063 particles as per cent of D. > 1.006 fraction in sera of 9 healthy persons and 9 survivors of myocardial infarction after ingestion of 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat).

both groups; this was the case also with V.-A in the high density lipoproteins.

After 6 hours the content of V.-A in particles D. 1.006—1.019 began to diminish in both groups, the disappearance rate in the late phase being faster in coronary patients than in healthy subjects. The disappearance rate of V.-A from D. 1.019—1.063 particles, on the contrary, was markedly slower in the coronary group than in the healthy group and slower also in comparison to the D. 1.006—1.019 particles. After 6 hours the rate of disappearance of V.-A from D. > 1.063 particles was faster in the healthy group than in the coronary group. Thus the V.-A content of D. > 1.063 particles in the healthy group was exceeded by coronary patients at 10 hours, after which the coronary patients showed a faster disappearance rate than the healthy group.

The percentile distribution of vitamin A in the different lipoproteins showed that fraction D. 1.006—1.019 initially dominated in both groups. However, the highest V.-A content at 24 hours in the healthy group was

present in the high density lipoproteins. In the coronary group, on the other hand, the contents in D. 1.006—1.019 and D. 1.019—1.063 at 24 hours were almost identical and greater than in the high density lipoproteins.

The percentile distribution curves of the D. 1.006—1.019 particles had a parallel course in both groups. A »crossing-over» occurred in the percentile distribution curves of D. 1.019 —1.063, showing a dominance of this fraction at a later phase in the coronary group as compared to the healthy group. Simultaneously with this, the percentile distribution curves of the D. > 1.063 fraction diverged, indicating a higher proportion of this fraction in the healthy group.

These data are in many respects inadequate and do not have the weight of statistically corroborated evidence. Thus it is not possible on the basis of these few observations to demonstrate the kinetics of vitamin A lipoprotein, nor the presence of a possible defect in this respect in coronary heart disease. However, some tentative conclusions may be justified. These data suggest that the newly absorbed vitamin A is carried primarily in the particles D. < 1.006 and D. 1.006—1.019. The major difference between the coronary and control groups existed initially in the two fractions of lowest density (D. < 1.006 and D. 1.006—1.019). In the later phase, however, the kinetics of vitamin A in the more dense fractions were dissimilar. The disappearance rate from particles D. 1.019—1.063 was slower in the coronary group, and the percentile increase of vitamin A in the D. > 1.063 fraction was higher in the healthy group. At 24 hour the latter fraction contained most of the vitamin in the healthy group.

DISCUSSION

SERUM BASAL LIPID LEVELS

There is probably good reason to assume that the blood donors represent in regard to the plasma lipid pattern a fairly unselected Finnish urban population. The samples for the cholesterol and tocopherol determinations of these subjects were taken in the non-fasting state. The plasma tocopherol level, however, is affected neither by an ordinary meal nor by a fat load, as was demonstrated in the present study and by other investigators (McCormick *et al.* 1960). Only a slight elevation of the serum total cholesterol has been observed after a fat meal (Havel 1957). No significant difference was found in the serum total cholesterol and tocopherol levels of blood donors and healthy subjects of the same age studied in the fasting state.

All the coronary patients had electrocardiographic evidence of a recent and/or old myocardial infarction. Most of the blood samples of coronary patients were taken in the fifth week after the acute attack of myocardial infarction or angina pectoris. Therefore, it cannot be concluded whether or not the high plasma lipid level found in the coronary patients existed also prior to the acute event. However, if the changes in the serum cholesterol values occurring at the acute stage of myocardial infarction are omitted, no essential differences in this respect have been found before and after the event (Gordon *et al.* 1959, Page and Lewis 1959).

The serum cholesterol level has been observed to decrease at the acute stage of myocardial infarction by many authors (Welin 1948, Björck *et al.* 1957, Dodds and Mills 1957, Pomerantz 1962) and the present results confirm this. A similar trend has been reported in the serum triglyceride level (Hauss and Böhle 1955, Albrink *et al.* 1961). In the present investigation, however, no significant fall was found in the serum triglyceride level.

A decrease of serum lipids has also been observed after a surgical operation (Man *et al.* 1946). In many respects the postinfarctive and postoperative states are analogous, with tissue destruction, fever and leukocytosis. The similar behavior of the serum cholesterol and tocopherol levels observed in the present study — the latter being purely an exogenous lipid — makes a defect in the lipid synthesis unlikely as a cause of the fall of the

serum lipid. The changes are probably too acute to be due to a poor intake, and thus the best explanation seems to be the increased catabolism or excretion of these lipids, as has been suggested by Pomerantz (1962).

The present coronary population showed, on the average, a significantly higher serum content of all the four lipids — total cholesterol, triglyceride, tocopherol and vitamin A — than the control population. The elevation of serum cholesterol, triglyceride and tocopherol in the coronary series was more marked in the younger persons and there appeared to be an almost continuous decrease in the plasma level of these three lipids with increasing age of the population from the 4th to the 7th decade. However, when the coronary series was divided into two age groups aged below and over 50 years, no significant difference was found in the lipid levels in these groups. This may be explained by a marked decline in the lipid values after the age of 40 years. The vitamin A series was too small to permit a study of the possible age trend, but also here the two age groups showed similar mean vitamin A values.

The control subjects, on the other hand, showed an opposite age trend. A continuous increase of the serum cholesterol and tocopherol levels occurred up to the 7th decade, while the triglyceride and vitamin A series were too small to allow such treatment. When the control groups were divided into two age groups aged below and over 35 years, the younger group had a significantly lower mean content of all the lipids with the exception of vitamin A. Thus, with respect to the serum cholesterol and tocopherol values, which were analyzed from a sufficient number of samples, no difference between the coronary and control groups existed any longer in the 7th decade.

The influence of aging on the plasma lipid or lipoprotein levels is a matter of great importance. Evidently there exists a higher death rate among subjects with high lipid levels, which partly explains the diminution of the serum cholesterol level with aging in the coronary population. However, as Lawry *et al.* (1957) emphasized, there must be another more important mechanism responsible for this phenomenon. According to Albrink *et al.* (1961), a marked rise in the serum triglyceride level with aging occurs in healthy men in the thirties or fourties, preceding by about twenty years the peak incidence of coronary heart disease and thus supporting the hypothesis that a serum lipid elevation is the first detectable evidence of the slowly developing chronic process. Supporting this view it has been shown that the increase of serum cholesterol with aging is not an obligatory one, but that the cholesterol level increases only in some persons (Sperry and Webb 1950, Man and Peters 1953). The young coronary patients, who show a particularly high incidence of hyperlipidemias, may probably derive from a somewhat different population than the aged coronary patients (Björck *et al.* 1957). Among the old patients, on the other hand, the high lipid levels may

partly be due to the »physiological» rise of serum lipids with age, as is the case concerning the cholesterol content of the aorta. It has been shown by Faber (1946) that the cholesterol content of autopsied aortas increased with age in normocholesterolemic subjects but not in hypercholesterolemic subjects.

Serum Cholesterol Level. — The present observation of a higher mean serum total cholesterol level in persons who had had a myocardial infarction as compared to healthy persons of comparable age has also been reported by numerous authors, as was stated in the review of the literature. In longitudinal studies, also, the persons who developed coronary heart disease during the time of observation have been reported to exhibit a higher serum cholesterol level than the base population (Gofman *et al.* 1956, Kannel *et al.* 1961).

The increase in the serum cholesterol level with aging up to the 7th decade in the normal population found in this study has been observed in population studies by numerous investigators (Gertler *et al.* 1950 b, Keys *et al.* 1950, Jones *et al.* 1951, Nikkilä 1955, Lawry *et al.* 1957, Lewis *et al.* 1957), but not by all (Little *et al.* 1956, Oliver and Boyd 1956).

Serum Triglyceride Level. — The incidence of hypertriglyceridemia in the coronary population in the present study was somewhat higher than that of the other hyperlipidemias. This is in accord with the data in the literature (Hauss and Böhle 1955, Schrade *et al.* 1959, 1960, 1961, Antonis and Bersohn 1960, Albrink *et al.* 1961, Berkowitz and Croll 1962). On the other hand, in a recent paper by Nikkilä and Pelkonen (1963) the incidence of hypertriglyceridemia in a somewhat different Finnish coronary population was lower than in the present study. However, a large proportion of that coronary population showed serum triglyceride values at the upper limit of the normal range (120—140 mg/100 ml), the normal maximum being 140 mg/100 ml.

The present data concerning the influence of age on the serum triglyceride level in the normal population is also in agreement with earlier reports (Hauss and Böhle 1955, Schrade *et al.* 1960, Antonis and Bersohn 1960, Carlson 1960 a, Albrink *et al.* 1961, Cramér 1962). In the coronary population, on the other hand, Carlson (1960 b) stated that the metabolism of triglyceride was more often disturbed in men below the age of 50 years than after this age, while the situation was the opposite in the study of Albrink *et al.* (1961). In the present study the highest mean level was observed at 30 to 39 years of age, but the mean levels in the age groups below and over 50 years were similar. Accordingly, the incidence of hypertriglyceridemia was almost equally high in the two age groups, being 70 per cent in the younger and 65 in the older group, if the normal limits are defined according to the distribution of values in the young population.

Plasma Tocopherol Level. — In the present study the mean plasma tocopherol level in the blood donors, repre-

senting broadly a Finnish »normal urban population», appeared to be lower than the earlier reported data from Holland (Engel 1949), Hungary (Kramer 1955), Italy (Rindi and Perry 1957), England (Leitner *et al.* 1960 b) and the United States (Harris *et al.* 1961), but of the same order of magnitude as that in the report of Postel (1956) also from the United States. The only data reported from Finland, 5.1 mg/L for women and 4.2 mg/L for men (Rauramo 1946), are considerably lower than the present averages.

The present observations concerning the age dependence of the plasma tocopherol content in the normal population and in women and men separately are in good accordance with the data reported earlier (Darby *et al.* 1949, Lemley *et al.* 1949, Chieffi and Kirk 1951, Leitner *et al.* 1960 b).

There is no data available on the plasma level of tocopherol in coronary heart disease. However, Vannotti and Gervasoni (1957) and McCormick and McCluer (1960) observed that tocopherol was present in human atheromas. The latter finding is interesting in the light of the present study, which definitely revealed a significantly higher mean plasma tocopherol level in patients with myocardial infarction than in control subjects. The presence of a high content of tocopherol in atheromas and in plasma in atherosclerosis is thus analogous to that of cholesterol, triglyceride and vitamin A.

The serum cholesterol and tocopherol levels appeared to be in close correlation of a high statistical significance. The possibility of a methodological error was excluded in this study, as it had been in the study of Postel (1956). A positive correlation between the serum cholesterol and tocopherol levels has been reported in diabetic patients (Bensley *et al.* 1950, Vanzetti *et al.* 1956) and in thyroid disorders (Postel 1956). In addition, high serum tocopherol levels have been observed to exist in hypercholesterolemic states (Darby *et al.* 1949). Darby and co-workers postulated that the simultaneous occurrence of hypercholesterolemia and hypertocopherolemia was due to »an increased lipid carrying power of serum». This means, in modern terminology, hyperlipoproteinemia, which, indeed, evidently exists in coronary heart disease. Furthermore, the distribution of cholesterol and tocopherol in the plasma lipoproteins is very similar (Bragdon *et al.* 1956, McCormick *et al.* 1960). Postel also considered it unlikely that the changes in the serum tocopherol content observed in thyroid disorders were determined by the serum cholesterol content. In his opinion the thyroid activity appears to dictate the rate of synthesis, degradation and excretion of cholesterol, while the regulation of serum tocopherol is determined primarly by the intake, absorption and rate of disposal without the component of synthesis. A common excretion pathway as well as a similar enterohepatic circulation of cholesterol and tocopherol have also been suggested by some authors (Klatskin and Molander 1952 a, Popper *et al.* 1949, Simon *et al.* 1956 a). With the exception of the endogenic synthesis, the metabolism of serum

tocopherol and cholesterol thus have so many similarities that the correlation between the serum levels does not seem to be unexpected.

A highly significant correlation between the serum triglyceride and tocopherol levels was found in the coronary group but not in the healthy group. According to the known lipid composition of lipoprotein, most of the triglyceride appears to be in particles D. < 1.019 (Bragdon *et al.* 1956). On the other hand, in the study of McCormick *et al.* (1960) the major part of the plasma tocopherol was in the Sf 3—9 particles and in the present study in the D. 1.019—1.063 particles. In coronary heart disease, the lighter beta-lipoproteins Sf 12—400 have been found to be elevated more than the Sf 0—12 lipoproteins (Gofman *et al.* 1954). According to the present observations, relatively more tocopherol was present in D. 1.006—1.019 particles in the coronary patients than in healthy persons. The significant correlation between the serum triglyceride and tocopherol levels in the coronary group may thus partly be due to a shift of tocopherol in the lipoprotein spectrum toward lighter particles, in analogy to the shift of cholesterol in the lipoprotein spectrum in hypertriglyceridemias (Albrink 1961).

Plasma Vitamin A Level. — The mean vitamin A level in healthy persons in the present study was lower than the earlier reported mean levels, this concerning also the data published from Finland (Saksela 1940, Pitkänen 1944). The data on the influence of age on the plasma vitamin A level are controversial. In the studies of Saksela (1940) and Vetter (1958) the plasma vitamin A level was not age-dependent, while Leitner *et al.* (1960 a) reported an increase with age. In the present study the older healthy individuals (age over 35 years) had a higher mean level than the younger ones, but the difference was not significant.

Carotenoids have been found to be present in human atheromas (Thomson 1934, Blankenhorn *et al.* 1956). A higher mean plasma vitamin A level has been seen in coronary patients than in control ones, supporting the present observation (Beaumont *et al.* 1958, Beaumont and Lenègre 1959). In various hyperlipidemic states hypercarotenemia and hypervitaminosis A have been frequently observed, as was shown in the review of the literature. Many theories have been presented to explain this phenomenon. A defect in the conversion of beta-carotene to vitamin A has been postulated as the reason for the hypercarotenemia (Ralli *et al.* 1936, Cohen 1958), but the evidence is poor, at least in diabetes (Kimble *et al.* 1946). In the case of hypervitaminosis A a »pathologic affinity to serum» or an »increased solubility in the serum» were suggested in the early studies (Lindqvist 1938, Popper *et al.* 1948). These suggestions alluded thus to an increased lipid or lipoprotein level in plasma, to an abnormal vitamin A-lipoprotein linkage, to an increased influx into or a defect in the removal of vitamin A from circulation. The latter has been nicely shown by Kagan *et al.* (1950) in nephrotic child-

ren, in a case of hyperlipemia associated with coronary heart disease by Martt and Connor (1956), and in patients with angina pectoris by the French investigator group (Beaumont *et al.* 1958).

In the present study the younger control group (age below 35 years) was used as the »normal» population for comparison because of the rather high incidence of clinically undetected atherosclerotic lesions at a later age (Dock 1959). The plasma content that was exceeded only by 10 per cent of the younger control subjects was defined as the normal value.

On the basis of these criteria of normality, hypertriglyceridemia appeared to be the most common lipid abnormality of plasma. In 44 per cent of the coronary group all the lipids (cholesterol, triglyceride, tocopherol) were simultaneously abnormally high, and 17 per cent of the cases showed a completely normal serum lipid pattern. On the other hand, 27 per cent of the patients has only one abnormally high serum lipid level.

Two basic considerations seem to be at hand on the basis of the present lipid measurements. Firstly, there exists in the coronary population no typical serum lipid pattern or single metabolic defect affecting only one serum lipid, but a universal tendency to the occurrence of hyperlipidemias. Secondly, without regard to the quality of the lipids, young coronary patients show a slightly higher frequency of hyperlipidemia than those of more advanced age.

KINETIC ASPECTS OF PLASMA LIPIDS AND THEIR TRANSPORT

A prolonged lipemia is frequently found in patients with coronary heart disease. However, the disappearance rate of lipids administered intravenously seems to be normal in coronary heart disease. This apparent discrepancy may probably be explained by the recently expressed view that artifical fat emulsions and alimentary chylomicra are removed from the blood circulation in a different manner. The reticulo-endothelial system seems to be more important in phagocytizing the artificial oil emulsions, while the chylomicra of alimentary origin are removed by the parenchymal cells of the liver (DiLuzio 1960, Dole and Hamlin 1962).

In the present study the great majority of the members of the coronary group showed a more marked and prolonged tocopherolemia than the control group after oral administration of the vitamin. A similar abnormality was found in the vitamin A studies. Furthermore, the peak level of plasma vitamin A tended to occur later in the coronary patients than in the healthy subjects. Comparison of the results obtained in the loading tests and in the serum fasting lipid measurements revealed further that the metabolic

abnormality was best disclosed by the loading tests. Of some importance is probably the observation that the plasma tocopherol levels at 24 hours were significantly correlated to the fasting serum cholesterol, triglyceride and tocopherol levels. This may suggest that a slow clearance rate of lipids is one of the important factors in the genesis of hyperlipidemias.

The plasma tocopherol and vitamin A are both carried completely by the lipoproteins, but in a somewhat different manner. The lightest particles D. < 1.006 seemed to be quantitatively less important in the tocopherol transport than in the vitamin A binding. On the other hand, the more long-lived D. 1.019—1.063 particles accounted for the major portion of the plasma tocopherol, while only 10—25 per cent of vitamin A was found in these particles. However, since no actual turnover studies were done, the possibility cannot be excluded that the relatively low tocopherol content of the short-lived D. $<$ 1.006 particles may in fact reflect only a higher turnover rate of tocopherol in these particles.

In spite of the absence of an endogenous synthesis of these two vitamins, there are still too many unknown factors that preclude an accurate interpretation of lipoprotein-vitamin kinetics on the basis of the results obtained. However, some more or less speculative considerations may be justified.

It seems unlikely that an increased absorption from the intestine is the major cause of the abnormal lipemia in coronary subjects. The difference in the plasma lipid content in healthy subjects. and coronary patients tends to become the more apparent the longer the time since the ingestion of the fat. On the other hand, a deficient lipolysis in the gastrointestinal tract has been postulated by Marks *et al.* (1962) as the reason for the high postprandial lipemia. A low blood lipase activity has also been reported in association with high postprandial lipemias (Tietz *et al.* 1960).

In discussing the causes of hyperlipidemia, most authors agree that one of the defects is in the removal of lipids from the circulation. Undoubtedly the lipoprotein lipase liberated into the circulation after the administration of heparin is capable of clearing the lipemic postprandial plasma. However, the role of endogenous lipoprotein lipase in the normal lipid metabolism is still a matter of controversial opinion (Engelberg 1960, Olson and Vester 1960, Dole and Hamlin 1962). The viewpoint that endogenous lipoprotein lipase has a minor role has originated from the low amount present in the plasma (Gates and Gordon 1958). Studies of the clearing effect of heparin on the postprandial lipemia in coronary heart disease have led to contradictory results (Block *et al.* 1951, Mitchell and Bronte-Stewart 1959). That hyperchylomicronemia actually follows a deficiency of this enzyme has been demonstrated by Havel and Gordon (1960) in describing a family with a lipoprotein lipase defect.

The injection of heparin has been shown to decrease the total plasma vitamin A content, with a concomitant increase of the free vitamin A alcohol

(Schrieck and Kunkel 1956). In the study of Beaumont and Beaumont (1961) the response to vitamin A load diminished also after the administration of heparin to 3 hyperlipidemic patients. They observed in addition a decrease in the vitamin A content of the chylomicra, while the vitamin content of the beta-lipoproteins separated by dextran sulphate increased. Therefore, theoretically a defect in an enzyme system sensitive to heparin, analogous to lipoprotein lipase, may be responsible for the abnormal vitamin A metabolism in coronary heart disease and in hyperlipidemia. In the light of this consideration the observation of Popper *et al.* (1948) that the elevated plasma vitamin A level in nephrotic sera was chiefly due to the vitamin A ester is of interest. In this connection it is also wortwhile to note that in the present study most of the plasma vitamin A at 24 hours was in the D. > 1.063 particles in healthy persons, but that in coronary patients this fraction contained the least vitamin A. The D. > 1.063 particles have been found to be the chief carriers of the free vitamin A alcohol (Krinsky *et al.* 1958). The increase in vitamin A alcohol observed after the injection of heparin occurred in the alpha lipoprotein fraction (Schrieck and Kunkel 1956) that corresponds to the D. > 1.063 fraction in the present study. A defect in the vitamin A »clearing factor» (esterase) may thus result in a low vitamin A content in high density lipoproteins.

According to Nikkilä and Pelkonen (1962 a), the administration of heparin did not influence the plasma tocopherol level during the tocopherol loading test. The reason for the insensitivity of tocopherol to heparin may lie in the fact that, contrary to vitamin A, tocopherol is absorbed as free alcohol (Week *et al.* 1952).

The role of the various organs in the removal of alimentary chylomicra is not adequately known. However, the liver evidently is one of the central organs in this process. It has been shown that the newly absorbed vitamin A esters are phagocytized directly by Kupffer's cells of the liver, while the free vitamin A alcohol is deposited after hydrolysis into the parenchymal cells of the liver (Glover and Morton 1948). The data available concerning tocopherol removal are few, but in studies with labeled tocopherol the maximum radioactivity in the liver was obtained one hour after ingestion of the label and a second peak occurred at 32 hours (Sternberg and Pascoe-Dawson 1959). A recent study by DiLuzio (1960) indicated that the reticulo-endothelial system has an essential role in the cholesterol metabolism. He was able to obtain a profound lowering of the serum cholesterol level in rats after inducing hyperfunction of the reticulo-endothelial system. On the other hand, blockage of the reticulo-endothelial system has been found to manifest in an increase of the plasma cholesterol and vitamin A levels and in a slowing down of the disappearance rate of vitamin A in experimental animals (Brown *et al.* 1952). A common metabolic pathway of serum cholesterol and vitamin A may also be sug-

gested on the basis of the present results, according to which there was a significant correlation of the serum cholesterol and 24-hour vitamin A levels. An insufficient function of the reticulo-endothelial system should thus probably be considered to be one of the possible mechanisms in the development of hyperlipidemia.

The half-life of circulating plasma lipoproteins has been shown to increase with increasing density (Fredrickson *et al.* 1958, Gitlin *et al.* 1958, Edgren 1960). Conversion of Sf 10—100 lipoproteins to Sf 3—9 lipoproteins has been observed (Gitlin *et al.* 1958). On the other hand, a similar protein has been found to be present both in the chylomicron fraction and in the high density lipoproteins (D. 1.063—1.21), which therefore have been thought to comprise one metabolic unit (Rodbell *et al.* 1959).

The present studies of tocopherol kinetics showed in coronary heart disease a high percentage of tocopherol in the D. 1.006—1.019 particles and a significantly decreased disappearance rate of tocopherol from the D. 1.019—1.063 particles. A defect in the conversion of Sf 10—100 lipoproteins to Sf 3—9 lipoproteins has been presented by Gitlin *et al.* (1958) in nephrosis. It has also been emphasized by George *et al.* (1961) that a slow disappearance rate of labeled neutral fats in coronary heart disease is more compatible with a defect in the metabolism of the Sf 20—400 lipoproteins than in that of the chylomicra. Tocopherol is insensitive to heparin and thus the present results support the finding of George *et al.* and Gitlin *et al.* showing a retention of tocopherol in the D. 1.006—1.019 particles.

In this connection it is noteworthy that according to the observation of Hanig *et al.* (1956) the Sf 12—100 lipoprotein is always present in substantial amounts in aortas where there is atherosclerotic activity, while the Sf 0—12 lipoprotein is absent almost completely in the aorta extracts.

Vitamin A studies showed in coronary patients an accumulation of the vitamin in the D. < 1.006 and D. 1.006—1.019 particles. The consideration presented above is compatible also with the abnormal vitamin A kinetics in the D. 1.006—1.019 particles. On the other hand, a high content of vitamin A in the D. < 1.006 particles and a low content of the vitamin in the D. > 1.063 particles possibly suggest a defect in the heparin-sensitive system.

Hyperlipidemia may theoretically be the result of an overproduction of the plasma lipids or of a defect in the removal of the lipids from the circulation. The present studies with purely exogenous lipids demonstrate that the lipid removal, at least, may be impaired. The present results support the view that the defect in fat disposal may lie in various steps of the complex process, which is in a good accordance with the heterogeneity of the hyperlipidemias found by measurement of the fasting plasma lipid values in coronary heart disease.

SUMMARY

The object of the present investigation was to study the metabolism of vitamins A and E, representing purely exogenous lipids, in coronary heart disease. In order to classify the lipid disorder of the subjects the serum total cholesterol and triglyceride levels were also determined.

The control subjects were 176 male and 146 female blood donors and 88 male and 13 female healthy subjects. The blood samples from blood donors were taken in the non-fasting conditions. The blood donors underwent no medical examination. The healthy subjects were either medical students, all subjectively healthy, or patients under medical observation in the hospital. The patients included in the healthy group were admitted to the hospital for a minor congenital heart disease or the medical examination revealed no organic disorders.

The coronary group was comprised of 110 male and 14 female survivors of myocardial infarction. All the patients were admitted to the hospital because of an acute attack of myocardial infarction or of angina pectoris and all had electrocardiographic evidence of an old or recent myocardial infarction. The blood samples were not taken before at least 3 weeks had elapsed from the acute attack, and most of the samples were taken 4 weeks after admission.

Serum Total Cholesterol Level. — The blood donors showed a continuous increase of the mean cholesterol levels from the 3th to 7th decade. In the coronary group, on the other side, definite decreases of serum cholesterol levels occurred from the 4th to 5th and from the 6th to 7th decade. The mean levels of coronary patients below and over 50 years did not, however, differ significantly.

The coronary group showed a significantly higher mean level than the blood donors. The difference was also significant when the age groups of 30—39, 40—49, and 50—59 years in the two populations were compared. Aged persons in the 7th decade, however, sowed no difference.

Serum Triglyceride Level. — The older healthy subjects over age 35 showed a significantly higher mean level than the younger subjects. In the coronary group there was a tendency to decreased levels with increasing age. However, no significant difference was found in the mean values in the two age groups (age below and over 50 years).

The coronary group showed a signi-

ficantly higher mean level than the healthy subjects. The difference was also significant when the age groups 30—39 and 50—59 years of the two populations were compared.

Plasma Tocopherol Level. — The blood donors showed an almost continuous increase of the mean levels from the 3th to the 7th decade. An opposite age trend was seen in the coronary population.

The coronary group had a significantly higher mean level than the blood donors. The difference was also significant in the age groups from the 4th to 6th decade, but not in the 7th decade.

Plasma Vitamin A Level. — In the healthy population no significant difference existed between the mean levels in the two age groups (age over and below 35 years). The two coronary age groups (age below and over 50 years) showed also similar mean levels. The coronary group had a significantly higher mean level than the control group.

Incidence of Hyperlipidemia in Survivors of Myocardial Infarction. — The plasma content that was exceeded only by 10 per cent of the younger control subjects (age below 35 years) was used as the normal value. The normal values of the serum total cholesterol, triglyceride, tocopherol and vitamin A contents were 290 mg/100 ml, 125 mg/100 ml, 15 mg/L, and 156 I.U./100 ml, respectively.

The incidence of hypercholesterolemia in the whole coronary population was 60 per cent, being 70 per cent for the younger (age below 50 years) and 50 per cent for the older patients. The corresponding percentages of hypertriglyceridemia were 68, 70 and 65 per cent; those of hypertocopherolemia 53, 55 and 50 per cent, and those of hypervitaminosis A 59, 64 and 55 per cent, respectively.

Interrelationships of the Serum Lipid Levels. — The regression analyses revealed a significant regression between the serum cholesterol and tocopherol levels in the coronary group and in the combined control group consisting of the blood donors and the healthy subjects. The serum triglyceride and tocopherol levels were significantly correlated in the coronary group only. The serum triglyceride and cholesterol levels also showed a significant correlation in both groups, the correlation being closer in the coronary group.

The plasma vitamin A level did not correlate significantly with the serum cholesterol or triglyceride levels.

The lipid levels were followed in 9 patients after the acute event until at least 5 months had elapsed. A significant fall in the serum cholesterol and tocopherol levels was observed during the first week. After at least 5 months had elapsed from the acute attack the initial values were reached. The variations observed in the serum triglyceride levels were not significant.

Tocopherol Loading Test. — To 38 healthy subjects and 64 survivors of myocardial infarction 2 gm of tocopherol acetate and 100 ml of cream (40 per cent fat) were given, after which blood samples for tocopherol

determination at 4, 6, 10, and 24 hours were taken.

The healthy subjects showed a higher initial increase than the coronary subjects. However, a significantly higher concentration curve was found in the coronary group than in the healthy group. The difference was as significant when the older healthy and the younger coronary groups of similar mean ages were compared. The 24-hour tocopherel level was found to have a significant correlation to the plasma basal tocopherol, cholesterol and triglyceride values in both groups. The plasma 24-hour tocopherol content of 24.5 mg/L was exceded by 10 per cent of the younger healthy subjects, by 33 per cent of the older healthy persons, and by 83 per cent of the coronary group.

Vitamin A Loading Test. — To 36 healthy subjects and 34 survivors of myocardial infarction 1,500,000 units of vitamin A palmitate and 100 ml of cream (40 per cent fat) were given. In the complete test the blood samples for vitamin A determination were taken 2, 3, 4, 6, 10, 24 hours after administration of the vitamin.

The peak level occurred from 4 to 10 hours after administration of the vitamin and was significantly higher in the coronary group than in healthy persons. Furthermore the peak levels tended to occur later in the coronary than in the healthy group. The healthy persons showed a more rapid initial increase. Later, however, the mean increase from the basal level as well as the mean levels were significantly higher in the coronary group than in the healthy group.

None of the subjects had reached the initial plasma content at 24 hours. A significant correlation between the plasma basal and 24 hour vitamin A levels was present in the healthy group. The basal cholesterol and the 24-hour vitamin A levels were in a significant correlation in both the groups.

The plasma 24-hour vitamin A content of 450 I.U./100 ml was exceded only by 10 per cent of the younger healthy subjects, by 2 of 10 older healthy subjects; only 3 of the 29 patients in the coronary group were below this limit.

In order to study the *Vitamin A and E kinetics,* both of these were analyzed from 5 lipoprotein fractions. The plasma lipoproteins were isolated in a Spinco Model L preparative ultracentrifuge by two successive runs. In the primary separation D. < 1.006 and D. > 1.006 fractions were obtained. In the secondary separation the D. > 1.006 fraction was separated further, and D. 1.006—1.019, D. 1.019—1.063 and D. $>$ 1.063 fractions were obtained.

The primary separation for further determination of the tocopherol content was performed from fasting plasma and from samples taken from 25 healthy subjects and 17 survivors of myocardial infarction 4, 6, 10, and 24 hours after administration of the tocopherol. The secondary separation was performed from fasting plasma and from samples taken from 7 healthy subjects and 8 survivors of myocardial infarction 4, 10, and 24 hours after the tocopherol load.

In vitamin A studies the primary separation of plasma lipoproteins was performed from blood samples taken from 9 healthy subjects and 12 survivors of myocardial infarction 4, 6, 10, and 24 hours after the administration of vitamin A. The fraction D. > 1.006 was separated further of the plasma of all the healthy subjects and 9 survivors of myocardial infarction.

Tocopherol in Plasma Lipoproteins. — Tocopherol was found to be present in all the fractions isolated, most of it being in the D. 1.019—1.063 particles. The most characteristic finding in the coronary group was the high percentage of tocopherol in the D. 1.006—1.019 particles.

The newly absorbed tocopherol was carried primarily in the D. < 1.006 and D. 1.006—1.019 particles; later, however, the major part of the newly absorbed tocopherol was found in the D. 1.019—1.063 particles. The D. > 1.063 particles were quantitatively less important. The coronary group showed a marked retention of tocopherol in the D. 1.006—1.019 particles and a significantly slow disappearance of tocopherol from the D. 1.019—1.063 particles.

Vitamin A in Plasma Lipoproteins. — The newly absorbed vitamin A appeared to be carried primarly in the D. < 1.006 and D. 1.006—1.019 particles. At 24 hours in the healthy group, however, most of the vitamin A was found in the D. > 1.063 particles. The major abnormality in the coronary group was the high vitamin A content in the D. < 1.006 and D. 1006—1.019 particles. The disappearance rate of vitamin A from the D. 1.019—1.063 particles was significantly slow and the percentile increase of vitamin A in the D. > 1.063 particles was also low in the coronary group.

The error of lipid metabolism in coronary heart disease was discussed. The possible role of the endogenous »clearing factor» and of the reticuloendothelial system in the pathogenesis of hyperlipidemias was emphasized.

It was concluded that the disappearance rate of exogenous lipids from the blood circulation may be impaired in patients with coronary heart disease and that there exists no single defect in the lipid metabolism but a universal tendency to hyperlipidemia.

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