STUDIES ON BLOOD LIPIDS IN ATHEROSCLEROTIC INDIVIDUALS

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SUMMARY

In the recent literature on atherosclerosis, two subjects have become of great importance. Firstly, whether the frequent association between elevated serum lipid levels and atherosclerosis is of any predictive value, and secondly, which if any of the many antilipemic agents are of therapeutic value. Both of these subjects have been reviewed in the text.

In the experimental work in this thesis the antilipemic action of various drugs was assessed by determining the blood lipids of various groups of individuals by established methods. In addition, the technique of paper electrophoresis was utilized to determine the relative amount of lipoprotein in the Beta globulin fraction of the serum. Finally, a procedure called the chloroform turbidity test was performed on all sera. Since elevated values obtained by this method have been described to have an empirical correlation with atherosclerosis, an attempt was made to verify this finding.

A study was made of three groups of atherosclerotic individuals treated with various anticoagulants, phenylindanedione (Danilone), and the coumarin derivatives Sintrom and Tromexan over an eight week period. No change was noted in the blood lipids of the phenylindanedione group but significant elevations of the total serum cholesterol

and the total cholesterol phospholipid ratio occurred at various periods after the commencement of treatment in both the Tromexan and Sintrom treated groups. No explanation can be offered to explain the difference in response of the blood lipids to these two types of anticoagulants.

Five individuals treated with Heparin every 48 hours who were compared to a control group showed a significant fall in the Beta percentage of the total lipoprotein lipid and the neutral fat in accord with the findings of other workers.

The blood lipids of a group of five individuals treated with triiodothyroacetic acid did not differ significantly from those of a control group of patients. This result does not corroborate the results of most other workers.

The average chloroform turbidity values of a group of young normal individuals did not differ significantly from the values of a group of atherosclerotic controls. This finding is at variance with the observations of the originator of this procedure.

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I. INTRODUCTION

While a causal relationship between elevated serum lipids and clinical atherosclerosis has not been unequivocally demonstrated to this date, sufficient evidence has been accumulated to suggest this possibility. It has therefore become of great interest to determine the effect of various dietary and pharmacological regimes on the serum lipids, with the hope that any lowering of the lipid livels would be accompanied by some degree of arrest of the atherosclerotic process, or an amelioration of existing vascular disease. The purpose of this research has been primarily to assess the effect of various agents on the circulating lipids. Since the therapy of many individuals suffering from atherosclerotic diseases involves anticoagulation, often for long periods of time, it was felt worthwhile to determine the effect on the serum lipids of some of these anticoagulants. Phenylindanedione (Danilone), and two coumarins, an oxycoumarin derivative (Sintrom) and ethyl biscoumacetate (Tromexan) were chosen as being fairly representative of two classes of anticoagulants which are in common clinical usage.

Frequent reports in the literature concerning the antilipemic effect of heparin, even in doses insufficient to produce anticoagulation, stimulated our investigation of this drug.

The use of thyroxin and other thyroid hormone derivatives by other investigators, for their effects on blood cholesterol, prompted our investigation of the effects of some of the newer thyroid hormone derivatives on the serum lipids. The acetic acid analogue of triiodothyronine, known as Triac, was chosen for this study.

The material in the introduction of this thesis will be arranged in the following manner. First, an attempt will be made to demonstrate some of the evidence which has been accumulated to show the relationship between elevated serum lipids and clinical atherosclerosis. The various lipid measurements will be discussed and some of the present controversy as to which lipid measurement gives the best correlation with atherosclerotic diseases, will be presented.

Secondly, the historical background of various drugs in their specific application against lipemia will be reviewed. Special emphasis will be given to those drugs used in the present study, namely, heparin and the thyroid hormone derivatives. Some mention will also be made of the low fat and vegetable fat diets, of gonadal hormone therapy, and of other suggested antilipemic agents.

Finally, the clinical results of these regimes in preventing or modifying the atherosclerotic process will be discussed at the end of each appropriate section.

II. REVIEW OF THE LITERATURE

Studies on Blood Lipids

1. The correlation of various lipid measurements with atherosclerosis.

The total cholesterol was the first serum measurement to arouse a substantial degree of interest. Since this substance was found in the atherosclerotic plaques of blood vessels it seemed reasonable that circulating blood cholesterol might be incriminated in this disease. A correlation between clinical atherosclerosis and elevations of the total serum cholesterol over those levels found in control groups of comparable age and sex has been established (1, 2).

Ancel Keys, interested in this measurement as a possible means of predicting clinical atherosclerosis, noted that both the total serum cholesterol and certain lipoprotein fractions afford differentiation between groups of men who are clinically healthy and groups of men who are likely to develop coronary heart disease. He observed, however, that "these measurements of total cholesterol or of the Sf 12 to 20 lipoprotein concentration in the serum have very little practical value for individual prognosis, the index of forecasting efficiency being of the order of 20%" (3).

In 1949, Ahrens and Kunkel (4) observed that certain high lipid sera did not have the usual turbid appearance and this

clarity was associated with elevated levels of serum phospholipids. In contrast, other high lipid sera of turbid appearance had lower levels of serum phospholipids. Previous experimental work in the production of atherosclerosis in dogs by high cholesterol, fat and thiouracil feedings, and in rabbits by high cholesterol feedings, had revealed that the hypercholesterolemia which preceded the development of atherosclerosis was not followed by a concomitant rise of serum phospholipids. Thus a fall in the phospholipid cholesterol ratio occurred. This led Ahrens and Kunkel to advance the idea that a decrease in the amount of phospholipid in proportion to the amount of cholesterol might augment the deposition of cholesterol in the vessels, because the loss of the solubilizing action of the phospholipids would allow it to precipitate.

Wilkinson (5) minimizes the importance of the cholesterol phospholipid ratio, stating that with diseases which are the end result of atherosclerosis the ratio is no different than that of a large miscellaneous group of subjects. He claims that one can predict the phospholipid total cholesterol ratio within narrow limits if given the total cholesterol, and states that the ratio is of no more value as an index of atherosclerosis than the total cholesterol itself.

Investigators, already aware of the inadequacy of the total serum cholesterol or the phospholipid cholesterol ratio as an

index of atherosclerosis, were interested in other lipid measurements. Since the work of Macheboeuf in 1928 it had been realized that the lipid fractions of the serum are carried in combination with proteins. In 1946 Cohn (6) developed an exacting but workable method for separating the serum into its constituent lipoprotein fractions. Employing ethanol fractionation methods at low temperatures he was able to isolate two main fractions, namely 3 - 0 and 4 - 1, which, on the basis of subsequent work by other investigators, were found to be equivalent to the beta and alpha lipoproteins, electrophoretically separated. Another technical advance was the use of the ultracentrifuge by Gofman and the Donner group (7) as a means of separating the serum into its lipoprotein constituents. This technique allowed not only the separation of the low density or beta lipoproteins from the high density, or alpha lipoproteins, but also permitted further fractionation of these main lipoprotein fractions.

In 1951, Jones (8) employing the ultracentrifuge technique, reported the relationship of certain lipoprotein fractions to clinical atherosclerosis. He noted a strong correlation between elevations of the Sf 10 - 30 class of lipoproteins, a fraction of the beta lipoproteins, and the rate of development of atherosclerosis. A critical comparison was made in which an analysis of the total

serum cholesterol and the Sf 12 - 20 lipoproteins, a subfraction of the beta lipoproteins, was done on aliquots of the same serum of both normals and atherosclerotics using the latter as a test group. They found that the overall correlation of Sf 12 - 20 lipoprotein levels with atherosclerosis is two to four times as great as that for serum cholesterol levels with atherosclerosis.

With this stimulus a general interest arose in the separation of the blood serum into its lipoprotein fractions. In 1952, Kunkel and Slater (9) using zone electrophoresis, noted that this method gave very comparable results with the method of chemical fractionation used by other workers. They noted that variations from the normal pattern were found in pathological sera with elevated lipid concentration, (in individuals suffering from atherosclerosis), there being a diminution of the alpha lipoproteins and an increase of the beta lipoproteins.

The introduction of improved methods of electrophoresis, particularly paper electrophoresis, about 1953, offered the simplest possible tool for lipoprotein analysis. Swahn's (10) description of a simple staining technique with Sudan Black lipid stain enabled experimenters to readily identify the main alpha and beta lipoprotein bands. His data and that of Nikkila (1) in 1953 corroborated the work of Kunkel and Slater (9) concerning the abnormal pattern found in

the majority of atherosclerotic individuals, and in the sera of individuals suffering from those diseases often associated with elevations of the blood lipids and known to predispose to the development of the atherosclerotic process. Rosenberg (11) further confirmed this work in 1954.

Analysis of the lipoprotein lipids for cholesterol revealed that this constituent of the beta lipoprotein is increased and that the cholesterol in the alpha lipoprotein is usually decreased in individuals suffering from atherosclerosis.

Jencks (12) in 1955, introduced a method employing a lipid dye known as Sudan 3, or Oil Red-O, which he claimed was superior to the Sudan Black recommended earlier by Swahn. Using a technique introduced by Swahn, he compared the dye uptake by the lipoprotein bands to a stained spot of a known amount of fat, and he was able to determine quantitatively changes in the alpha and beta lipoprotein bands. His work confirmed that of previous investigators but in addition he made two interesting observations. The first was that despite the difference in the distribution of the lipoprotein lipids between the serum of normal and atherosclerotic individuals, the distribution of serum proteins was not significantly different in these two groups (13). The significance of this observation is probably that the alteration in distribution of lipoprotein lipids in these abnormal

states is not due to an underlying alteration of protein distribution. The second observation of interest made by Jencks was that ultracentrifugal analysis of lipoproteins of the Sf 12 - 20 class were less often abnormal than were electrophoretic analyses of the ratio of alpha to total lipoprotein in sera from atherosclerotic individuals.

It is clear that at the present date (1957) none of the serum lipid measurements or ratios give a sufficiently satisfactory correlation with atherosclerosis to be of much value in individual prognosis. Careful scrutiny of the data of investigators comparing so-called normal individuals with those suffering from known atherosclerotic diseases reveals that there is a range of values in which overlap occurs between both groups. This is readily admitted by most workers engaged in these studies. The greatest controversy has arisen over which particular measurement is the most useful in giving a reasonable correlation with atherosclerotic disease.

Recently a very large survey was conducted by a cooperative effort of the Donner Laboratory group in Los Angeles and various other laboratories in the eastern United States (14). The purpose of this study was to "compare and contrast the lipoprotein findings with those for serum cholesterol determination with respect to coronary disease". Their method involved the clinical and laboratory investigation of some 16,000 individuals who were selected

as being healthy at the beginning of the survey. Two years later the group was re-examined and those who had suffered from any attack attributable to atherosclerosis of the coronary vessels were carefully noted. These individuals who had suffered "new events" as they termed the atherosclerotic sequelae, were indicated on curves where their original total serum cholesterol and lipoprotein values were plotted.

Unfortunately, the results from this survey are not clear. Firstly, the Donner group altered their method of ultracentrifuge analysis during the study. Further, they introduced a correction factor to their method, which altered the results. Secondly, the Donner group maintained that only cases with definite clinical, laboratory and EKG findings of myocardial infarction should be used in the study, and that lesser degrees of disease such as angina pectoris should not be included.

The Eastern laboratories would not agree to change the method during the course of the experiment. Secondly, they argued that not only cases of definite myocardial infarction, but also other cardiac evidence of the atherosclerotic process must be evaluated in the series of cases examined.

"Both groups agree that atherosclerosis as manifested by clinical signs of coronary artery disease is associated with a disorder

of lipid metabolism and there is some predictive value in the various lipid measurements examined". However, they disagree with regard to the degree and specificity of this predictive value.

The Donner group maintain that "the mean value for measured standard of Sf 12 - 20 lipoproteins and for the measured atherogenic index is significantly elevated in those with definite new events as compared with the base population for which such measurements were available. The serum cholesterol for the same group of cases cannot be shown to be significantly elevated in the definite new events as compared with the base population. There exists a predictive relationship of various blood lipid measures with de novo coronary disease". (The combined measure of lipoproteins predicting coronary disease risk has been designated the Atherogenic index. It is obtained from the standard Sf 0 - 12 and the combined standard Sf 12 - 400 lipoprotein measurements, (concentration in mgm./100 ml.) as follows. The Atherogenic index equals the (standard Sf 0 - 12) plus 1.75 (standard Sf 12 - 400).

The Eastern laboratories conclude that "Atherosclerosis, as manifested by definite evidence of coronary artery disease is associated with an antecedent elevation of the serum Sf 20 - 100 (and possibly Sf 12 - 20) lipoproteins, and of the serum cholesterol. The elevation of serum lipoproteins and cholesterol was not of clinical

use in predicting those individuals who would develop coronary heart disease during the observation period. The use of Sf 12 - 20 and Sf 20 - 100 lipoproteins or the related Atherogenic index had no advantage over the simple measurement of cholesterol in the characterization of men prone to develop coronary heart disease".

In general it would seem safe to say that at the present time there is general disagreement as to which lipid measurement is the most valuable in providing a good correlation with those diseases which occur as a result of atherosclerosis. The main body of opinion is generally agreed that no lipid measurement is very useful as a tool for individual prognosis.

2. (a). The use of heparin as an antilipemic agent.

Investigators have been interested for a long time in drugs which can produce a lowering of serum lipids. The possible usefulness of heparin in this respect was first suggested by the accidental discovery of Hahn (15) in 1943, that heparin injected into a dog was capable of clearing the turbidity of lipemic serum of alimentary origin. He noticed that heparin, added to the same lipemic serum in vitro produced no clearing. In 1950 Anderson and Fawcett (16) demonstrated that lipemic plasma could be cleared in vitro by the addition of plasma from another subject who had received an injection of heparin. He suggested that heparin was

capable of stimulating the clearing activity but was not per se responsible for it.

Anfinsen, Boyle and Brown (17) noted that heparin plus a tissue factor extracted from rats' hearts was capable of clearing a turbid serum when added in vitro. It seemed that heparin and tissue factor were necessary to provide the active clearing factor. Surprisingly, this combination would not act on purified lipoprotein until serum was added. They therefore postulated that pure clearing factor and a co-factor found in the plasma are necessary before clearing activity can occur.

In 1951 Graham (18) utilized the ultracentrifuge technique to determine what physical changes occur in the serum during this clearing of turbidity after heparin administration. He noted that "heparin injected into rabbits and man caused a profound reorientation in the distribution of the low density lipoproteins characterized by a shift of the lipoproteins of high Sf rates to those of successively lower Sf rates". He also observed that heparin administered to cholesterol fed rabbits prevented the development of high levels of Sf 10 - 50 lipoproteins and retarded the development of atherosclerosis in such animals.

Boyle, Bragdon and Brown (19) confirmed Graham's findings by observing that post heparin plasma shows an increase in

high density lipoproteins and a concomitant decrease in certain low density lipoproteins. The authors suggested an enzymatic conversion of one lipoprotein class to another.

In 1953, Nikkila (1) using electrophoretic techniques, demonstrated, in accord with Boyle, Bragdon and Brown, that the clearing of lipemic serum due to heparin was accompanied by a decrease of the area under the beta globulin peak and an increase of that under the alpha globulin, in the Tiselius pattern. The lipoprotein patterns by paper electrophoresis stained with Sudan Black lipid dye also showed a transfer of beta to alpha lipoproteins. This shift of stainable lipid was accompanied by a decrease of cholesterol in the beta lipoprotein area and an increase in the alpha lipoprotein area. In many fasting sera, the clearing factor brought about a transfer of some cholesterol from the beta to the alpha globulin areas, whereas in some cases, no effect was noted.

Nikkila advanced the theory that the increased amounts of alpha lipoprotein lipid are due to this lipoprotein taking the excess lipids from the lipoproteins of the lowest density including possibly particles not attached to protein. Hence the density of these latter particles increases, their flotation rate decreases in a medium of constant density, and the change is detected on the ultracentrifuge low density lipoprotein pattern as an increase of the lower Sf classes

(alpha lipoproteins). This sequence of events, according to Nikkila, explains Graham's observations without it being necessary to assume an actual successive transformation of higher Sf classes to those of lower Sf rates.

Nikkila observed that the clearing factor chiefly affected the triglycerides. This concept received support from the work of Nichols (20) who showed that clearing factor causes a release of fatty acids from egg yolk lipoproteins.

Heparin is capable of altering the distribution of lipids, including cholesterol, from one lipoprotein fraction to another. Most authors report that the total serum cholesterol remains unchanged, despite heparin therapy both in humans (21, 22) and in animals (23).

As evidence that endogenous heparin normally has an antilipemic function Bragdon and co-workers (24) showed that heparin antagonists such as toluidine blue and protamine sulfate are capable of producing turbidity in clear serum when injected in vivo. The changes in the serum lipids which occur are exactly opposite to those described by other workers when heparin is injected. The action of protamine and toluidine blue is likely specific since other chemically related basic amines which are not heparin antagonists do not have this effect. This work gives further evidence that

heparin is in some way necessary for clearing activity.

In 1955 Korn (25) isolated clearing factor from rats' hearts. He found that this factor was capable of hydrolysing protein bound triglycerides without the presence of serum, thus refuting Anfinsen's earlier claims that serum was necessary as a co-factor. Korn (26) found that this clearing factor activity could be greatly increased by heparin and inhibited by protamine.

Secondly, Korn demonstrated that clearing factor, or lipoprotein lipase, as he termed it, is substrate specific. It is capable of acting on lipoproteins or chylomicrons in which the fat is bound to protein, but is ineffective against a pure solution of triglycerides. He noted, however, that even a very small amount of serum added to the triglycerides would allow enzymatic activity to occur. Korn concluded that a conjugation between serum proteins and lipids in the fat solution must occur before the substrate specific enzyme, lipoprotein lipase, can act upon the triglyceride fraction. It was further noted that the alpha lipoprotein fraction of the serum combining with fat allowed the greatest clearing activity to occur, but that the beta lipoprotein fraction also allowed a lesser amount of enzyme activity.

Korn outlined a possible mechanism by which such processes could function in normal fat transport. It is known that

fat absorbed from the gastrointestinal tract is found in the triglyceride form in the lymphatics of the bowel. It is also known that these triglyceride particles combine with small amounts of protein to form visible fat particles of chylomicrons. Korn suggests that alpha lipoproteins may conjugate with these triglycerides. They would act as a substrate for the lipoprotein lipase, and glycerol and fatty acids would be released, again regenerating free alpha lipoprotein.

Korn has demonstrated the presence of clearing factor in animal tissues and according to a recent report by Anfinsen (27), Korn has now unpublished data that it also occurs in human adipose tissue. Interestingly, Nikkila (1) back in 1953, noted endogenous clearing activity in human sera in two cases.

Engleberg (28) has recently noted that endogenous clearing activity is more easily detected in plasma than in serum. He has suggested a method of measuring this activity by adding a small known quantity of a triglyceride solution, such as sesame oil to a fixed amount of plasma and observing the gradual clearing of turbidity by spectrophotometry.

The observations of Nikkila and Engleberg strengthen Korn's postulation that lipoprotein lipase is present normally in the human. Hueper (29) in his review of the pathogenesis of atherosclerosis suggests that heparin may be an important agent in human

lipid metabolism.

This raises the interesting possibility that if this substance is present normally it may be absent in certain individuals. Nikkila (1) had two patients with endogenous lipemia of unknown origin which did not clear on the administration of heparin. It did however, when post heparin serum from another individual was added in vitro. According to Anfinsen (27), Havel and co-workers also have two such unpublished cases of idiopathic hyperlipemia. The relationship that these cases bear to the general problem of atherosclerosis is not at present clear. There is no evidence as yet available to demonstrate whether atherosclerotics differ from so called normal individuals in their ability to clear lipemic serum, either endogenously or after the administration of heparin. It is intimated in the literature that this line of investigation is being pursued.

From a therapeutic point of view the definitive problem is whether or not treatment with heparin offers any protection against atherosclerosis. Surprisingly little work has been done in an attempt to elucidate this problem. Graham (18) in 1951, reported that intermittent heparin therapy to patients with severe angina pectoris resulted in the relief of symptoms in 55 out of 59 cases. On the other hand, two well controlled studies by Gruner (30)

and Rinzler (31) were unable to substantiate Graham's findings. They found heparin ineffectual in the prevention of angina pectoris. Engleberg (32) in 1956 published a study of 222 cases of individuals with pre-existing coronary artery disease, followed over a two-year period. One hundred and five cases received heparin in doses of 200 mgm. subcutaneously, twice weekly and the remaining 117 individuals were untreated. Engleberg reports 21 deaths out of 117 in the control group as compared with only four deaths of 105 individuals in the test series. The difference in the two groups is significant.

Some substances which are chemically related to heparin have similar antilipemic and anticoagulant properties. Waldron and Freedman (33) demonstrated in 1948 that clearing of lipemic plasma occurs with the administration of a synthetic heparin-like sulfonated polysaccharide. Levy and Swank (34) demonstrated a moderate clearing of lipemia in dogs after the injection of dextran.

Recently, Cohen and Tudhope (35) have reported that sulfonated dextran has even more prolonged anticoagulant activity than heparin. They also showed that this substance produces a temporary fall in the total blood cholesterol, which returns to pre-treatment levels within two weeks, coinciding with a reduction in the dosage level from 10,000 to 5,000 units daily. Heparin does

not appear to have this anticholesterolemic property in the dosages used by most investigators.

(b) The effect of the coumarin anticoagulants on the blood lipids.

Very little information is available in the literature concerning the effect of the coumarin or indanedione anticoagulants on the serum lipids. Bernard-Griffiths (36) and co-workers have studied the effect of a combined regime of 1.5 gm. of choline citrate and 40 mgm. of ethyl dicoumarol daily on the serum lipids of 15 chronic atherosclerotic individuals followed from 20 to 45 days. The dosage of anticoagulant produced varying changes in the prothrombin time of ten patients; in the remainder it was normal. Although the authors present no actual data, they claim a moderate increase in the total cholesterol in all cases, a diminution in the total lipids of 12 of 15, and a decrease in lipoproteins in nine out of 15 individuals.

Constantinides (23) noted that dicoumarol, unlike heparin, does not have any significant effect on the clearing activity in rabbits. This observation suggests that heparin and the coumarin drugs are dissimilar not only chemically and in their pharmacological mechanism of anticoagulation, but also in their effect on blood lipids.

(c) The effect of the thyroid hormone and its analogues on blood lipids.

It has been known for many years that myxoedema is usually associated with hypercholesterolemia, and hyperthyroidism with hypocholesterolemia (37). It has further been noted that myxoedema patients have a high incidence of atherosclerosis. Such patients, treated with thyroid hormone, are restored to normal metabolism and their total serum cholesterol also falls to normal levels. This observation provoked speculation that treatment with the thyroid hormone might offer to hypercholesterolemic euthyroid individuals some degree of protection from atherosclerosis.

This concept received encouragement from animal experimentation. Horlick and co-workers (38), using cholesterol fed chickens which normally develop early atherosclerosis, discovered that a significant difference existed between the control animals and those protected with varying amounts of thyroxin and others with large doses of potassium iodide.

Many years ago, Hurxthal (37) noted that thyroxin administered to three euthyroid individuals produced a fall in the total serum cholestero. In 1954, Stristower (39) completed a similar series utilizing a wide range of lipid measurements including the ultracentrifuge lipoprotein analysis. Thyroxin administered in doses of 10 grains daily to 19 schizophrenics for 39 weeks,

resulted in a significant lowering of the serum cholesterol, the standard Sf 0 - 12 lipoproteins and standard 12 - 20 lipoproteins. A similar response was noted in three normal individuals given thyroxin in doses of three grains daily. It was observed, however, that almost all patients suffered some weight loss and it became evident that it would not be possible to exclude this factor of negative caloric balance as the cause for the lowering of the serum lipids.

Stristower (40) later examined 30 male and 30 female patients treated with thyroxin over long periods. Only 39 completed the study. These individuals were placed on doses of 195 mgm. of thyroxin daily for 30 weeks, then 260 mgm. daily for 39 weeks and finally 325 mgm. for 36 weeks. A fall in the total cholesterol and in the levels of the Sf 0 - 12 and Sf 12 - 20 lipoproteins occurred at first with doses of 195 mgm. of thyroxin daily, but eventually the individuals escaped and the total cholesterol approached the baseline by 24 weeks. The authors conclude that dosages of 260 and 325 mgm. of thyroxin daily produced a sustained fall in certain serum lipids. This is open to dispute. It is obvious that the lipid measurements of the patients on the 260 mgm. dosage are returning toward the baseline close to the end of the 39 week period, and may have been prevented from doing so by the increase in dosage of thyroxin which again caused a downward fall. Despite the authors

claims it would appear from the data that 260 mgm. of thyroxin daily is not adequate to maintain a sustained fall of the serum lipids. Though the authors minimize the side effects of the drug, it is pointed out that only 39 out of 60 patients completed the treatment. While this in itself is no criticism of the work, it is notable that four of these 21 were dropped from treatment because of considerable weight loss. This places the incidence of this side effect at about 7%. The suggestion is made that dried thyroid is worthy of consideration as a prophylactic agent against coronary artery disease. This must be considered with the foregoing reservations.

The synthesis of the acetic acid analogue of thyroxin, tetraiodothyroacetic acid, (Tetrac) in 1952, and the acetic acid analogue of triiodothyronine (Triac) in 1953, revived interest in the thyroid hormone (41). Thibeault and Pitt-Rivers (42) showed that Triac differed from both thyroxin and triiodothyronine by increasing the oxygen consumption of kidney slices immediately in vitro. It also produced an immediate increase in oxygen consumption when injected into thyroidectimized rats. Its action in man, however, provided the most interesting observation. In two individuals with myxoedema treated with repeated injections of Triac, the patients improved clinically, the total cholesterol fell, but the BMR remained at a low level (43). This observation led to the speculation that perhaps

Triac might differ qualitatively in its effect from thyroxin and triiodothyronine. Subsequently, it was shown that Triac could affect the BMR, but the same individual treated with thyroxin had a relatively greater rise of BMR and proportionately less lowering of the total cholesterol (44)

Trotter (45) treated 15 euthyroid females for a short period of two weeks with Triac. Three groups of five individuals received 2, 3 and 4 mgm. respectively. A significant fall in the total serum cholesterol occurred in all groups. A similar degree of depression was produced in an identical group who were given .08 mgm. daily of triiodothyronine. One individual treated with Triac showed side effects from the drug, none of the seven patients treated with triiodothyronine did so. Very slight weight loss occurred in both groups. This work did not demonstrate any difference between the effects of Triac in doses of 2 - 4 mgm. and those of triiodothyronine in doses of .08 mgm. daily in euthyroid individuals.

Oliver and Boyd (46) treated 12 hypercholesterolemic males suffering from coronary disease with Triac, for longer periods. They noted a fall in the total serum cholesterol, the cholesterol phospholipid ratio, and the beta lipoprotein cholesterol without elevation of the BMR. The data shows that it was actually elevated in two men.

Two of the twelve men developed severe angina of effort without any noticeable change in the BMR or EKG. The authors postulate a selective requirement for oxygen by the myocardium over other tissue, which would therefore be consistent with a lack of elevation of the BMR. These authors also noted an escape from Triac despite increasing dosages, and the serum lipid re-approached the baseline levels. They conclude that Triac may not prove suitable for long term control of hypercholesterolemia in patients with clinical coronary disease.

(d) The sex incidence of atherosclerosis and the effect of sex hormones on the blood lipids.

One of the most interesting features of the problem of atherosclerosis is the peculiar sex incidence of some of its clinical complications. Coronary artery disease in particular, strikes the male more often, particularly in the younger age groups. The incidence of this disease in individuals under 40 years of age is estimated to be 25 times higher in the male (47, 48). In Epstein's series of garment workers (49) he reported that 10% of males over 40 were afflicted with coronary artery disease, while only 2% of females in the same age group were similarily affected. In diabetes mellitus, this peculiar sex incidence does not occur, and the ratio

is about one to one (50). Barker and Hines (51) have observed that peripheral vascular disease of the atherosclerotic type is six times more common in males than in females of all ages. Other figures place this ratio at about four to one (49). On the other hand, Epstein's figures for cerebral thrombosis occurring in individuals over 40 years of age, reveal that the incidence of the disease is about equal in the two sexes (49).

It might be expected that the remarkable disparity in the sex incidence of certain atherosclerotic sequelae might be explained on a pathological basis. Willius, Smith and Sprague (52) examined 5,000 hearts at autopsy on individuals of all ages and both sexes dying of many diverse causes. They found that the incidence of moderate to severe coronary sclerosis was about twice as common in males as females. Their figures for the occurrence of aortic sclerosis are similar.

Ackerman, Edwards and Dry (53) compared 600 male and 600 female hearts for evidence of atherosclerosis. Sclerosis was estimated at 16 locations in the coronary vessels of each heart. The severity of the sclerosis was estimated by grading from 0 to 4, 0 representing no atheroma and 4 denoting complete occlusion of the vessel. Averages were taken of all the gradings at each location. The mean degree of sclerosis of the coronary vessels of females

under 40 was 1.13 compared to 1.56 for males of the same age.

It does not appear that the peculiar sex incidence of coronary artery disease is explicable simply on the basis of the occurrence or the extent of atheromatosis occurring in the intima of the coronary arteries, particularly in the younger age groups.

Certain anatomical factors have also been investigated. Dock (54) studied the epicardial portions of the coronary arteries of 12 male and 12 female newborn infants dying of various causes. He reported that as a group there was a greater incidence of intimal thickening in eight out of 15 males, but in only four out of 15 female infants. They also report that the sites of intimal thickening were in areas which are usually associated with coronary thrombosis in adult life. Minkowski (56) examined the intima in the coronary arteries of 122 males and 82 females whose ages varied from newborn to one month. He could establish no significant sex difference. When he studied a sub group of infants dying within 24 hours of birth from trauma or asphyxia he was able to show a significantly greater degree of intimal thickness in the males. It is uncertain whether these intimal changes are temporary, or whether they are of a permanent nature. It will require more extensive studies to resolve this problem.

Endocrine factors may be important in the sex incidence of coronary artery disease. The occurrence of the disease is very uncommon in females below the age of 40 during the active sex life, but becomes increasingly prevalent during the post-menopausal period. Secondly, the blood lipid pattern in the females pre-menopausally differs considerably from that of the male of the same age. Nikkila (1) reports that females below 40 years of age have a smaller proportion of cholesterol in the form of beta lipoprotein and a greater proportion of cholesterol in the alpha lipoproteins when compared with men in the same age group. Above 40 the sex difference becomes insignificant. Page (57) has pointed out that the Sf 10 - 20 (beta lipoprotein) fraction is present in smaller amounts in young females than in their male counterparts. An exaggeration of this difference is often found in the serum of individuals developing clinical atherosclerotic disease. After the menopause the female blood lipoprotein pattern closely resembles that of the male. The range of concentration of cholesterol and phospholipid in the plasma is the same in males and females (58). The range of concentration of total lipids is also unaffected by the sex of the subjects.

The experimental work done on cockerels by Pick, Stamler and Katz (59) may be of considerable significance, since it tends to give support to the concept that endocrine factors might be

responsible for the relative freedom of young females from clinical coronary artery disease. These investigators noted that the control cockerels fed a cholesterol and fat supplemented diet developed both aortic and coronary sclerosis of considerable degree. The test groups were fed oestrogens in the diet for several weeks prior to the sacrificing of the animals. Much less atheroma occurred in the coronary arteries of the test group, and three of the animals had no evidence of the disease at this site. Interestingly, the aortas of the animals in the test group were not significantly different from those of the control groups. The results were suggestive of some selective protection of the coronary vessels over the aortas by the oestrogenic hormone. The total serum cholesterol tended to be higher in the test group, suggesting that the hormone might be mobilizing the lipids from the vessel walls and causing an increase in the circulating lipid levels.

Several clinical trials with gonadal hormones have been reported. Russ, Eder and Barr (60) treated seven survivors of myocardial infarction with typical abnormal blood lipid patterns, with estinyl (oestradiol), 0.5 mgm. daily. All the subjects showed a change in the distribution of the blood lipids. Using ethanol fractionation techniques, they showed that there was an increase in the fractions 4, 5 and 6 (alpha lipoprotein) and a corresponding

decrease in the beta lipoprotein of fractions 1 and 3. Usually a decrease also occurred in the total cholesterol, the total phospholipid and in the cholesterol phospholipid ratio. The serum lipid patterns of seven cases of hypercholesterolemia and xanthomatosis exhibited similar changes although the patterns were never completely restored to normal.

Methyl testosterone was administered to two normal and seventeen abnormal individuals, the latter suffering from various disease states. This drug produced almost exactly the opposite result to oestrogen. The most constant effect was a change in the distribution of the lipoproteins with an increase in the beta lipoprotein fraction.

Cooper (61) confirmed the results of Russ et al, both on the effect of oestrogens on the blood lipid levels of atherosclerotic males and on the "atherogenic" effect of androgens. He also noted that four castrated or eunuchoid males had lipoprotein patterns similar to those found in young pre-menopausal females. Patients with prostatic carcinoma, treated with oestrogens were noted to have "better lipoprotein spectra than would be anticipated for their age". Interestingly, in two post-menopausal females with atherosclerosis and hypertension who were treated with oestrogen, the lipoprotein pattern was not altered.

It seems obvious that the oestrogenic hormones play some role in modifying the serum lipids and perhaps in altering the susceptibility of the female to atherosclerosis. Unfortunately these hormones, used in doses employed in the above clinical trials were reported to have produced fairly severe feminizing effects. The male patients usually complained of some gastrointestinal upset, and loss of libido. In their present form at these doses, oestrogens seem to have limited application.

(e) The effect of diet on blood lipids and atherosclerosis.

Considerable emphasis is currently being placed on the dietary control of blood lipids, in the hope that this may inhibit the atherosclerotic process. Within the last few years, increasing evidence has been advanced to demonstrate a relationship between the diet and this disease. Observations made on large European populations during the recent war suggested that a fall in the dietary fat intake of these people coincided with decreased evidence of atherosclerotic disease during this period (62). Studies made on certain native populations in Asia revealed that those individuals who subsist normally on low fat diets have a low incidence of atherosclerotic disease compared to that found in North American populations. Steiner (63) in 1944 for example, noted a relative freedom from

degenerative heart disease in 155 autopsies done on Okinawans of all ages. Only one case of coronary sclerosis and seven cases of aortic sclerosis were found in the group. It would be oversimplifying a complex problem to conclude that the only variable to be considered in the pathogenesis of atherosclerosis in these studies was a low fat diet. Many other factors, such as the total caloric intake, protein consumption and other unknown stresses may have played a part. However, these observations gave great impetus to the study of the effect of diet on the development of atherosclerosis both in animals and in humans.

It was known that cholesterol fed rabbits will first develop hypercholesterolemia and later atherosclerosis. Dogs, fed large amounts of cholesterol and high doses of thiouracil, develop hypercholesterolemia and atherosclerotic aortic degeneration (64). This experimental work led to the concept that dietary cholesterol might be the causative factor of this disease. It was soon evident, however, that the doses of cholesterol used in these animal experiments were very high and far in excess of any comparable dose which could be tolerated in the human diet.

Clinical studies by Wilkinson (65), Gertler (66) and later by Keys (67) showed no relationship between the total serum cholesterol and the dietary cholesterol in humans.

Ancel Keys' very extensive studies in North America and in various parts of Europe were of major importance in incriminating dietary fat as the agent which seemed responsible for the high incidence of atherosclerotic disease.

In reviewing international vital statistics, Keys noted an excessively high death rate in the United States over the entire span of adult life, which was mainly due to degenerative heart disease (myocardial infarction, angina pectoris, coronary sclerosis). It can be shown that an increase in dietary fat from 30% to 45% of the total caloric intake in the American population since 1910 can be directly related to the rising incidence of degenerative heart disease in the 40 to 65 year old males in this population. Keys noted that "the different age specific death rates of men from 40 to 65 from degenerative heart disease in different countries are directly related to the difference in those countries in the proportion of the total calories derived from total fats. The relationship in women is much less clear". In his series on a large group of Minnesota males, which he considers typical of American males in general, Keys (68) noted that the serum cholesterol concentration changes directly with changes in the total fat content of the diet, and that this response begins in a few days, and reaches a plateau in a few weeks.

On the basis of personal investigation, Keys (69) compared populations in Italy (Naples) with those in Minnesota and England (London). He attempted to show that the level of the total serum cholesterol is not dependent on the total caloric intake or relative obesity as judged from relative body weight or subcutaneous fat thickness. Fats contributed about 35.4% of the caloric intake of the Englishmen, 40% of that of the Minnesotans, and 20% of that of the Neapolitans. Proteins provided about 12 to 13% of the total calories in all groups. The men studied in London were fairly lean when compared to those in Minnesota or in Naples, averaging only 91% of the relative obesity of the Minnesota and Neapolitan samples. On the other hand, the Neapolitans had the lowest average serum cholesterol, despite the fact that they were the fattest, averaging about 103% of the U. S. standards for relative obesity.

Keys concludes that in healthy young men the serum cholesterol seems to be independent of the diet until about age 30, but from that age on, in men on relatively high fat diets, as in England and the U. S., the serum cholesterol increases by small yearly increments to middle age. That this phenomenon is not an inevitable result of aging is shown by the fact that large groups of men in Italy and Spain on low fat diets do not have this same increase in total serum cholesterol beyond early adult life.

Adlersberg (70) on the other hand, studying 1,500 subjects of middle class incomes in Staten Island, New York, found that the serum cholesterols increase in males from 20 to 30, and then remain constant to age 60. This disparity with Keys' findings has not yet been explained.

Low fat diets.

There have been few reports of clinical trials of treatment with low fat diets. Lindquist (71) studied 17 individuals of both sexes from ages 50 to 77 who had suffered cerebro-vascular accidents, and who were in a static state with considerable mental and physical disability of some duration. Their pre-treatment caloric intake of 2,200 calories daily and their body weights remained unchanged during the treatment period.

These individuals were placed on diets containing 25 gm. of fat, less than 75 mgm. of cholesterol and 85 gm. of protein. The remaining calories were supplied as carbohydrate. Seven individuals received this diet alone, and the remainder received lipotrope supplements. The patients were assessed at five and 11 weeks after the institution of therapy.

The criteria for mental improvement were a disappearance of confusion and disorientation. An improvement in motility is defined as an improvement in voluntary movement at physiotherapy

and may be secondary to mental improvement.

Eleven of the total 17 patients showed mental improvement and eight of these 11 also had increased motility. These improvements occurred in patients with a moderate degree of mental impairment but the demented patients failed to respond to treatment. The improved cases were equally distributed through both treatment groups (diet alone and diet plus lipotrope). Decreases of the total serum cholesterol occurred in 10 of 11 cases whose pre-treatment values were over 200 mgm.%. There was no correlation with clinical improvement, however.

Thus, it would seem from Lindquist's work, that a low cholesterol and low fat diet is as effective as one supplemented with lipotropic factors and further, it was shown that the clinical result is independent of any changes occurring in the blood cholesterol.

Nelson (72) studied 175 individuals suffering from the sequelae of cardiac and cerebral atherosclerosis. These individuals were placed on diets containing 30-40 grams of fat daily. Unfortunately, no mention is made of the total caloric intake. The patients were followed from 36 to 72 months.

The figure for the post treatment lipid level was derived from the averaging of several serial determinations. Successful treatment is defined as a lowering of the blood cholesterol by 7%

or a decrease of the cholesterol phospholipid ratio by 12% over the pre-treatment values. The treatment failures were those who did not meet these requirements. Some individuals stopped treatment after a few months. These were often patients who had improved sufficiently that they no longer considered such a rigorous diet necessary.

The death rate in the successful treatment group was approximately 10% (11 patients). The death rate in the group of patients whose blood lipids did not meet the requirements for successful treatment (treatment failures and those who stopped treatment) was 32% (20 patients). The difference between the two groups is statistically significant.

The clinical response of Nelson's patients to treatment with a low fat diet is in accord with Lindquist's work, but the latter did not find that this clinical response was correlated with a change in the serum lipids. It will require further studies to resolve this point.

The use of vegetable oils.

An important discovery was accidentally made by Kinsell in 1952 (73). While treating a patient on a diet which was made up entirely of vegetable oils, he noticed a striking fall in the total and

esterified serum cholesterol and phospholipids. Further studies of individuals on such diets often revealed decreases averaging 100 mgm. in the total cholesterol in the normocholesterolemic individuals and more in the hypercholesterolemic ones. When iso caloric amounts of animal fat were substituted for the vegetable oils, a prompt rise to pre-treatment levels of cholesterol occurred. These findings were confirmed by Ahrens (74) and Beveridge (75). These properties of vegetable oils are apparently not due to some positive factor such as vegetable sterol or phospholipid, since diets with large amounts of vegetable oils are more effective in lowering the serum lipids than diets containing less vegetable oil and heavily supplemented with vegetable sterols and phospholipids. A popular concept in the literature is that this antilipemic property of vegetable fat is related to its content of unsaturated fatty acids (76), but there is some evidence to suggest that the ability of a vegetable fat to lower serum lipids is not entirely related to its unsaturated fatty acid content. Keys (77) has recently demonstrated that corn oil has superior antilipemic properties to certain other oils which contain a greater proportion of unsaturated fatty acids.

It must also be pointed out that it has been demonstrated that either vegetable or animal fat given to an individual on a very low fat diet causes an elevation of blood lipids. At the present time

the advantage of vegetable oil would seem to be that iso calorically it produces less of a rise in blood lipids than animal fat, but it is inferior to a very low fat iso caloric diet in this respect. From a practical point of view, however, it is difficult to maintain a very low fat diet, and the use of vegetable oils of certain types would seem to be a reasonable compromise if used in moderate amounts. The study of the effect of various diets on blood lipids is at present an active and popular one. Undoubtedly in a short time many of the current concepts may be rendered obsolete.

(f) The use of plant sterols as antilipemic agents.

Recently plant sterols have been introduced as antilipemic agents. The crude forms have been further refined to their various constituent sterols and the most popular of these at the present time is the beta sitosterol. The exact mode of action of the sitosterols is not positively established. Chemically, beta sitosterol is very similar to cholesterol except that it has an ethyl group on the 24th carbon atom. Both compounds possess a hydroxy group on the 3rd carbon atom and in the presence of bile salts and pancreatic esterase, they will form esters with fatty acids, which is a necessary step before absorption of either can occur. The sitosterols are poorly absorbed, however, only about one tenth as effect-

ively as is cholesterol. Those which are absorbed retain their structure and are not converted to cholesterol (78). The postulated mechanism of the sitosterols is that they compete with cholesterol for esterification with fatty acids in the gut and thus prevent this necessary step in cholesterol absorption. The sitosterols are said to be more effective than a low cholesterol diet because they not only interfere with exogenous cholesterol absorption, but also with cholesterol which has been secreted into bile and which is normally reabsorbed in the small bowel. The sitosterols are not known to have any other action than that mentioned above.

Interest arose in the possible therapeutic value of these compounds when various investigators demonstrated that they were capable of producing a reduction of the serum lipids and inhibiting atherosclerosis when fed as a constituent of the diet for prolonged periods of time to cholesterol fed animals (79).

The sitosterols have been tried extensively in humans. The general consensus of opinion seems to be that they are effective in lowering the total serum cholesterol by about 15% of the mean control values, when given in doses of from 8 to 20 gm. daily (80, 81, 82). They do not seem to be effective if the total serum cholesterol is below 200 mgm.%. Best (83) reports that beta sitosterol in doses of 20 to 25 gm. daily fed to patients on ad lib diets

results in a sustained fall of the total serum cholesterol, the total lipids and neutral fat, and, to a lesser degree, of phospholipids. He also reports that some decrease occurs in the Sf 3 - 10, 10 - 30 and 30 - 100 classes of lipoproteins during sitosterol administration, but this effect is less consistent than the reduction of serum cholesterol.

Wilkinson, (84) on the other hand, challenges the effectiveness of these compounds to produce a true fall of blood lipids. His group reported no success in several clinical trials using the various sterol preparations. Wilkinson postulates that one of the possible reasons for the disparity between his results and those of other workers may be due to unexplainable spontaneous fluctuations of the serum cholesterol occurring over a control period of one to two years, which may be erroneously attributed to treatment with sitosterols. Wilkinson also excludes from his series many of the hypercholesterolemic states with which other workers have reported good success.

It is interesting to note that Wilkinson maintains his patients on iso caloric amounts of food during control and treatment periods. Most other workers have allowed ad lib feedings during both periods. It is possible that the bulky and rather unpleasant sterols may depress the appetite and consequently effect the total caloric and total fat intake during the test period. This may secondarily affect the blood lipids.

BIBLIOGRAPHY TO THE REVIEW OF THE LITERATURE

1. Nikkila, E.: Studies on the lipid protein relationships in normal and pathological sera and the effect of heparin on serum lipoproteins. Scand. J. Clin. and Lab. Investigation. 5: Supp. 8, 1953.

2. Morrison, L.M., Hall, L., and Chaney, A.: Cholesterol and cholesterol ester levels in acute myocardial infarction. Am. Heart J. 35: 866, 1948.

3. Keys, A.: Atherosclerosis: A problem in newer public health. J. Mount Sinai Hosp. 20: 118, 1953.

4. Ahrens, E.H., and Kunkel, H.G.: The stabilization of serum lipid emulsions by serum phospholipids. J. Exp. Med. 90: 409, 1949.

5. Jackson, R.S., and Wilkinson, C.F.: The ratio between phospholipid and the cholesterols in plasma as an index of human atherosclerosis. Ann. Int. Med. 37: 1162, 1952.

6. Cohn, E.J., Strong, L.E., Hughes, W.L. Jr., Mulford, D.J., Ashworth, J.N., Melin, M., and Taylor, H.L.: Preparation and properties of serum and plasma proteins. IV. A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids. Am. Chem. Soc. J. 68: 459, 1946.

7. Gofman, J.W., Lindgren, F.T., and Elliott, H.: Ultracentrifuge studies of lipoproteins of human serum. J. Biol. Chem. 179: 973, 1949.

8. Jones, H.B., Gofman, J.W., Lindgren, F.T., Lyon, T.P., Graham, D. M. Stristower, B., and Nichols, A. F.: Lipoproteins in atherosclerosis. Am. J. Med. 11: 358, 1951.

9. Kunkel, H.G., and Slater, R.J.: Lipoprotein patterns of serum obtained by zone electrophoresis. J. Clin. Investigation. 31: 667, 1952.

10. Swahn, B.: Studies on blood lipids. Scand. J. of Clin. and Lab. Investigation. 5: Supp. 9, 1953.

11. Rosenberg, I.N., Young, E., and Proger, S.: Serum lipoproteins of normal and atherosclerotic persons studied by paper electrophoresis. Am. J. Med. 16:818, 1954.

12. Jencks, W.P., Durrum, E.L., and Jetton, M.R.: Paper electrophoresis as a quantitative method: The staining of serum lipoproteins. J. Clin. Investigation. 34: 1437, 1955.

13. Jencks, W.P., Hyatt, M.R., Jetton, M.R., Mattingly, T.W., and Durrum, E.L.: A study of serum lipoproteins in normal and atherosclerotic patients by paper electrophoretic techniques. J. Clin. Investigation. 35: 980, 1956.

14. Gofman, J.W., Hanig, M., Jones, H.B., Lauffer, M.A., Lowry, E.Y., Lewis, L.A., Mann, G.V., Moore, F.E., Olmsted, F., Younger, F., Andries, E.C., Barach, J.H., Beams, J.W., Fertig, J.W., Page, I.H., Shannon, J.A., Stare, F.J., and White, P.D.: Evaluation of serum lipoprotein and cholesterol measurements as predictors of clinical complications of atherosclerosis. Circulation. 14: 691, 1956.

15. Hahn, P.F.: Abolishment of alimentary lipemia following injection of heparin. Science. 98: 19, 1943.

16. Anderson, N.G., and Fawcett, B.: An antichylomicronemic substance produced by heparin injection. Proc. Soc. Exper. Biol. and Med. 74: 768, 1950.

17. Anfinsen, C.B., Boyle, E., and Brown, R.K.: The role of heparin in lipoprotein metabolism. Science. 15: 583, 1952.

18. Graham, D.M., Lyon, T.P., Gofman, S.W., Jones, H.B., Yankley, A., Simonton, J., and White, S.: The influence of heparin on lipoprotein metabolism. Circulation. 4:666, 1951.

19. Boyle, E., Bragdon, J.H., and Brown, R.K.: The role of heparin in 'in vitro' production of alpha lipoproteins in human plasma. Proc. Soc. Exper. Biol. and Med. 81: 475, 1952.

20. Nichols, A.V., Freeman, N.K., Shore, B., and Rubin, L.: The interaction of 'heparin active factor' and lipoproteins. Circulation. 6: 457, 1952.

21. Soffer, A., and Murray, M.: Prolonged observations of the cardiovascular status in essential hyperlipemia with special reference to serum lipid response to heparin. Circulation. 10: 255, 1954.

22. Kuo, P.T., Joyner, C.R. Jr., and Reinhold, J.G.: Effects of fat ingestion and heparin administration on serum lipids of 'normal', hypercholesterolemic, hyperlipemic and atherosclerotic subjects. Am.J. Med. Sci. 232: 613, 1956.

23. Constantinides, P.: Personal communication.

24. Bragdon, J.H., and Havel, R.T.: In vivo effect of anti-heparin agents on serum lipids and lipoproteins. Am. J. Physiol. 177: 128, 1954.

25. Korn, E.D.: Clearing factor, a heparin activated lipoprotein lipase. Isolation and characterization of the enzyme from normal rat heart. J. Biol. Chem. 215: 1, 1955.

26. Korn, E.D.: Clearing factor, a heparin activated lipoprotein lipase. Substrate specificity and activation of coconut oil. J. Biol. Chem. 215: 15, 1955.

27. Anfinsen, C.B. Jr.: Biochemical aspects of atherosclerosis. Federation Proc. 15: 894, 1956.

28. Engleberg, H.: Human endogenous lipemia clearing factor (active factor). Am. J. Physiol. 181: 309, 1955.

29. Hueper, W.C.: The pathogenesis of atherosclerosis. Am. J. of Clin. Path. 26: 559, 1956.

30. Gruner, A., Hilden, T., Raaschore, F., and Vogelius, H.: Heparin treatment of angina pectoris. Am.J. Med. 14:433, 1953.

31. Rinzler, S.H., Travell, J., Bakst, H., Benjamin, Z.H., Rosenthal, R.L., Rosenfield, S., and Hirsch, B.B.: The effect of heparin in effort angina. Am.J. Med. 14:438, 1953.

32. Engleberg, H., Kuhn, R., and Steinman, M.: A controlled study of the effect of intermittent heparin therapy on the course of human coronary atherosclerosis. Circulation. 13: 489, 1956.

33. Waldron, J.M., and Friedman, M.H.F.: The relationship between anticoagulants and lipemia. Federation Proc. 7: 130, 1948.

34. Levy, S.W., and Swank, R.L.: Relationship of native heparin to clearing of alimentary lipemia. Proc. Soc. Exp. Biol. Med. 82: 553, 1953.

35. Cohen, H., and Tudhope, G.R.: Dextran sulphate: Use as an anticoagulant, and action in lowering serum cholesterol. B. M. J. 1: 1023, 1956.

36. Bernard-Griffiths, C., Cuvelier, R., Sebald, Mlle., Berger, J. A., and Andraud, G.: Considerations sur les lipoproteines du sujet age. Influence d'une medication lipotrope et anti-coagulante. Therapie. 10: 756, 1955.

37. Hurxthal, L.M.: Blood cholesterol and thyroid disease: Myxoedema and hypercholesterolemia. Arch. Int. Med. 53: 762, 1934.

38. Horlick, L., Paulier, D., and Katz, L.N.: Thyroid and experimental atherosclerosis in the chicken. Am. Heart J. 35: 863, 1948.

39. Stristower, B., Gofman, J.W., Galioni, E., Almada, A.A., and Siman, W.: Effect of thyroid extract on serum lipo-proteins and serum cholesterol. Metabolism. 3: 218, 1954.

40. Stristower, B., Gofman, J.W., Galioni, E.F. Rubinger, J.H., Poteau, J., and Guzvich, P.: Long term effect of dried thyroid on serum lipoprotein and serum cholesterol levels. Lancet. 1: 120, 1957.

41. Pitt-Rivers, R.: Physiological activity of the aceticacid analogues of some iodinated thyronines. Lancet. 2:234, 1953.

42. Pitt-Rivers, R., and Thebeault, O.: Immediate effects of thyroxine analogues on biological oxidation in vitro. Lancet. 1: 285, 1955.

43. Lerman, J., and Pitt-Rivers, R.: Physiologic activity of triiodothyroacetic acid. J. Clin. Endocrin. and Metab. 15: 653, 1955.

44. Trotter, W.R.: Effect of triiodothyroacetic acid in a case of myxoedema. Lancet. 2:374, 1955.

45. Trotter, W.R.: Effect of triiodothyroacetic acid on blood cholesterol levels. Lancet. 1:885, 1956.

46. Oliver, M.F., and Boyd, G.S.: The influence of triiodothyroacetic acid on the circulating lipid and lipoproteins on euthyroid men with coronary disease. Lancet. 1:124, 1957.

47. Gertler, M.M., Garn, S.M., and White, P.: Young candidates for coronary heart disease. J. A. M.A. 147: 621, 1951.

48. Glendy, R.E., Levine, S.A., and White, P.D.: Coronary disease in youth. J. A. M. A. 109: 1775, 1937.

49. Boas, E.P., and Epstein, F.H.: Prevalence of manifest atherosclerosis in a working population. Arch. Int. Med. 94: 94, 1954.

50. Leibow, L.M., and Hellerstein, H.K.: Cardiac complications of diabetes mellitus. Am. J. Med. 7: 660, 1949.

51. Allen, E.V., Barker, N.W., and Hines, E.A.: Peripheral vascular disease. Second edition. W. B. Saunders and Co. Philadelphia, 1955.

52. Willius, F.A., Smith, H.L., and Sprague, P.H.: A study of coronary and aortic sclerosis. Incidence and degree in 5,060 consecutive post mortem examinations. Proc. Staff Meet., Mayo Clin. 8:140, 1953.

53. Ackerman, R.F., Dry, T.J., and Edwards, J.E.: Relationship of various factors to the degree of coronary atherosclerosis in women. Circulation. 1: 1345, 1950.

54. Dock, W.: The predilection of atherosclerosis for the coronary arteries. J.A.M.A. 131: 875, 1956.

55. Frangman, R.J., and Hellwig, C.A.: Histology of coronary arteries in newborn infants. Am. J. Path. 23: 901, 1947

56. Minkowski, W.L.: The coronary arteries of infants. Am. J. Med. Sci. 214: 623, 1947.

57. Page, I.H.: The Lewis A. Connor Memorial Lecture: Atherosclerosis - an introduction. Circulation, 10:1, 1954.

58. Peters, J.P., and Man, E.B.: The interrelation of serum lipids in normal persons. J. Clin. Investigation. 22:707, 1943.

59. Pick, R., Stamler, J., Rodbord, S., and Katz, L.N.: Estrogen induced regression of coronary atherosclerosis in cholesterol fed chicks. Circulation, 6: 858, 1952.

60. Russ, E.M., Eder, H.A., and Barr, D.P.: Influence of gonadal hormones on protein lipid relationships in human plasma. Am. J. Med. 19: 4, 1955.

61. Cooper, E.E.: Sex hormones and atherogenesis. Am. Pract. Digest Treat. 7: 436, 1956.

62. Malmros, H.: Relation of nutrition to health: Statistical study of effect of wartime on atherosclerosis, cardio-sclerosis, tuberculosis and diabetes. Acta Medica Scand. 246: 137, 1950.

63. Steiner, P.E.: Necropsies on Okinawans. Arch. Path. 42: 359, 1946.

64. Steiner, A., and Kendall, F.E.: Atherosclerosis and arteriosclerosis in dogs following injection of cholesterol and thiouracil. Arch. Path. 42: 433, 1946.

65. Wilkinson, C.F. Jr., Blecha, E., and Reimer, A.: Is there a relation between diet and blood cholesterol. Arch. Int. Med. 85: 389, 1950.

66. Gertler, M.M., Garn, S.M., and White, P.D.: Diet, serum cholesterol and coronary artery disease. Circulation. 2: 696, 1950.

67. Keys, A., Mickelsen, O., Miller, E.O., and Chapman, C.B.: The relation in man between cholesterol levels in the diet and in the blood. Science. 112: 79, 1950.

68. Keys, A., Anderson, J.T., Fidanza, F., Keys, M.H. and Swahn, B.: Effect of diet on blood lipids in man. Clin. Chem. 1: 34, 1955.

69. Keys, A., and Keys, M.H.: Serum cholesterol and the diet in clinically healthy men at Slough near London. Brit. J. Nutrition. 8: 138, 1954.

70. Adlersberg, D., Schaefer, L.E., Steinberg, A., and Wang, C.: Age, sex, serum lipids and atherosclerosis. J.A.M.A. 162: 619, 1956.

71. Linquist, G., and Isaksson, B.: Studies on the effect of a low lipid diet and lipotropic agents in cerebral atherosclerosis. Acta Med. Scand. 156: 11, 1956.

72. Nelson, A.M.: Treatment of atherosclerosis by diet. Results in patients followed 36 - 72 months. Northwest Med. 55:643, 1956.

73. Kinsell, L.W., Partridge, J., Boling, L., Morgen, S., and Michaels, G.: Dietary modification of serum cholesterol and phospholipid levels. J. Clin. Endocrin. 12: 909, 1952.

74. Ahrens, E.H. Jr., Blanhenhorn, D.H., and Tsaltas, T.T.: Effect on human serum lipids of substituting plant for animal fat in diet. Proc. Soc. Exper. Biol. and Med. 86: 872, 1954.

75. Beveridge, J.M.R., Connell, W.F., Mayer, G., Firstbrook, J. B., and De Wolfe, M.: The effects of certain vegetable and animal fats on plasma lipids of humans. Circulation, 10:593, 1954.

76. Kinsell, L.W., and Michaels, G.D.: Hormonal nutritional lipid relationships. Fed. Proc. 14: 661, 1955.

77. Keys, A., Anderson, J.T., and Grande, F.: Essential fatty acids, degree of unsaturation and effect of corn (maize) oil on the serum cholesterol level in man. Lancet. 1:66, 1957.

78. Gould, R.G.: Absorbability of beta sitosterol. Trans. N.Y. Acad. Sci. 18:129, 1955.

79. Siperstein, M.D., Nichols, C.W. Jr., and Chaikoff, I.L.: Prevention of plasma cholesterol elevation and atheromatosis on the cholesterol fed bird by the administration of di hydro cholesterol. Circulation. 7:37, 1953.

80. Best, M. M., and Duncan, C.H.: Modification of abnormal serum lipid patterns by administration of sitosterol. Ann. Int. Med. 45: 614, 1956.

81. Pollack, O.J.: Reduction of blood cholesterol in man. Circulation. 7:702, 1953.

82. Steiner, A., and Riley, F.P.: The effect of beta and dihydro beta sitosterol in the serum lipids of patients with coronary atherosclerosis. Circulation. 12: 483, 1955.

83. Best, M.M., Duncan, C.H., Van Loon, E.J. and Wathen, J.D.: The effects of sitosterol on serum lipids. Am. J. Med. 19: 61, 1955.

84. Wilkinson, C.F. Jr., Jackson, R.S., Bozian, R.C., Benjamin, M.R., Levere, A.H., Croft, G., and Davidson, N.W.: Clinical experience with sitosterols. Trans. N.Y. Acad. Sci. 18: 119, 1955.

III. EXPERIMENTAL STUDIES

1. The Effect of Anticoagulants on the Blood Lipids in Atherosclerotic Subjects.

METHOD

The effect of heparin in vivo on blood lipids is well documented (15, 18). Very little information is available, however, on the effect of other anticoagulants in this regard. Since phenylindanedione and many of the coumarin derivatives are widely used in the treatment of atherosclerotic diseases, it was felt worthwhile to determine their effect on the blood lipids.

Six groups of patients were studied.

A control group of seven middle aged atherosclerotic subjects who were untreated, had blood taken for study at two week intervals for eight weeks. None of these individuals had suffered a recent vascular accident. The blood serum was either analyzed immediately or quickly frozen and stored for future analysis.

In addition, the blood lipids of a group of young, healthy adults were examined at weekly intervals for a period of four weeks.

Atherosclerotic patients treated with certain anticoagulant drugs formed three groups of subjects. Of these three groups, the first consisted of eight individuals treated with phenylindanedione (Danilone), the second included ten cases who received an oxycoumarin derivative, (Sintrom), and the third group was composed of six patients anticoagulated with ethyl biscoumacetate (Tromexan). The age

range in each group was from 50 to 70 years and each contained one female patient. The initial total serum cholesterols of the individuals studied ranged from approximately 200 to 350 mgm. percent. Because the majority of the subjects who were studied had experienced recent myocardial infarctions or in a few cases cerebral thromboses, it was only possible to obtain one blood sample before the institution of treatment with anticoagulants. Sera were taken subsequently at two week intervals for eight weeks. The dosages of all three anticoagulants varied from one individual to another, but all were given sufficient to produce therapeutic levels of anticoagulation.

The anticoagulants were administered orally in divided dosages daily. All patients had daily prothrombin times determined by the Quick one stage method for the first two to three weeks until a constant prothrombin level was obtained. Thereafter, prothrombin times were determined at one or two week intervals. The prothrombin time was maintained at about 25 to 30% of normal in all cases.

Finally, six atherosclerotic individuals were treated with heparin in doses insufficient to produce anticoagulation. All subjects had control sera taken for study before the initiation of treatment.

The first group of t wo elderly males received heparin in doses of 20 mgm. subcutaneously every 72 hours for one week. The dose was then increased to 200 mgm. subcutaneously every 72 hours.

Blood was drawn for study 72 hours after the preceding injection of heparin at approximately weekly intervals for six weeks.

The second group of four individuals consisted of a 60 and a 40 year old male, and a 40 and a 65 year old female. Heparin was administered to these individuals in dosages of 200 mgm. subcutaneously every 48 hours. Blood was drawn for study 48 hours after the preceding injection of heparin at approximately weekly intervals for six weeks.

Since many of the atherosclerotic subjects in the groups studied had shown previous evidence of vascular disease, many of these had been placed on diets of moderate fat and caloric intake months previous to the present study. In any case, as far as is known, the diets of all patients in this series were unchanged immediately prior to and during the period of observation.

The following lipid measurements were made on the sera of the individuals of all the groups studied.

The total serum cholesterol was determined on each sample of serum using the method of Zlatkis, Zak and Boyle (1). The variation on duplicate determinations of the same serum was about 15 mgm. %. The total phospholipid phosphorous was determined on each sample according to a modification of the method of Youngberg and Youngberg (2). The variation on duplicate determinations was 1 mgm. %. The total

cholesterol phospholipid ratio was calculated from the results.

The total serum lipids were determined on the sera of the patients studied using the turbidimetric method of Kunkel, Ahrens and Eisenmenger (3). The variation on duplicate determinations of the same sera was 50 mgm. %.

The lipoproteins were determined by paper electrophoresis after the method of Durrum (5). The separated lipoproteins were stained with either Sudan Black or Oil Red-O dye. In some cases duplicate strips of the same serum were obtained with Brom phenol blue for proteins to check the accuracy of the lipoprotein separation.

The stained lipoproteins were eluted with methanolic chloroform and the solutions read with a Beckman spectrophotometer at a wave length suitable to the appropriate dye.

The B lipoprotein was expressed as a percentage of the sum of the optical densities of the total Alpha and Beta lipoprotein lipid. The greatest variation encountered with repeated determinations of the same serum subjected to paper electrophoresis on different occasions and stained with either dye was 7%.

RESULTS

It will be seen in Fig. 1 that significant elevations occurred in the total serum cholesterol in the Sintrom and Tromexan treated groups ($p < .05$). In the Sintrom treated group the mean cholesterol rose becoming significant eight weeks after the initiation of treatment. At this time, nine of the ten individuals had elevations over pretreatment values; the one exception had a fall of 4 mgm. %.

In the group of subjects treated with Tromexan, significant elevations in the total serum cholesterol were noted at four, six and eight weeks. Five of six subjects had increases of their total cholesterol over pretreatment values at these time periods. The one exception showed a rise in the total cholesterol at only the four and six week periods and was back to pretreatment levels at eight weeks.

In the groups of normal subjects, atherosclerotic controls and Danilone and Heparin treated individuals, the average total serum cholesterols varied from one occasion to another but no significant changes occurred.

No significant changes occurred in the B % of the total lipoprotein lipid in any group except those subjects treated with Heparin in doses of 200 mgm. every 48 hours. (The two subjects treated with 200 mgm. of Heparin every 72 hours showed no change in the B % of the total lipoprotein lipid and were not illustrated graphically).

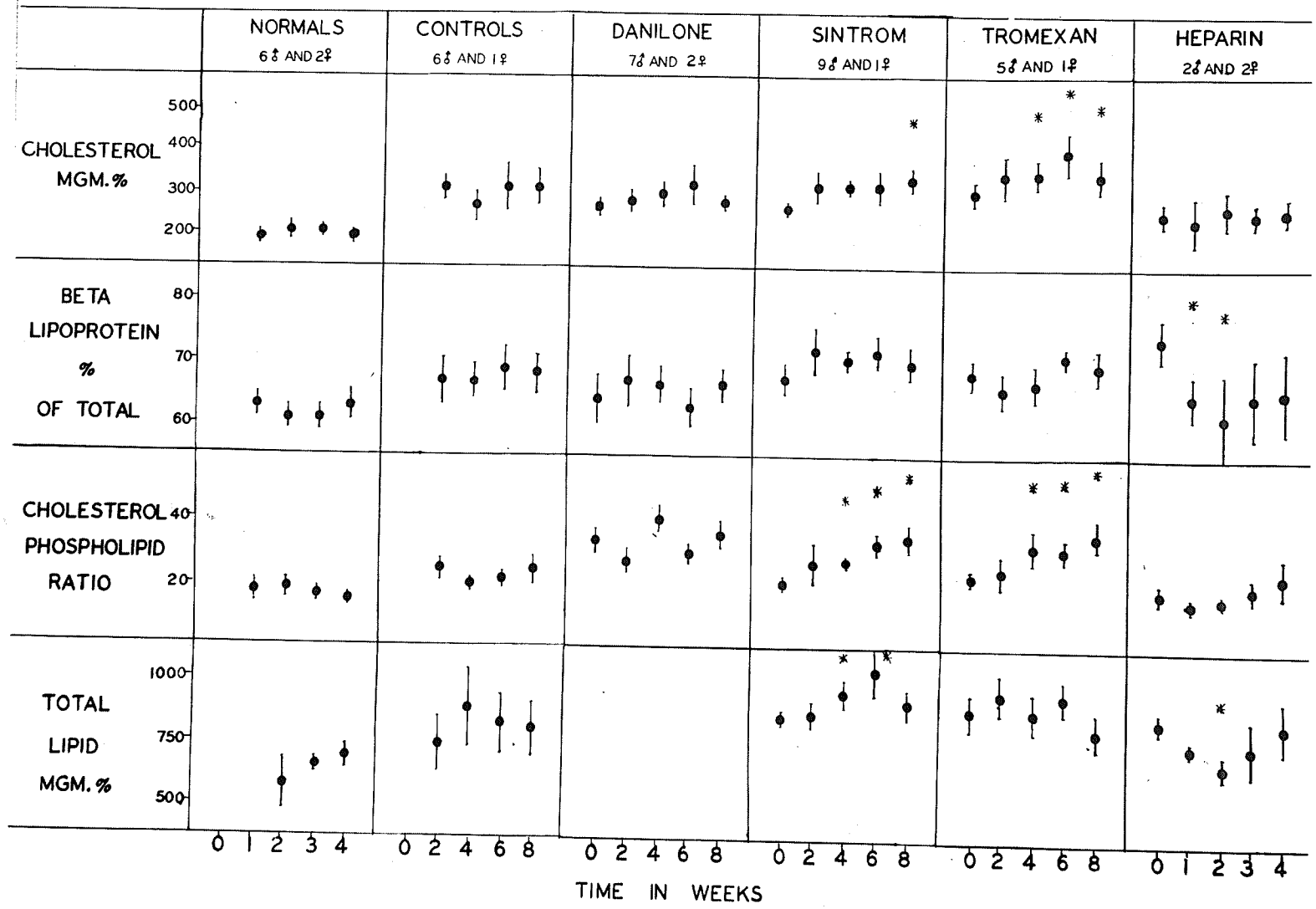

Fig. 1. **The Blood Lipids of Normal Individuals and Untreated and Treated Atherosclerotic subjects.**

Mean values ± S. D.

* Significant differences from time 0 [illegible]

In the former group the B % of the total lipoprotein lipid decreased in all five individuals at one week after the start of treatment and in four of the five at two weeks. This was significant at both time periods ($p < .05$). Thereafter only two individuals had values lower than pretreatment levels. In the remainder these values returned to the control level despite continued treatment.

The cholesterol phospholipid ratio was significantly elevated in the Sintrom and Tromexan treated groups at four, six and eight weeks ($p < .01$ and $p < .05$ respectively). It will be noted that significant elevations of the cholesterol phospholipid ratio corresponded approximately to significant elevations of the total serum cholesterol in these two groups and in addition, at two other time periods in the Sintrom treated group. (At these two time periods the average total cholesterols were increased over pretreatment levels but not significantly).

The total serum lipids were significantly elevated in the Sintrom treated group at four and six weeks. The total serum lipids were also significantly decreased at two weeks in the Heparin treated group which corresponds with a significant fall in the B % of the total lipoprotein lipid at that time period.

DISCUSSION

There is an apparent difference between the lipid values of the groups anticoagulated with the coumarin derivatives, Sintrom and Tromexan, and the group treated with phenylindanedione. In the former two groups significant elevations of the total serum cholesterol and cholesterol occurred. These were at 8 weeks in the Sintrom-treated individuals and at 4, 6 and 8 weeks in the Tromexan treated group.

No significant changes occurred in the total serum cholesterol of the individuals treated with Danilone.

The B percentage of the total lipid lipoprotein lipid was not significantly altered in any of the three groups. Since the main portion of the total serum cholesterol is carried by the B lipoprotein (6) it might be expected that a significant rise in the total serum cholesterol would be paralleled by a rise in the B percentage of the total lipoprotein lipid. Cholesterol, however, is not stainable in the free form and is poorly stained in the esterified form. The method employed in this study involves the staining of the triglyceride components of the lipoprotein lipid and it is, therefore, possible that an increase in the cholesterol lipid in the B lipoprotein band could go undetected.

Bernard-Griffiths (7) reported that a combination of 40 mgm. of dicoumarol and 1.5 gm. of choline citrate given to athero-

sclerotic individuals produces a slight increase in the total serum cholesterol. Although not strictly comparable with our studies, since he employed two drugs and his patients were not well controlled at therapeutic levels of anticoagulation, this increase of the total serum cholesterol is compatible with our findings in the Sintrom and Tromexan treated groups. He reported a decrease of B lipoprotein in nine of 15 cases, but gave no information as regards the α lipoprotein. Thus, his claims are not necessarily inconsistent with our findings of no significant change in the ratio of B to the total lipoprotein lipid.

There are several possible explanations for the difference in the total serum cholesterol values of patients under treatment with phenylindanedione and the coumarin derivatives. The coumarins may have caused a rise in the total serum cholesterol by a decreased catabolism or an increased synthesis of cholesterol, or they may have caused an acceleration of the mobilization of cholesterol from vascular plaques. Alternatively, the coumarin drugs may have had no effect on the total serum cholesterol but phenylindanedione may have tended to depress the total serum cholesterol and prevented a possible rise after infarction. No data was found in the literature to show the effect of infarction on the total serum cholesterol in untreated individuals.

Further study of a group of atherosclerotics who have had recent myocardial infarctions or cerebral thromboses and who have received no anticoagulant therapy will ne necessary to resolve this problem.

In the patients treated with Sintrom and Tromexan in this series an interesting finding was that the total cholesterol phospholipid ratio was significantly elevated at time periods which corresponded to elevations of the total serum cholesterol. This is a reflection of the failure of the total phospholipids to increase simultaneously with the total serum cholesterol at these time periods. In the present state of knowledge of lipid metabolism, the interpretation of this result is difficult. Certain investigators such as Hueper (8), however, have maintained that an elevation of certain serum lipids without a simultaneous increase of the serum phospholipids produces a state of "plasmatic instability" which may contribute to the deposition of lipid deposits within the vessel walls.

The total serum lipid increased simultaneously with increases of the total serum cholesterol only in the Sintrom treated group at 4 and 6 weeks. Similar increases of the total serum fat did not occur in the Tromexan treated group at those time periods when significant elevations of the total cholesterol occurred. The failure of these two lipid measurements to parallel each other in these

instances, suggests that the fatty acids and the phospholipids do not parallel the total serum cholesterol.

The response of the blood lipids of individuals to treatment with Heparin varied in the present series. It was not possible to demonstrate changes in the lipids of two individuals who received this drug in dosages of 200 mgm. every 72 hours, while such changes were apparent in the blood lipids of others who received Heparin in the same dosage every 48 hours. Many possibilities for this difference are apparent. The first two individuals treated with this drug every 72 hours may have been refractory to it. Nikkila (6) has described two such individuals. On the other hand, a more likely possibility would seem to be that the frequency of administration was an important factor. Finally, the blood may have been taken at too long an interval after the administration of Heparin in these two cases. Graham (9) has demonstrated that the B lipoprotein fraction is only reduced for a period of 24 hours in the sera of rabbits treated with intravenous Heparin.

The lack of a significant response of the total serum cholesterol in either of the two groups treated with Heparin is in accord with the findings of many authors (10, 11, 12).

The total serum lipid in this heparin treated group (200 mgm. every 48 hours) was reduced coincidentally with the B percent-

age of the total lipoprotein lipid at one and two weeks after the institution of treatment. Soffer (10) has also recorded a fall in the total serum lipid in individuals treated with Heparin. In his work, however, the drug was given intravenously and the changes in the total lipid were reported to be of only 24 hours duration. It was interesting to note that in the present series the changes in the blood lipids which were noted initially in all the subjects were only transitory in three of the five individuals. The lipid values of these latter individuals were observed to be back at the pretreatment baseline on blood taken at four weeks after the institution of treatment. Thus, it would seem reasonable to speculate that three of the individuals who were successfully treated initially became refractory to the drug.

Finally, it is interesting to note that the young normal group of individuals in this series could be separated from the atherosclerotic controls more easily on the differences in their average total cholesterol values than by the average B percentage of the total lipoprotein lipid values. This division is of little importance in itself since it may be due to a difference in ages rather than an indication of the atherosclerotic process. It does suggest, however, that the determination of the B percentage of the total lipoprotein lipid by the methods used in this study is not a superior lipid measurement to older, simpler determinations for accomplishing such a division.

CONCLUSIONS

In the group of eight Danilone treated patients, no signifant changes occurred in the total serum cholesterol.

In the group of ten patients treated with Sintrom, significant elevations of the total serum cholesterol occurred at eight weeks after treatment was started.

In the Tromexan treated group of six individuals, significant elevations of the total serum cholesterol occurred at 4, 6 and 8 weeks.

No significant changes occurred in the B percentage of the total lipoprotein lipid at any time period in any of the three groups treated with these drugs.

Significant elevations of the total cholesterol phospholipid ratio occurred at 4, 6 and 8 weeks in both the Sintrom and Tromexan treated groups.

The blood lipids of two atherosclerotic males, ages 64 and 71, who were treated with Heparin in dosages of 200 mgm. every 72 hours remained unchanged.

In the other group of five atherosclerotic individuals treated with a similar dose of Heparin every 48 hours, it was noted that the B percentage of the total lipoproteins was significantly reduced at one and two weeks after the institution of treatment. The total serum

lipid was correspondingly reduced in four of the five individuals at one week and in all of them at two weeks. The total serum cholesterol and the cholesterol phospholipid ratio were unchanged.

THE EFFECT OF TRIIODOTHYROACETIC ACID (TRIAC) ON BLOOD LIPIDS IN ATHEROSCLEROTIC SUBJECTS

Although it was noted many years ago that the thyroid hormone was capable of reducing the total serum cholesterol of euthyroid subjects (37), it was only recently that this was suggested as a possible therapeutic tool in the management of atherosclerotic individuals (39, 40). The synthesis of various thyroxin analogues intensified interest in this possibility, particularly since it had been suggested that some of these substances might be metabolically inactive but selectively retain their anticholesterolemic properties (43). Several studies have been performed which indicate that certain thyroxin analogues, such as triiodothyroacetic acid (TRIAC), may be capable of reducing both the total serum cholesterol (45, 46) and also the B lipoprotein cholesterol (46). Despite the speculation which arose early in the development of the thyroxin analogues, it is now apparent that they have the same metabolic properties as thyroxin (46).

The present study was undertaken to confirm the findings of other workers regarding the efficacy of TRIAC in reducing the blood lipids.

METHOD

Six atherosclerotic patients treated with triiodothyroacetic acid (TRIAC) were studied. Blood was drawn before the initiation of treatment and at regular weekly intervals for four weeks after the initiation of treatment. Two of these individuals received 2 mgm. daily for approximately four weeks. The remaining four received 4 mgm. daily. With the exception of one individual who suffered a myocardial infarction soon after starting treatment, the remainder were also followed for four weeks. All individuals were from 50 to 70 years of age and were male with one exception. A control group of untreated atherosclerotic individuals of the same age and sex as those in the test group were studied at two week intervals for eight weeks. The diets of the individuals of both groups were unchanged during the treatment period.

The following lipid measurements were made on the sera of all individuals studied.

The total serum cholesterol was determined on each sample of serum according to the method of Zak, Zlatkis and Boyle (1).

The total phospholipid phosphorous was performed using a modification of the method of Youngberg and Youngberg (2).

The total cholesterol phospholipid ratio was calculated from the foregoing results.

The total serum lipids were performed by the turbidimetric method of Kunkel, Ahrens and Eisenmenger (3).

The chloroform turbidity of the serum was determined by the method described by Green (18).

RESULTS

The results were analyzed as paired data, comparing the pre-treatment values at time O with the values at each time period in the graph.

The lipid values of a group of six untreated middle aged atherosclerotic males and one female are illustrated in Tables I and II. These were used for controls for comparison with the two groups of individuals treated with triiodothyroacetic acid (TRIAC).

Two atherosclerotic patients, a 72 year old male and a 41 year old female, were treated with TRIAC in dosages of 2 mgm. daily. Their lipid measurements are illustrated in Tables III A, IV A, and V A. No notable changes occurred in the serum lipid values of the male. In the female, (designated as N), however, there was a decrease in the total cholesterol from a pre-treatment level of 460 mgm. to 331 mgm. in blood drawn one week after the initiation of treatment (Table III A). This decrease was maintained throughout the treatment period of four weeks. No changes were noted in the B percentage of the total lipoprotein lipid. The cholesterol phospholipid ratio fell with the reduction of the total cholesterol (Table IV A). The fall in the total cholesterol was paralleled by a decrease in the level of the total serum lipid from a pre-treatment level of 1232 mgm. to 837 mgm. at one week. This decrease of the total serum lipid was

not well sustained since the total lipid of this individual varied just under pre-treatment levels despite continued therapy (Table IV A). The chloroform turbidity values were sharply reduced on blood drawn at all time periods after the initiation of treatment in this individual (Table V A).

Three middle aged atherosclerotic males were treated with TRIAC in dosages of 4 mgm. daily. Their results are illustrated in Tables III B, IV B, and V B. All of these had initial total serum cholesterol and total lipid values under 250 and 700 mgm. respectively.

There was some fluctuation in the levels of the total serum cholesterol (Table III B) of these three individuals, but no trends occurred. The variation in their lipid values was similar to that observed in the control group. (Table I). There was a slight decrease of the B percentage of the total lipoprotein lipid in two of three, which was maintained throughout the treatment period. Similarly, there was a slight decrease of the chloroform turbidity values in all three patients after the initiation of treatment.

Neither of these reductions was of statistical significance.

None of the patients treated with TRIAC in the present series with either dosage of 2 or 4 mgm. daily, developed any clinical evidence of hypermetabolism during the four weeks of study.

TABLE I

TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF A GROUP OF UNTREATED MIDDLE AGED ATHEROSCLEROTIC CONTROLS

CONTROLS

	CHOLESTEROL mgm. %				B-LIPOPROTEIN % total			
No Rx.	2 wks.	4 wks.	6 wks.	8 wks.	2 wks.	4 wks.	6 wks.	8 wks.
M.	321	308	384	337	77	77	80	78
G.	308	260	200	263	64	61	62	62
P.	298	283	264	352	67	68	74	65
R.	198	170	207	178	67	68	68	60
C.	292	258	350	285	59	63	59	72
H.	566	453	545	533	80	74	78	79
Pl.	193	180	224	193	54	60	63	63
Mean	311	273	311	314	67	67	69	68
S.D.	±54	±39	±52	±49	±8.7	±2.6	±3.4	±3.3

TABLE II

THE TOTAL FAT VALUES AND THE TOTAL CHOLESTEROL PHOSPHOLIPID RATIOS OF A GROUP OF UNTREATED MIDDLE AGED ATHEROSCLEROTIC CONTROLS

CONTROLS

	CHOLESTEROL/PHOSPHOLIPID				TOTAL FAT mgm.%			
	2 wks.	4 wks.	6 wks.	8 wks.	2 wks.	4 wks.	6 wks.	8 wks.
M.	30	21	24	21	1060	1022	1167	1097
G.	20	16	14	21	774	840	634	882
P.	22	20	21	28	1087	997	1044	997
R.	18	15	20	20	517	640	552	517
C.	26	18	21	20	636	651	621	-
H.	39	27	30	44	-	1527	1177	912
Pl.	21	23	23	22	408	494	574	433
Mean	25	20	22	25	747	881	824	806
S.D.	±3	±1.7	±2.0	±3.5	±106	±149	±118	±106

TABLE III

(A) THE TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF TWO ATHEROSCLEROTICS TREATED WITH 2 MGM. OF TRIAC DAILY

(B) THE SAME LIPID MEASUREMENTS OF THREE ATHEROSCLEROTICS TREATED WITH 4 MGM. of TRIAC DAILY

TRIAC

		B - LIPOPROTEINS - % total				CHOLESTEROL mgm. %				
	Pre Rx	1 wk.	2 wks.	3 wks.	4 wks.	Pre Rx.	1 wk.	2 wks.	3. wks.	4 wks.
A.	G 70	59	71	75	66	300	310	325	279	329
	N 69	73	66	61	71	460	331	334	351	324
B.	F 69	67	70	64	74	223	229	215	237	226
	M 64	62	54	57	55	186	166	170	188	177
	S 64	52	58	56	59	156	174	141	167	150
	Mean 66	60	60	59	63	188	190	175	230	184
	S. D. 2.0	5.4	6	3	7	23	29	26	39	27

TABLE IV

(A) THE TOTAL FAT VALUES AND THE TOTAL CHOLESTEROL PHOSPHOLIPID RATIOS OF TWO ATHEROSCLEROTICS TREATED WITH 2 MGM. OF TRIAC DAILY

(B) THE SAME LIPID MEASUREMENTS OF THREE ATHEROSCLEROTICS TREATED WITH 4 MGM. OF TRIAC DAILY

TRIAC

	CHOLESTEROL/PHOSPHOLIPID					TOTAL FAT - mgm. %				
	Pre Rx	1 wk.	2 wks.	3 wks.	4 wks.	Pre Rx	1 wk.	2 wks.	3 wks.	4 wks.
A.	G 33	34	39	33	55	714	734	737	682	684
	N 37	19	18	22	19	1232	837	1107	1092	1052
B.	F 16	20	17	24	21	593	555	599	555	651
	M 18	13	19	20	21	654	639	431	493	541
	S 18	16	9	19	17	480	450	487	525	467
Mean 17		16	15	21	19	575	548	505	524	553
S. D. .59		2.5	3.8	1.8	1.7	63	67	60	23	67

TABLE V.

(A) THE CHLOROFORM TURBIDITY VALUES OF TWO ATHEROSCLEROTICS TREATED WITH 2 MGM. OF TRIAC DAILY

(B) THE CHLOROFORM TURBIDITY VALUES OF THREE ATHEROSCLEROTICS TREATED WITH 4 MGM. OF TRIAC DAILY

TRIAC

CHLOROFORM TURBIDITY UNITS

		Pre Rx.	1 wk.	2 wks.	3 wks.	4 wks.
A.	G 90	22	20	11	26	
	N 65	34	34	38	27	
B.	F 24	18	11	12	10	
	M 12	7	7	6	8	
	S 10	6	8	9	6	
	Mean 15	10	8.6	9	8	
	S.D. 5.4	4.7	1.5	2.1	1.5	

DISCUSSION

It has been well established that thyroxin and the various thyroid hormone analogues are capable of depressing the level of certain of the blood lipids; particularly the total serum cholesterol (13, 14, 16) and various subfractions of the B lipoprotein (15, 17).

Trotter (16) has demonstrated that doses of 2, 3 and 4 mgm. of triiodothyroacetic acid (TRIAC) are capable of decreasing the total cholesterol values of euthyroid females when treated for a short period of time. Similarly, Oliver and Boyd (17) observed that the total serum cholesterol, the cholesterol phospholipid ratio and the B lipoprotein cholesterol were all decreased in the serum of 12 hypercholesterolemic males with coronary artery disease treated with TRIAC in dosages of 2 to 5 mgm. daily.

The results of the present series show no significant difference between the pre- and post-treatment lipid values at any time period. Although this series was composed of only a few individuals, the results do not indicate that significant changes would have occurred in a larger group of similar subjects.

A hypercholesterolemic female on 2 mgm. of TRIAC daily was the only individual of five subjects who exhibited a sustained fall in the total cholesterol, the cholesterol phospholipid ratio and the chloroform turbidity values. Two of the three males treated with

TRIAC in dosages of 4 mgm. daily demonstrated a slight fall in the B. percentage of the total lipoprotein lipid and in the chloroform turbidity values, but these decreases were not any greater than variations which occurred spontaneously in the control group.

The failure of the total cholesterol to fall under treatment in four of five atherosclerotic patients is difficult to reconcile with reports of other investigators, (15, 16) who treated euthyroid normocholesterolemic and hypercholesterolemic individuals with similar doses of TRIAC to those used in the present study, and noted a decrease in the level of total cholesterol, the cholesterol phospholipid ratio and the B lipoprotein cholesterol or Sf 0-12 value. No explanation is apparent for the disparity in the results of this series and those obtained by other workers.

CONCLUSIONS

A group of six male and one female atherosclerotic subjects who were untreated were used as controls for the present series.

Two atherosclerotic individuals, a 72 year old male and a 41 year old female, were treated with TRIAC in dosages of 2 mgm. daily for four weeks.

The serum lipid values of the male remained unchanged. There was a marked sustained decrease in the total serum cholesterol, the cholesterol phospholipid ratio and the chloroform turbidity values of the female, after the initiation of treatment. This differed remarkably from the usual fluctuation of the lipid values on blood taken periodically from a group of atherosclerotic controls. No demonstrable changes occurred in the B percentage of the total lipoprotein lipid of the female.

Three middle aged atherosclerotic males were treated with TRIAC in dosages of 4 mgm. daily for four weeks. No differences of statistical significance were noted between the pre-treatment and post-treatment values at any time period. The results did not differ appreciably from those obtained in the group of untreated atherosclerotic controls.

Except for the case of one hypercholesterolemic female the results of the present series are not in accord with those of other workers who treated similar subjects with similar dosages of TRIAC. No explanation is apparent for this disparity of results.

THE CHLOROFORM TURBIDITY TEST IN NORMAL AND ATHEROSCLEROTIC SUBJECTS

INTRODUCTION

In 1955, Paul Green (18) described a relatively simple procedure known as the chloroform turbidity test, which may be of value in the diagnosis of atherosclerosis. This procedure consists essentially of the interaction of a very dilute solution of serum in normal saline with a small amount of chloroform. The solution is shaken and allowed to stand for 20 minutes. The opacity of the milky supernatant is then measured in the spectrophotometer by setting it to 100% transmission with a blank of serum and saline. The optical density times 100 is arbitrarily considered to be the number of units of turbidity of the solution.

Although the author is uncertain as to the exact significance of the test, he claims that there is an empirical correlation between the number of chloroform turbidity units in the serum and the amount of Gofman's atherogenic B lipoprotein fraction. He also reports that the number of units is only roughly proportional to the total serum cholesterol, or to the total fat, and can change independantly of these. No data is presented in his paper as evidence for these claims of correlation. Dr. Green states that the range of normality of this test is from 14 to 25 units, but he does not give the ages, or the criteria for normality, or the conditions of the test for these so called "normal" individuals. Merrick (14) has recently demonstrated that the chloroform turbidity values alter markedly after a

high fat meal. Thus, an individual whose chloroform turbidity value on fasting sera may have been in the normal range, may have a value in the atherogenic range after a fat meal.

In Green's series of 26 patients with myocardial infarctions who were not suffering from either hypertension or diabetes mellitus, only three of 26 had normal chloroform turbidity values. Thus, it seemed that this test might be of value in selecting normal individuals from those with myocardial infarctions, or indeed in predicting those individuals who would untimately suffer from this disease.

An attempt was made in this paper to corroborate some of Green's findings by performing this test on all subjects simultaneously with the other lipid measurements.

METHODS

The chloroform turbidity test was performed on six groups of predominantly male patients, including six to ten patients in each group.

Three groups were composed of middle aged atherosclerotic subjects who were under treatment with either Danilone (phenylindanedione) Sintrom or Tromexan (coumarin derivatives). All of the individuals in these groups had suffered relatively recent myocardial infarctions or cerebral thromboses.

The fourth group consisted of four individuals treated with Heparin at a dosage of 200 mgm. every 48 hours. This dosage was insufficient to produce constant anticoagulation.

The fifth group was composed of untreated middle aged atherosclerotics, and the sixth group of eight untreated, healthy, young individuals.

Blood was taken in the fasting state, the serum was separated and quickly frozen and stored. In the cases of the young untreated normal, the middle aged atherosclerotic control, and the heparin treated groups, the blood was taken at weekly intervals for four weeks. In all other groups blood was taken before the institution of treatment and at regular intervals of two weeks on at least four occasions.

The detailed method followed for the determination of the chloroform turbidity was done according to Green.

The variation in the chloroform turbidity values on repeated determinations of the same serum was approximately five units.

RESULTS

It will be noted in Figure 1 that there was considerable fluctuation of the average chloroform turbidity values from one time period to the next in all groups studied. This occurred because of the variation of the values of any given individual over several determinations at different time periods. The variation in the values of the normal individuals was somewhat less than in other groups.

There was also considerable variation of the chloroform turbidity values from one individual to another within the groups. This accounts for the rather large standard deviations.

No significant changes occurred in the groups treated with Danilone, Sintrom and Tromexan. Although there was a decrease in the average chloroform turbidity of the Heparin treated group at two, three and four weeks, this was not significant. The average chloroform turbidity of the control group fluctuated from one occasion to another.

This test offered no differentiation between the group of young healthy normals in our series and the middle aged atherosclerotic controls since there is no significant difference between the mean chloroform turbidity values of either of these groups at any time period.

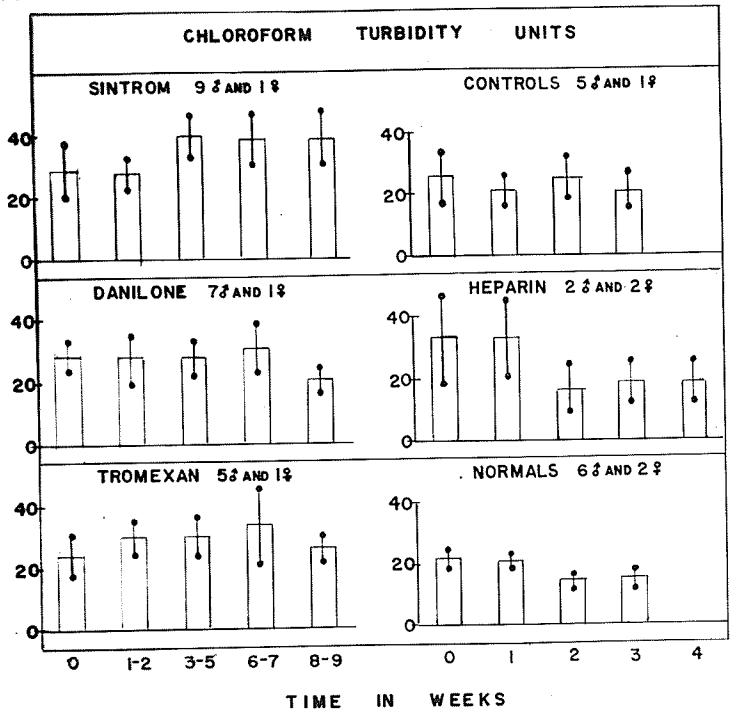

Fig. 1 The chloroform turbidity values (units) of four groups of treated atherosclerotics, a group of controls, and a group of normals.

Mean values ± S.D.

Eleven of the 24 subjects who had suffered an acute thrombotic episode had "normal" chloroform turbidity values eight to nine weeks after infarction. This test was of little value in separating such individuals from young normal subjects.

In those groups of patients in which myocardial infarction occurred, no significant differences were noted in the chloroform turbidity values done on blood taken immediately after infarction, and eight to nine weeks later. Thus, Green's observations that the chloroform turbidity values decrease in patients who have suffered myocardial infarction and rise gradually to restore a "steady state", were not confirmed in this short term study.

An attempt was made to correlate the chloroform turbidity values of 65 individuals in all groups with the other serum lipid measurements. It was noted in plotting these that the members of normal, control and treated groups had a similar distribution. There was a significant correlation between the chloroform turbidity measurement and the total cholesterol and the total fat, but not between the B percentage of the total lipoprotein lipid and the chloroform turbidity measurement.

DISCUSSION

The widest range of chloroform turbidity values performed on the fasting serum of normal individuals in this series was between six and 36 units. This is roughly in accord with Green's range of normal values (14 - 25 units). Since Green (18) neither states the ages, the criteria of normality, or the conditions of the test for his normal individuals in his article, the two groups may not be strictly comparable.

Green claims that in patients who are not ill, the chloroform turbidity values do not vary greatly from one occasion to another, but stay roughly at the same level and consequently in the same range of normality or abnormality. He describes this as the "steady state". In this study this was found to be approximately true in the group of normal individuals. Variations occurred within the group, but these remained in Green's (18) range of normality. In the group of atherosclerotic controls however, the chloroform turbidity values varied from the normal to the atherosclerotic range. In this group the chloroform turbidity values tended to be on the high "normal" range in some cases, and a magnitude of variation, no greater than that which occurred in the so called "normal" group, was sufficient to place these patients in the atherosclerotic range.

Green has observed that the chloroform turbidity measurements tend to decrease soon after infarction has occurred, and after

an unstated period of time return to "the steady state". If the chloroform turbidity values done on blood drawn from patients shortly after infarction has occurred in this series are compared to the values obtained on blood drawn eight to nine weeks later, no significant differences are noted. Indeed, almost one half of the individual patients have lower values at this time. It is notable that all the patients in this series were anticoagulated, and Green (18) does not state whether his patients were treated in a similar manner. Secondly, it is possible that eight to nine weeks is not sufficient time after infarction for the chloroform turbidity values to regain the steady state, although it seems reasonable to assume that a trend toward increased values would be occurring by this time.

The differences between the mean chloroform turbidity values of the normal and middle aged atherosclerotic control groups were not significant and therefore the test was of little value in distinguishing such individuals.

In Green's series of patients suffering from myocardial infarctions without diabetic or hypertensive complications, 23 of 26 had values in the atherosclerotic range. In the present series of 24 similar cases of acute thrombotic episodes, 90% of which were myocardial infarctions, 11 of 24 had values in the so called "normal" range. The values selected in this series for the comparison were those obtained

eight to nine weeks after infarction had occurred, so that these values were theoretically the highest obtainable. Thus, in this series, the chloroform turbidity test was of little value in selecting the proven atherosclerotics with myocardial infarction from a young, normal population.

It was noted in the present series that the total serum, fat and the total cholesterol were significantly correlated with the chloroform turbidity measurement. This would seem to be not altogether at variance with Green's observation that these lipid values are roughly proportional to the chloroform turbidity values. In the present work, the B percentage of the total lipoprotein lipid is not well correlated with the chloroform turbidity value. Green has observed that there was a good correlation of the chloroform turbidity value with Gofman's B lipoprotein fraction. This latter measurement is a subfraction of the B lipoprotein and theoretically can undergo a relatively greater increase than the total B lipoprotein, thus forming a different relationship with the chloroform turbidity value.

Despite the observation in this series, that the chloroform turbidity test correlates with both the total serum cholesterol and the total serum fat, it was noted that the chloroform turbidity measurements were not sufficiently altered to parallel significant changes in these or other lipid measurements or ratios studied. Thus, despite

the statistical validity of the observed correlations between certain of the blood lipids and the chloroform turbidity test in large numbers of patients, this correlation would not appear to be sufficiently good to affect a relatively small group of patients.

CONCLUSIONS

The range of values of the chloroform turbidity test performed on the fasting sera of a group of eight young, normal individuals of both sexes was six to 36 units.

There was some variation of the chloroform turbidity values of one individual from one occasion to another in all the groups of patients studied. In the group of atherosclerotic controls this was sufficient in some cases to vary their values from the atherosclerotic range to the normal range.

In this study no significant differences were noted between the average chloroform turbidity values of the group of young normal individuals and the group of atherosclerotic controls.

In those groups of patients in which myocardial infarction occurred, no significant differences were noted in the chloroform turbidity values done on blood taken immediately after infarction, and eight to nine weeks later. Thus, Green's observation that the chloroform turbidity values decrease in patients who have suffered myocardial infarction and rise gradually to restore a "steady state" were not confirmed in this short term study.

Eleven of the 24 patients who had suffered an acute thrombotic episode had "normal" chloroform turbidity values eight to nine weeks after infarction. This test was of little value in separating such individuals from young normal subjects.

BIBLIOGRAPHY TO EXPERIMENTAL STUDIES

1. Zlatkis, A., Zak, B., and Boyle, A.J.: New methods for direct determination of serum cholesterol. J. Lab. and Clin. Med., 41: 486, 1953.

2. Hawk, P.B., Oser, B.L., and Summerson, W.H.: Practical Physiological Chemistry, 12th edition, 1947, Blakiston Company, Toronto, page 540.

3. Kunkel, H.G., Ahrens, E.H. Jr., and Eisenmenger, W.J.: Application of turbidimetric methods for estimation of gamma globulin and total lipid to the study of patients with liver disease. Gastroenterology. 11: 499, 1948.

4. Swahn, B.: Studies on blood lipids, Lund. Scand. J. of Clin. and Lab. Investigation. 5: Supp. 9, 1953.

5. Jencks, W.P., Durrum, E.L., and Jetton, M.R.: Paper electrophoresis as a quantitative method: The staining of serum lipoproteins. J. of Clin. Investigation. 34: 1437, 1955.

6. Nikkila, E.: Studies on the lipid protein relationships in normal and pathological sera and the effect of heparin on serum lipoproteins. Scan. J. of Clin. and Lab. Investigation. 5: Supp. 8, 1953.

7. Bernard-Griffiths, C., Cuvelier, R., Sebald, Mlle., Berger, J.A., and Andraud, G.: Considerations sur les lipoproteines de sujet age. Influence D'une medication lipotrope et anticoagulante. Therapie. 10:756, 1955.

8. Hueper, W.C.: The pathogenesis of atherosclerosis. Am. J. of Clin. Path., 26: 559, 1956.

9. Graham, D.M., Lyon, T.P., Gofman, S.W., Jones, H.B., Yankley, A., Simonton, J., and White, S.: The influence of heparin on lipoprotein metabolism. Circulation, 4:666, 1951.

10. Soffer, A., and Murray, M.: Prolonged observations of the cardiovascular status in essential hyperlipemia with special reference to serum lipid response to heparin. Circulation, 10: 255, 1954.

11. Kuo, P. T., Joyner, C.R., and Reinhold, J.G.: Effects of fat ingestion and heparin administration on serum lipids of "normal", hypercholesterolemic, hyperlipemic and atherosclerotic subjects. Am. J. of the Med. Sciences. 232: 613, 1956.

12. Constantinides, P.: Personal communication.

13. Hurxthal, L.M.: Blood cholesterol and thyroid disease: Myxoedema and hypercholesterolemia. Arch. Int. Med., 53: 762, 1934.

14. Stristower, B., Gofman, J.W., Galioni, E., Almada, A.A., and Siman, A.: Effect of thyroid extract on serum lipoproteins and serum cholesterol. Metabolism. 3: 218, 1954.

15. Stristower, B., Gofman, J.W., Galioni, E., Rubinger, J.H., Poteau, J., and Guzvich, P.: Long term effect of dried thyroid on serum lipoprotein and serum cholesterol levels. Lancet. I: 120, 1957.

16. Trotter, W.R.: Effect of triiodothyroacetic acid on blood cholesterol levels. Lancet. I: 885, 1956.

17. Oliver, M.F. and Boyd, G.S.: The influence of triiodothyroacetic acid on the circulating lipid and lipoproteins in euthyroid men with coronary disease. Lancet. I: 124, 1957.

18. Green, P.: Preliminary report on a simple laboratory test that appears to be of value in the detection of coronary artery atherosclerosis. Manitoba Medical Review. 35: 351, 1955.

19. Merrick, W.G.: Evaluation of normal serum chloroform turbidity. Unpublished.

APPENDIX

THE TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH DANILONE

DANILONE

	CHOLESTEROL					LIPOPROTEINS				
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
G.	333	286	417	291	276	62	65	62	66	70
Fa. (f)	356	378	266	516	340	70	70	71	65	70
Fi.	269	335	321	296	320	60	69	66	56	60
Fe.	257	282	196	276	300	72	78	64	70	67
A.	336	354	355	-	350	78	77	76	-	75
An.	249	212	260	243	267	51	53	61	58	58
W.	234	210	218	-	202	58	58	60	-	60
F.	202	294	367	-	256					
Mean	279	294	300	324	289	64.4	67.1	66.6	63	66.6
S. D.	±21	±21.3	±25.7	±48.5	±18.4	±3.95	±3.91	±2.54	±2.9	±2.3

T test for paired data - No significant difference between pre-treatment and post-treatment values at any time period.

THE TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH SINTROM

SINTROM

	CHOLESTEROL					BETA PERCENTAGE OF TOTAL ALPHA AND BETA LIPOPROTEIN LIPID					
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.		0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
W. (f)	264	575	340	347	318	W.	70	86	74	65	64
T.	236	275	244	193	260	T.	66	66	72	68	70
T.	206	270	343	257	317	P.	67	87	65	72	75
P.	320	344	290	240	316	F.(f)	64	62	68	74	70
E. (f)	288	438	418	420	407	L.	57	73	75	66	65
L.	267	250	303	371	325	S.	72	75	72	77	62
S.	278	237	328	288	364	F.	67	65	64	72	72
B.	294	282	318	272	301	B.	69	60	73	74	73
F.	227	258	295	278	247	H.	75	75	72	79	84
H.	354	273	317	540	533	Mean	67.4	72.0	70.5	71.9	70.05
Mean	273	320	320	321	339*	S. D.	±2.08	±3.45	±1.4	±2.27	±2.4
S. D.	±14.8	±34.8	±15.4	±36.6	±27.5						

T test for paired data - *Significant difference between pre-treatment values and those at 8 week time period. $P < .05$.

T test for paired data - No significant difference between pre-treatment and post-treatment values at any time period.

THE TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH TROMEXAN

TROMEXAN

	CHOLESTEROL						BETA PERCENTAGE OF TOTAL ALPHA AND BETA LIPOPROTEIN LIPID				
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.		0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
S.	328	555	428	374	432	S.	64	66	66	69	69
R.	342	372	427	590	375	R.	72	63	67	75	64
T.	391	289	406	466	450	T.	74	65	62	70	65
L.	228	312	259	256	224	L.	70	75	78	71	76
T. (f)	238	206	243	292	263	T.(f)	65	66	65	69	71
P.	285	326	346	378	328	P.	66	73	73	73	74
Mean	304	343	352*	395*	346*		68.5	66	67	71.3	69.8
S. D.	±28.4	±46	±37.2	±47.4	± 40		±1.86	±2.4	±2.98	±1.18	±2.4

T test for paired data - *Significant difference between pre-treatment values and those at 4 6 and 8 weeks. $P < .05$ at each of these periods.

T test for paired data - No significant difference between pre-treatment and post-treatment values at any time period.

A. THE TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF TWO ATHEROSCLEROTICS TREATED WITH 200 MGM. OF HEPARIN EVERY 72 HOURS

B. THE SAME LIPID MEASUREMENTS OF A GROUP OF ATHEROSCLEROTICS TREATED WITH 200 MGM. OF HEPARIN EVERY 48 HOURS

A. Subcutaneously every 72 hours.

	LIPOPROTEINS						CHOLESTEROL					
Subj.	Pre Rx	1 wks.	2 wks.	3 wks.	4 wks.	5 wks.	Pre Rx	1 wks.	2 wks.	3 wks.	4 wks.	5 wks.
G.	56	57	57	55	67	59	340	350	345	506	344	345
L.	71	66*	68**	69	62	68	355	415	-	365	356	395

* 20 mgm. dosage every 72 hours for one week
** 200 mgm. dosage every 72 hours for the remainder of the treatment period.

B. 200 mgm. subcutaneously every 48 hours

Gi.	71	62	60	66	71	232	181	186	227	287
T.	76	73	65	69	77	320	366	375	335	355
Gu. (f)	74	57	52	50	56	212	215	236	252	241
S.	67	61	47	58	50	235	193	187	183	177
Sp.	84	71	85	83	78	335	247	366	300	283
Mean	74	65	62	65	66	269	240	270	259	269
S.D.	±3.2	±3.4*	±7.2*	±6.2	±6.3	±31	±58	±43	±30	±34

* T test for paired data - Significant difference between pre-treatment values and those at the one and two week time periods.
P < .05 at each of these periods.

No significant difference between pre-treatment and post-treatment values at any time period.

THE TOTAL CHOLESTEROL AND THE BETA PERCENTAGE OF THE TOTAL LIPOPROTEIN LIPID VALUES OF A GROUP OF YOUNG "NORMAL" SUBJECTS

NORMALS
25-35 yrs.

	LIPOPROTEINS				CHOLESTEROL			
Subject	1 wk.	2 wks.	3 wks.	4 wks.	1 wk.	2 wks.	3 wks.	4 wks.
M.	62	64	63	63	145	170	206	227
B.	61	64	63	-	171	186	200	-
V.	56	55	60	64	209	180	206	212
W.	72	65	64	64	248	344	244	229
K.	66	62	66	56	219	214	236	232
F.	64	62	56	70	177	179	171	146
I. (f)	61	65	59	70	176	182	205	154
H. (f)	62	53	53	57	174	197	197	179
Mean	63	61	61	63	190	207	208	197
S. D.	$\pm$ 1.8	$\pm$ 1.8	$\pm$ 1.9	$\pm$ 2.3	$\pm$ 12	$\pm$ 22	$\pm$ 9	$\pm$ 15

THE TOTAL CHOLESTEROL PHOSPHOLIPID RATIOS OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH DANILONE

CHOLESTEROL/PHOSPHOLIPID

Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
G.	50	-	43	29	33
Fa. (f)	36	42	28	38	38
Fi.	28	30	46	29	51
Fe.	28	25	28	28	35
A.	42	35	48	-	39
An.	32	18	41	30	40
W.	22	28	-	-	16
F.	30	20	50	-	36
Mean	34	28	41	30	36
S. D.	$\pm$3.4	$\pm$3.9	$\pm$3.7	$\pm$2.1	$\pm$3.7

T test for paired data - No significant differences between pre-treatment and post-treatment values at any time period.

THE TOTAL FAT VALUES AND THE CHOLESTEROL PHOSPHOLIPID RATIOS OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH SINTROM

SINTROM

CHOLESTEROL/PHOSPHOLIPID

Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
W. (f)	19	52	32	29	27
H.	26	16	31	41	31
T.	18	31	29	33	32
P.	27	33	24	21	35
F.	26	-	33	32	31
L.	20	19	32	50	40
S.	22	17	29	26	62
B.	19	26	20	31	44
Fi.	12	-	24	32	13
Mean	21	28	28*	33*	35*
S. D.	±1.7	±5.1	±1.6	±3	±4.7

T test for paired data - *Significant differences between pre-treatment values and those at the 4, 6 and 8 week time periods. $P < .01$ at each of these periods.

TOTAL FAT (MGM)

0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
879	1112	1042	997	1107
1012	930	1159	1782	950
847	897	738	843	792
832	813	1082	1017	802
919	972	1107	1050	1127
877	801	898	791	898
813	817	-	1072	930
765	897	842	-	737
717	589	812	857	937
851	870	960*	1051*	920
±31	±55	±59	±119	±48

*Significant differences between pre-treatment values and those at the 4 and 6 week time periods. $P < .05$ at each of these periods.

THE TOTAL FAT VALUES AND THE CHOLESTEROL PHOSPHOLIPID RATIOS OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH TROMEXAN

TROMEXAN

	CHOLESTEROL/PHOSPHOLIPID					TOTAL FAT (MGM)				
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
S.	23	39	36	29	41	921	1057	-	923	529
R.	26	37	39	38	38	914	1057	825	890	1011
T. (f)	21	78	32	40	32	1122	912	1011	1107	982
L.	28	23	50	30	27	810	932	822	-	727
T.	20	13	15	26	21	682	649	697	823	662
P.	20	19	23	-	55	812	1117	1077	1074	950
—	—	—	—	—	—	—	—	—	—	—
Mean	23	25	33*	32*	36*	880	954	886	973	810
S. D.	±1.5	±4.7	±5.5	±2.9	±5.3	±71	±75	±76	±60	±61

T test for paired data - *Significant differences between pre-treatment values and those at the 4, 6 and 8 week time periods. $P. < .05$ at these periods.

No significant differences between pre-treatment and post treatment values at any time period.

THE TOTAL FAT VALUES AND THE CHOLESTEROL PHOSPHOLIPID RATIOS OF A GROUP OF ATHEROSCLEROTICS TREATED WITH 200 MGM. OF HEPARIN EVERY 48 HOURS

HEPARIN

	CHOLESTEROL/PHOSPHOLIPID					TOTAL FAT				
Subj.	0 Pre Rx	1 wks.	2 wks.	3 wks.	4 wks.	0 Pre Rx	1 wks.	2 wks.	3 wks.	4 wks.
Gi.	17	14	14	13	33	755	581	682	827	808
T.	20	19	-	21	41	1122	917	731	932	1097
Gu. (f)	13	16	17	15	19	833	853	750	768	902
S.	17	15	16	18	19	682	669	577	501	576
Sp.	27	17	19	31	14					
Mean	19	16	17	20	24	848	750	685*	757	845
S. D.	±2.6	±.97	±1.21	±3.55	±6.3	±39	±21	±48	±106	±102

T test for paired data -

*Significant difference between pre-treatment and post-treatment values at 2 week time period. $P < .05$.

THE TOTAL FAT VALUES AND THE CHOLESTEROL PHOSPHOLIPID RATIOS OF A GROUP OF YOUNG NORMAL SUBJECTS

NORMALS
25-35 yrs.

	CHOLESTEROL/PHOSPHOLIPID				TOTAL FAT			
Subject	1 wk.	2 wks.	3 wks.	4 wks.	1 wk.	2 wks.	3 wks.	4 wks.
M.	16	15	15	15	598	610	626	611
B.	15	16	15	-		645	669	671
V.	11	15	13	16	727	648	599	847
W.	16	24	18	12		819	787	812
K.	30	53	19	17		544	705	721
F.	17	17	10	18		414	581	525
I. (f)	21	15	20	16		517	629	705
H. (f)	21	20	15	12		484	637	679
	—	—	—	—	—	—	—	—
Mean	18	19	16	15		585	654	689
S. D.	± 2.4	± 2.4	± 1.25	± 1		± 108	± 25	± 40

A. THE CHLOROFORM TURBIDITY VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTIC CONTROLS

B. THE CHLOROFORM TURBIDITY VALUES OF A GROUP OF YOUNG "NORMAL" SUBJECTS

Chloroform Turbidity

CONTROLS					NORMALS				
Subj.	2 wks.	4wks.	6 wks.	8 wks.	Subj.	1 wk.	2 wks.	3 wks.	4 wks.
Mc.	22	37	40	-	M.	36	-	21	20
C.	30	15	18	21	B.	25	22	10	-
G.	46	21	22	39	V.	14	14	10	16
Pi.	33	28	48	28	W.	22	24	22	25
R.	10	10	7	9	K.	30	18	13	7
P.	12	15	12	10	F	12	21	15	16
Mean	26	21	25	21	I. (f)	22	21	13	18
S. D.	±8	±4.4	±7	±5.6	H. (f)	13	24	10	6
					Mean	22	21	14	15
H.	100	104	120	104					
					S. D.	±3.2	±1.6	±1.8	±2.8

THE CHLOROFORM TURBIDITY VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH DANILONE

	CHLOROFORM TURBIDITY				
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
G.	36	60	-	22	27
Fa. (f)	48	9	41	69	20
Fi.	14	16	24	30	29
Fe.	18	16	15	32	14
A.	30	18	51	29	27
F.	26	22	26	22	18
An.	16	14	16	8	12
W.	46	59	21	-	17
Mean	29	28	28	31	21
S. D.	$\pm$5.1	$\pm$7.6	$\pm$5.4	$\pm$7.7	$\pm$2.7

T test for paired data - No significant difference between pre-treatment and post-treatment values at any time period.

THE CHLOROFORM TURBIDITY VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH SINTROM

	CHLOROFORM TURBIDITY				
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
W. (f)	48	49	37	42	44
T.	-	20	54	24	34
Ta.	14	14	12	16	36
P.	12	33	54	70	34
F.	48	-	71	46	59
L.	14	34	25	25	14
S.	32	33	53	36	39
B.	7	15	20	20	20
F.	10	12	12	23	12
H.	75	40	67	85	95
Mean	29	28	40.5	38.7	38.7
S. D.	$\pm$8.3	$\pm$4.6	$\pm$7.4	$\pm$7.5	$\pm$8.0

T test for paired data - No significant difference between pre-treatment and post-treatment values at any time period.

THE CHLOROFORM TURBIDITY VALUES OF A GROUP OF MIDDLE AGED ATHEROSCLEROTICS TREATED WITH TROMEXAN

	CHLOROFORM TURBIDITY				
Subj.	0 Pre Rx	2 wks.	4 wks.	6 wks.	8 wks.
S.	14	32	37	30	32
R.	28	34	51	83	24
T.	51	50	35	12	31
L.	22	27	21	18	15
P.	20	23	21	-	20
T. (f)	11	12	16	27	34
	—	—	—	—	—
Mean	24	30	30	34	26
S. D.	$\pm$6.4	$\pm$5.7	$\pm$5.9	$\pm$14	$\pm$3.4

T test for paired data - No significant difference between pre-treatment and post treatment values at any time period.

THE CHLOROFORM TURBIDITY VALUES OF A GROUP OF ATHEROSCLEROTICS TREATED WITH 200 MGM. OF HEPARIN EVERY 48 HOURS

	CHLOROFORM TURBIDITY				
Subj.	Pre Rx	1 wk.	2 wks.	3 wks.	4 wks.
Gi.	24	-	9	33	34
T.	70	34	30	24	13
Gu. (f)	16	12	8	8	14
S.	21	54	16	9	12
	—	—	—	—	—
Mean	33	33	16	19	19
S. D.	14	15	5.8	7	6

T test for paired data - No significant differences between pre-treatment and post-treatment values at any time period.

Sp.	135	100	255	350	260