THE CONTROL OF BLOOD LIPIDS

by

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A thesis submitted for the degree of

DOCTOR OF MEDICINE

of the

UNIVERSITY OF GLASGOW

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I N D E X

PART I

PART I

CHAPTER 1

INTRODUCTION

"The aetiology of atherosclerosis is of prime importance if anything is to be done to prevent this common disability of declining years, but in spite of a vast amount of work the problem remains unsolved."

So writes Boyd (1947) in his Textbook of Pathology. Thirteen years later and after an even vaster amount of work this statement is not only still true, but more urgent, for atherosclerosis and its complications of ischaemia and thrombosis are no longer regarded as disabilities only of the declining years. Their greatest toll in terms of death, invalidism and economic suffering is imposed on the active middle-aged male afflicted with occlusive coronary atherosclerosis, with or without subintimal haemorrhage or thrombosis.

Cholesterol deposits were first recognised in arteries by Vogel (1847) but nearly 70 years elapsed before Anitschkow and Chalatov (1913), by the production of atheroma in rabbits given a cholesterol enriched diet, demonstrated a relationship between these cholesterol lesions and the serum lipids. This work on a herbivorous animal having normally low cholesterol levels though not strictly relevant to the problem as it presents in the omnivorous human with much higher normal cholesterol levels, nevertheless released a flood of research which flows just as strongly today and at times comes near to engulfing medical literature.

As a result of this great body of research there can be little doubt that the circulating blood lipids are in some way or ways involved in the problem of atherosclerosis. It is impossible in what must be no more than a concise introductory chapter to give an exhaustive account of the accumulated knowledge of this topic. But it is necessary to outline the framework on which this knowledge has been built. The brief account which follows is amplified in subsequent chapters.

LIPIDS AND THE LESION

The atherosclerotic plaque contains not only cholesterol, but other lipids also, namely phospholipids, triglycerides and fatty acids (Buck and Rossiter, 1951; Duff and McMillan, 1951). More recent work with silicic acid and gas-liquid chromatography has further characterised the nature and proportion of these lipids. Normal intima has a lipid distribution almost identical with the plasma lipids, as indeed has the early intimal lesion, but in the fibrous and ulcerous lesions the cholesterol content is much higher (Hirsch and Weinhouse, 1943). This may be due to the selective removal of the other lipids by phagocytosis and autolytic changes accompanying necrosis and ulceration. But cholesterol can be synthesised by aorta (Siperstein et al., 1951) and this may contribute to its deposition, for although phagocytes and foam cells at the plaque can slowly remove other lipids by engulfment and oxidation, it is not certain that they can degrade and remove cholesterol which cannot be oxidised to completion, in mammalian tissue, to any significant extent. (Lehninger, A. L., 1954).

LIPID TRANSPORT

Although early studies had suggested some relationship between blood lipids and the plasma proteins (Chick, 1914; Theorell, 1926) it was not until 1929 that Macheboeuf isolated a well defined serum lipoprotein. Virtually all lipids found in tissues and body fluids in a normal state exist in the form of lipoproteins or lipid-protein complexes. (Oncley et al., 1947; Gurd et al., 1949). Possible exceptions to this statement may be the neutral fat droplets in adipose tissue or the large chylomicrons, but even here there is some evidence that protein plays a part in stabilising these finely emulsified droplets of fat. Plasma lipoproteins constitute the transport system for the circulating lipids and comprise cholesterol (free and esterified), phospholipids and fatty acids conjugated within the protein shell - beta globulin in the case of the beta lipoproteins and alpha-1 globulin in the case of the alpha lipoproteins. Dervichian (1949) has explained the physico-chemical theory underlying this association of non-ionic lipids (cholesterol, cholesterol esters and glycerides) with ionic lipids (phospholipids and fatty acids).

Lipoproteins have shape and size, and can permeate not only capillary membranes but the intimal and medial layers of arteries (Pappenheimer, 1953; Page, 1954). It was thought at first that the membranes acted as semi-permeable structures containing pores which permitted the restricted diffusion of protein molecules and Pappenheimer who first advanced this Pore Theory calculated that the pores had a mean diameter of 60-90 Å. But more recent work (Grotte, 1956) indicates that there may be a smaller number of pores with a calculated radius of from 116 Å to 350 Å and this is adequate for the passage of the large beta-lipoprotein, a sphere with a diameter of 185 Å. That lipoproteins can and do

diffuse from the blood into the tissue fluid through such leaks or pores has been shown indirectly by the analysis of lymph (Page et al., 1953). The chylomicrons with a diameter of 0.5-1μ are much larger than the molecules of beta-lipoprotein, but there is evidence that even they may gain restricted access to the tissue fluid and lymph by pore leakage (Courtice and Morris, 1955).

There is however another metabolic pathway for chylomicrons suggested by the heparin-clearing reaction first demonstrated by Hahn (1943). Following the injection of heparin intravenously an enzyme appears in the blood, probably a lipoprotein lipase, which hydrolyses the chylomicron triglyceride to glycerol and unesterified fatty acids which combine with the serum albumin (Robinson and French, 1953). It is not proven that this hydrolytic breakdown of chylomicrons occurs normally, for chylomicron clearing can still take place when hydrolysis is inhibited (French and Morris, 1957). Nevertheless it seems that an enzyme with the properties of clearing factor must have a function in lipid transport.

It seems established therefore that normal blood vessels contain the various plasma lipids and that the transport of lipids through, and their metabolism within the vessel wall, is a normal occurrence. What happens then to transform this physiological process into a pathological one? This is where we enter the complex and often confusing realm of hypothesis. Nevertheless the theories of atherogenesis merit some mention if a full understanding of the problem is to be achieved and a rational, rather than an empirical approach

to therapy adopted. Themain possible aetiological factors have been collated in one theory by Page (1954) a description and brief exposition of which now follows.

FILTRATION THEORY OF ATHEROGENESIS

Atherogenesis depends on the following factors: -

1. The anatomy, biochemistry and physiology of the vessel wall, all of which are hereditarily conditioned.
2. The composition of the plasma filtrate. When the circulating plasma contains increased amounts of the biochemically less stable beta-lipoprotein,increased breakdown of this lipoprotein is liable to occur in the vessel wall with increased deposition of lipid.
3. The height of the lateral pressure and the amount filtered. That is, the higher the blood pressure the greater the amount of lipid forced through the wall. This partly accounts for the increased incidence of atherosclerosis in hypertension.
4. The metabolic capacity of the vessel wall. The vessel is no longer regarded merely as a passive membrane but as an organ. Lehninger (1954) postulates the release of excess lipoprotein splitting enzymes in vascular tissue, stimulated by anoxia.
5. The response of intimal tissue to filtered products and their metabolites.
6. Changes in the ability of the vessel wall to transport filtered substances. This might result from age, or hypertensive and metabolic disease such as diabetes. It might equally result from the organisation of mural thrombi (Duguid, 1954) and may also involve disorders in fibrinolysis, the normal

process by which clot is broken down in the body (Kwaan and Mc Fadzean, 1957).

Having brought our survey of the problem to this point two rather different but extremely relevant fields remain to be charted.

LIPIDS AND COAGULATION

There are many in vivo and in vitro studies on both normal and abnormal coagulation. Fullerton and Anastasopoulos (1949) showed that fatty meals given to human subjects caused a definite and consistent shortening of the clotting time of plasma in the presence of Russell's viper venom. These observations have been fully confirmed by other workers (Fullerton et al., 1953; O'Brien, 1955; Maclagan and Billimoria, 1956; and Merskey and Nossel, 1957). But against this the evidence for the shortening of the whole blood clotting time in silicone coated tubes is contradictory and in any case if the change is real, it is not a large one. (Fullerton et al., 1953; O'Brien, 1955; Manning and Walford, 1954). Present knowledge about changes in blood coagulation in man following fatty meals seems to indicate that there is indeed a definite shortening of the plasma clotting time in the presence of Russell's viper venom, but no other clotting test shows any unequivocal change. The work of Robinson et al., (1955) suggests that it is the rise in the free fatty acids of plasma after a fatty meal which contributes to the shortened stypven clotting time.

The in vitro studies of Poole (1955) showed that the presence of chylomicra shortened the clotting time of plasma. Subsequent studies (Poole and Robinson, 1956; Marcus and Spaet, 1958) have shown that the phospholipids, phosphatidyl

ethanolamine, a constituent of chylomicrons, and phosphatidyl serine, hasten plasma coagulation. These phospholipids are two of the lipids normally present in platelets.

Although the evidence presented is tenuous and indirect it probably implicates lipids in the mechanism of coagulation, but not necessarily of course in thrombosis, which is an intravascular phenomenon.

LIPIDS AND THE EPIDEMIOLOGY OF ISCHAEMIC HEART DISEASE

"In the triangular relationship between diet, serum cholesterol levels, atherosclerosis and ischaemic heart disease ..... there is strong evidence for a direct association between diet and serum cholesterol trends in man. Far less convincing is the strength of the other links in this triple association" (Bronte-Stewart, 1958). The evidence is vast however and what is given here is a highly selected and radically summarised account of some of the major and representative observations which have been made in this field.

Perhaps the most important single factor which stimulated interest in the role of diet in the production of ischaemic heart disease was the sharp fall in death rate from circulatory disease in many European countries during the Second World War (Malmros, 1950; Strom and Jensen, 1951). This was accompanied by a fall in the consumption of milk, butter, cheese and eggs. Changes in serum cholesterol were reported from severely rationed areas. (Brull et al., 1945). It must be remembered that many other environmental changes occurred during this time, including for example longer hours of work, and more walking as well as a reduction in the total amount of calories consumed.

In groups consuming little fat, such as the South African Bantu (Bronte-Stewart et al., 1955) and the Japanese (Keys et al., 1958) severe atherosclerosis is rare and the rise in serum cholesterol with ageing is insignificant. At birth Bantu and European serum cholesterol levels are the same (Bersohn and Wayburne, 1955). Differences appear early in life, and by the age of 20 significant differences are seen not only between races but between income classes within each race. The incidence of severe atherosclerosis is different, also, in the same race living under different environmental conditions. It is much higher in Japanese who have emigrated and sampled the American way of life (Larsen, 1957).

The association between abnormalities in the serum lipoproteins and coronary atheroma has been the subject of much discussion in recent years (Gofman et al., 1950). The ultracentrifugal studies of this group and others have shown an increase in the low density (mainly beta) lipoproteins in individuals with ischaemic heart disease. Oliver and Boyd (1953), in this country, have demonstrated a significant increase in the serum lipids of men and women with coronary artery disease at all ages, with the exception of women in the sixth decade, and Besterman (1957) found a highly significant increase in the pre-beta lipoprotein in 198 out of 200 cases with coronary arterydisease.

It seems reasonable to condense the data outlined above as follows. Lipids occur in the atheromatous lesion, they have access to the lesion, they are found in increased amounts in the blood of many (but not all) individuals with clinical or post-mortem evidence of the lesion and they may participate

in intravascular coagulation or act as inhibitors of normal fibrinolysis. For these reasons, and others given later, it is a widely held opinion that some form of control of serum lipids is therapeutically desirable, and that this should achieve lowering of the serum cholesterol and beta-lipoproteins, without unwelcome side effects.

AIMS AND PLAN OF THE THESIS

This study is primarily concerned with the nature, effectiveness and feasibility of certain methods of controlling the serum lipids. In Part I the role of oestrogens, certain thyroid analogues and the unsaturated fatty acid, linoleic acid, is studied in this context, both in man and in the experimental animal. During this work some ideas were conceived which although pertinent were slightly out of the mainstream of the investigation. They are examined experimentally in Part II. These include an appraisal of the effect of post-prandial lipaemia on blood viscosity, the relationship of the tissue mast cell to hyperlipaemia and lipidosis and the effect of long term impairment of fat absorption on the lipids.

It was not possible to study phospholipids, cholesterol and lipoproteins in every investigation, but with one exception, the lipoproteins have been followed throughout, and all analyses carried out by the author personally. In all other studies, apart from those reported in Chapters 3 and 4, the cholesterol estimations were a personal task. These statements are made partly in recognition of technical help received in some of the work but partly also to underline the continuity of technique throughout the major part of this work. (See Acknowledgements.)

Three methods are generally used for the separation of the plasma lipoproteins, zone electrophoresis (Kunkel and Slater, 1952; Swahn, 1953), ultracentrifugal flotation (described in detail by De Lalla and Gofman, 1954) and chemical fractionation by salting out procedures (Cohn et al., 1950). For a number of reasons paper electrophoresis is the method which commends itself to the clinical investigator and was used in this group of studies. It is important to understand not only the technical details of the method, but some of the theory underlying its different stages and also to have a clear notion of its limitations. For these reasons an account of the method, with a note of the structure and composition of the serum lipoproteins and an indication of the inter-relationships between the lipoprotein nomenclatures employed in the different methods available, should be included now, rather than postponed to an Appendix.

CHAPTER 2

THE ESTIMATION AND COMPOSITION OF SERUM LIPOPROTEINS

Lipoproteins are complex molecular substances. The general reader is much less familiar with them than with, by comparison, the relatively straightforward lipid substances cholesterol and phospholipid. Neither is their measurement so simple, or if simplified so accurate a procedure. Because some understanding of the main technical and physico-chemical details about them is essential if much of the discussion in the following chapters is to be fully comprehended, it seems desirable to discuss them now rather than relegate them to an appendix.

1. The Estimation of Lipoproteins

The method of lipoprotein analysis by paper electrophoresis throughout this work is essentially that described by Swahn in 1952 and amplified in his monograph of 1953. In many respects it corresponds to the method of Kunkel and Slater (1952) with however one major difference. They used Sudan III as the staining agent. At the outset of this study I used all three of the fat stains suggested by various authors, namely Sudan Black B (Swahn, 1952), Sudan III (Kunkel and Slater, 1952), and Oil Red O or Sudan II (Durrum et al., 1952). I had no doubt that Sudan Black B was the best for this particular purpose and thereafter used it solely. Partly for this reason and because all the lipoprotein analyses have been done personally a certain degree of quantitative comparison between lipoprotein data of separated studies is permissible.

TABLE I. The results of 18 lipoprotein analyses on a single serum sample (Case No.77, Chapter 13). The average deviation from the mean is ± 0.11 or ± 6.5 per cent.

BETA:ALPHA LIPOPROTEIN RATIO
1.36
1.52
1.54
1.60
1.62
1.63
1.63
1.65
1.66
1.67
1.69
1.73
1.78
1.80
1.81
1.83
1.86
1.93
Mean 1.68

However the method is not especially accurate for reasons which will be mentioned but it is simple and inexpensive and is suitable for the preliminary separation of lipoprotein fractions which may then be analysed by more accurate biochemical techniques.

At different times during the four years of this work the reproducibility of the method was tested by 12 to 20 estimations of different serum samples. The average experimental error for the beta:alpha lipoprotein ratio ranged from ± 6 to ± 10 per cent. The results of 18 analyses on the serum from patient no. 77 of Chapter 13 are shown in Table I. The average percentage error in this test group is ± 6.5.

Instruments and Materials

1. Horizontal Electrophoresis Tank of conventional design with a tightly fitting heavy glass lid and platinum electrodes.

2. A Power Unit producing a stabilised voltage ranging from 110 to 200 Volts. The electrical conditions of the system were such that the usual working voltage of 120 Volts gave a current of 0.8 milliamperes (ma.) through each 5 cm. wide strip of filter paper.

3. A Double Beam Reflectance Densitometer, manufactured by Joyce, Loebl and Co. Ltd. of Newcastle. This instrument is used to "scan" the stained lipoprotein electrophoresis strips. A photoelectric cell measures the amount of light reflected from the paper; the more lipid, the blacker the stain on the paper, therefore the smaller the amount of reflected light and the greater the deflection of the recording point of the machine. The

information appears as waves or peaks corresponding to the different lipoprotein "bands" on the electrophoresis strip. Because the intensity of staining of the lipoproteins is a measure of the amount on the paper (Swahn, 1953) the areas under these curves are indirect measures of the relative amounts of lipoprotein present, and while not usually employed to give absolute values for these lipids they are suitable for comparisons of the individual lipoprotein complexes in any one serum. However, because of a number of experimental factors which are largely outwith the control of the investigator the lipid-dye uptake quotient is not so constant as Swahn suggests and this is the main reason for the experimental error previously referred to.

4. An Amsler Planimeter, a precision instrument used for the measurement of areas bounded by irregularly curving lines. This instrument can be used for the measurement of absolute units of area (e.g. square yards, acres, etc.) by reference to appropriate conversion tables. But here it is sufficient to get a number only, which is a "unit of area" and which can be compared with other numbers representing proportionately larger or smaller areas.

The instrument was used at the same setting continuously. Provided it is used on a flat smooth board, in a good light, with a steady hand and within the optimum angle of use, the margin of error is trivial being not more than ± 1 per cent.

5. Whatman No. 1 Filter Paper of Chromatography Grade and provided in 2" broad strips, - this is a finer grade of paper than Whatman 3 mm. which is widely used as the supporting medium in paper electrophoresis. It is slightly

more difficult to handle than the thicker paper since it tears more easily when wet, but this minor drawback is quickly overcome by experience and is offset by the greater definition of the serum bands and by the whiter background which can be achieved with lipid and protein stains.

6. Veronal Buffer of pH 8.6 made by dissolving sodium barbitone (10.3 G) and barbitone (1.84 G) in 1 litre of distilled water. The buffer was kept in stock bottles in a refrigerator. This prevented the growth of moulds.

7. Sudan Black B, 0.1 per cent in 55 per cent ethanol, prepared exactly as described by Swahn (1953). Not more than 1 litre of the stain was made up at a time. The effective life of this amount of stain, if in constant use, is about one month.

8. Bromo-Phenol Blue, 0.01 per cent in a solvent containing 5 per cent zinc sulphate in 5 per cent acetic acid, and used for the identification of the serum proteins.

9. A Hot Air Oven maintained at 100° C.

10. Pipettes, beakers, washing trays, etc.

Method

Preparing the Tank

The electrophoresis tank is charged with buffer, the reservoirs at either end being filled to equal levels. This limits the osmotic flow of buffer along the paper which tends to occur when the reservoirs are at different levels. The meticulous accuracy of siphon levelling, suggested by some workers, is hardly necessary however. The paper strips are placed in the tank with their

ends dipping into the buffer compartments and allowed to moisten by capillarity. This takes time but it is the best way of ensuring homogeneous wetting of the paper. The cathode end of the paper is slung over a glass rod which is 2 cms. higher than the anodal end. This imparts a slight slope to the paper sufficient to prevent the pooling of buffer which tends to occur in wet and therefore sagging filter paper.

Applying the Serum

Once the papers are completely moistened the current is switched on for about 1 hour before the serum is applied. The procedure for applying the serum is perhaps the most critical part of the entire method and requires considerable practice. Since at least 0.1 ml. of serum must be used for satisfactory lipoprotein analysis it is best applied directly from a pipette moved to and fro across the application point, and not in instalments from the edge of a glass slide, though this technique is suitable for the single application of small amounts of serum (0.015 ml.) used in protein electrophoresis.

The application zone of the wet paper (where it rests on the glass rod) is partially dried by firm pressure with a pad of dry filter papers, 5 or 6 strips thick. If serum is applied topaper which is saturated with buffer it cannot be absorbed by the paper and tends to run over its surface. It is then impossible to obtain clearly defined bands. Furthermore the serum should be applied to the centre of the paper only, avoiding the outside edges by at least 1 cm. on each side.

Conditions during Electrophoresis

After the samples (an electrophoresis tank can take up to 6 strips) have been applied, the glass lid is placed in position and the current switched on at once. A normal run lasts 16 hours from 5 p.m. overnight to 9 a.m. The usual voltage applied is 120 Volts. After a run the current is switched off, the paper strips are removed, rolled end to end in an open cylinder and stood on edge on clean filter paper. They are immediately dried at 100° C. for 30 minutes, before staining.

At first many papers were spoiled by buffer condensing on the glass lid and falling in large drops on to the underlying papers. This was overcome by applying a light coating of silicone grease to the glass surface. The buffer vapour then condenses in a fine film which adheres to the glass surface.

Staining

When the strips are dry they are divided longitudinally. One half is stained for protein with bromophenol blue and the other for lipoprotein with Sudan Black B. The lipoproteins are identified from their position alongside the corresponding protein band. However the lipoprotein pattern is simple and standard enough to be recognised in its own right and, in the main, strips were stained for lipoproteins only. The double staining procedure was carried out as a check from time to time, and always when a new investigation or experiment was begun.

Lipoprotein staining was carried out essentially as described by Swahn (1953). Dried paper strips, usually in batches of 12 rolled together, are

placed for three hours in an air tight jar containing 0.1 per cent ethanolic Sudan Black B. They are next washed three times in 50 per cent industrial spirit, but not necessarily for the rigid 15 minute periods described by Swahn. There is no point in doing this unless newly prepared stain of identical concentration is used each time, for repeated use of the stain lowers its strength so that the intensity of the lipoprotein staining diminishes. The principle adopted then was to rinse in such a way that the blank area of filter paper was brought to a fairly standard light blue-black tone. This is best done using visual judgement rather than employing a rigid methodology in the presence of important variables, such as the strength of the stain and room temperature, to mention two of the most important.

Selection and Measurement of Suitable Strips

After rinsing, the strips are dried and examined, unsatisfactory strips being discarded. The investigator's own judgement seems to be the simplest and yet most accurate way of maintaining a satisfactory quantitative standard. Strips which pass inspection are trimmed to a width which fits the sample-holder in the densitometer. They are "scanned", the lipoprotein peaks delineated and the areas corresponding to the lipoprotein bands demarcated by dependent lines drawn to the base line. Systems employed for the construction of the base line vary widely among different workers. It is for each investigator to establish his own practice and adhere to it. Mine was to draw a line joining the mean level of the blank areas at either end of the strips. A typical lipoprotein strip with its superimposed linear pattern divided into lipoprotein areas is shown in Fig. 1.

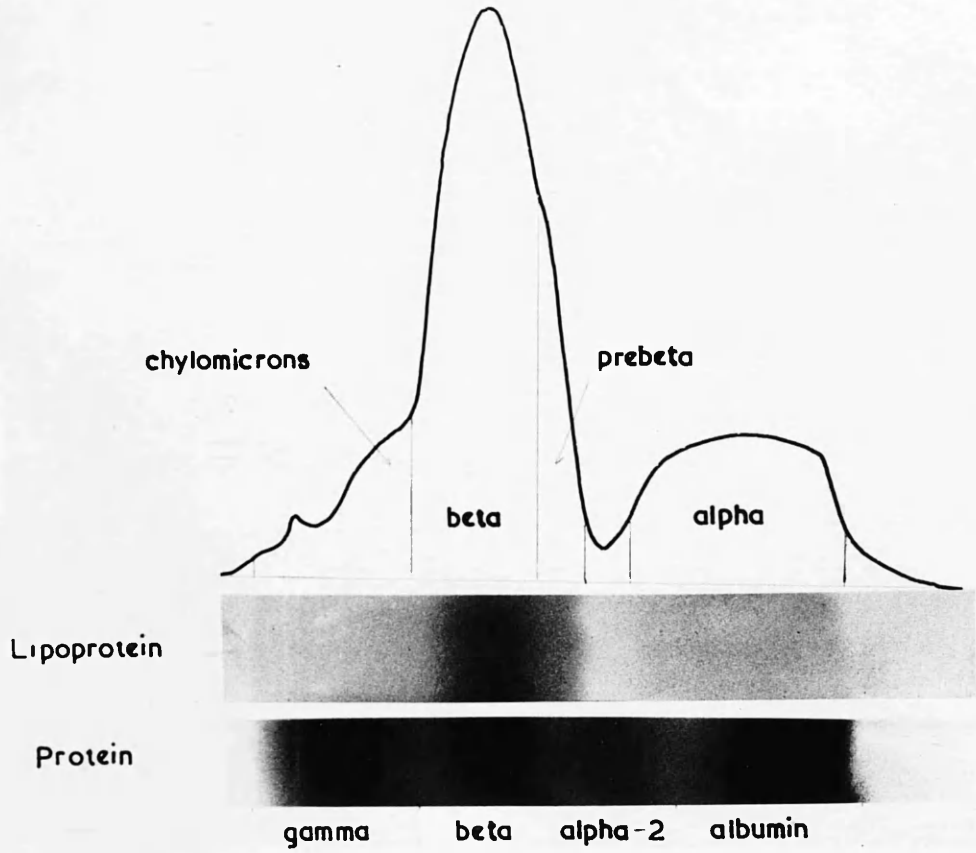


Fig. 1. An example of lipoprotein electrophoresis showing the neutral fat, beta, prebeta and alpha bands with the pattern obtained by densitometry. The protein pattern is shown alongside. The leading edge of the alpha-lipoprotein band is often called the lipalbumin zone.

Lipoprotein Nomenclature

Fig. 1. shows four distinct lipid staining areas. Beta-lipoprotein which is closely associated with beta-globulin is the predominant lipoprotein of adult serum. Alpha-lipoprotein runs in the alpha-1-globulin:albumin zone and is generally regarded as a relatively stable lipoprotein and less liable to qualitative or quantitative fluctuations than beta-lipoprotein. Its cholesterol content may however be affected by oestrogens (See Chaps. 3 and 4).

Prebeta-lipoprotein was first definitely recognised and described by Dangerfield and Smith (1954). It does not have a close relationship to any of the serum proteins and its characteristic position is adjacent to, but ahead of beta-lipoprotein and between beta and $alpha_2$-globulin.

The lipid zone between the point of serum application and beta lipoprotein consists of chylomicrons, lipomicrons and very low density beta-lipoprotein molecules, in that order. The chylomicrons are the lipid droplets, consisting mainly of neutral fat, which are responsible for the serum turbidity of post-prandial lipaemia. The lipomicrons, which also consist mainly of neutral fat, are much smaller and add little to this turbidity. In moving boundary or starch block electrophoresis the chylomicrons and lipomicrons occur in the $alpha_2$-globulin zone. They are strongly adsorbed to filter paper however and this force seems to be sufficient to disrupt their $alpha_2$-globulin linkage. This "neutral fat" zone of the electrophoresis strip is principally affected by fatty meals. It is also increased in such conditions as nephrosis, myxoedema, diabetes mellitus and essential hyperlipaemia where neutral fat as well as serum cholesterol is augmented.

Alpha$_2$-lipoprotein when present is associated with alpha$_2$-globulin This lipoprotein is encountered most frequently in the cholesterol-fed, oestrogenised chickens of Chapter 4. Its role in human beings is neither constant nor clear.

System for Reporting Lipoprotein Values

The lipoprotein value most frequently referred to in this thesis is the beta:alpha lipoprotein ratio. When prebeta lipoprotein is present it is included as part of the "beta" component of the ratio. Where a survey of prebeta-lipoprotein values is made the amount of prebeta-lipoprotein present is assessed as a fraction of the entire beta-lipoprotein complex. Besterman (1957) employs a scheme in which he relates each of the three main lipoprotein bands to their joint totals. In some studies only an estimated grading of the prebeta-lipoprotein band is used in which the values are 0, +, and ++. I tested this arbitrary judgement against the calculated ratios in a number of analyses and found that the prebeta: beta ratio 0.19-0.20 was the one at which transition from + to ++ almost constantly occurred.

Normal chickens do not show a beta-lipoprotein band corresponding to that in adult humans, although one does develop as a result of cholesterol feeding. (Chapter 4). But they do have a lipid zone which lies between the starting point and the region where beta-lipoprotein ultimately appears. So that a nominal value can be given to the beta:alpha lipoprotein ratio of normal chicken sera the "beta-lipoprotein area" is regarded (in the chickens only) as the whole area between the starting point and the distal margin of the actual beta-lipoprotein band.

TABLE II. Interrelationship in nomenclature of various methods of lipoprotein analysis.

DENSITY	GOFMAN ULTRACENTRIFUGATION (S_f)	STARCH ELECTROPHORESIS (globulins)	COHN FRACTIONATION
LOW DENSITY LIPOPROTEINS			
< 1.006 (chylomicrons)	> 20	alpha - 2	I + III
1.006 - 1.019	12 - 20	beta - 1	I + III
1.019 - 1.063	0 - 12	beta - 1	I + III
HIGH DENSITY LIPOPROTEINS			
1.063 - 1.125	HDL 2	alpha - 1	IV + V
1.125 - 1.21	HDL 3	alpha - 1	IV + V

Rats have a lipoprotein pattern quite distinct from that in humans or chickens and only qualitative variations in this animal's serum are described.

2. The Composition of Lipoproteins

Because most of the analytical biochemistry of lipoproteins has been performed in fractions obtained by methods other than electrophoresis it is important to understand clearly how the nomenclatures of the different fractionation techniques are related to one another. Table II gives the equivalent terminology for the lipoprotein fractions obtained by starch electrophoresis, ultracentrifugal flotation and Cohn fractionation. It is constructed according to the recommendations for lipoprotein nomenclature made by the Committee on Lipid and Lipoprotein Nomenclature of the American Society for the Study of Atherosclerosis (1956). The low density lipoproteins ($D < 1.063$) correspond to the Gofman Sf classes > 0. All these lipoproteins have the electrophoretic mobility of beta-globulins. Chylomicrons ($D < 1.006$) have the mobility of $alpha_2$-globulin on starch electrophoresis but run differently on paper. The lipids in Cohn fractions I and III correspond closely to those of density < 1.063. The lipoproteins of $D > 1.063$, the high density lipoproteins, have the mobility of $alpha_1$-globulin and are usually found in Cohn fraction IV and V.

There is good presumptive evidence that the Sf 12-20 class of lipoproteins and pre-beta-lipoprotein are identical. Gofman et al., (1950) reported a highly significant increase of the former and Besterman (1957) a similar increase of the latter in patients following myocardial infarction. In addition, the

TABLE III. The percentage composition of serum lipoprotein fractions.

LIPOPROTEIN FRACTION (by density)	TOTAL CHOLESTEROL	PHOSPHOLIPID	TRIGLYCERIDE	PROTEIN	CHOLESTEROL PHOSPHOLIPID RATIO
< 1.006	9.1	7.1	81.3	2.5	0.95
1.006 - 1.019	22.2	17.9	51.8	7.1	0.90
1.019 - 1.063	46.9	23.1	9.3	20.7	1.30
1.063 - 1.21	19.4	26.1	8.1	46.4	0.48

biochemical data for the Sf 10-20 lipoproteins shown in Table III are comparable to the more limited data obtained for prebeta-lipoprotein by Besterman.

Chemical Properties of the Lipoproteins

Table III is compiled from data obtained by Bragdon et al., (1956). It gives a detailed account of the normal lipid composition of the different lipoprotein classes. Chylomicrons consist mainly of triglyceride with much smaller amounts of cholesterol and phospholipid and only a trace of protein. The Sf 10-20 group of lipoproteins (prebeta-lipoprotein) contain major cholesterol and phospholipid components and about three times the amount of protein in the chylomicrons, as well as a large triglyceride fraction. Beta-lipoprotein is characterised by a high ratio of cholesterol to phospholipid (C/P ratio). Alpha-lipoprotein has the highest protein content of any of the lipoprotein fractions and it is probably this and its much lower C/P ratio which confer upon it the biochemical stability it is generally regarded as possessing.

Attempts have been made to characterise the major lipid fractions in greater detail. Steele and Kayden (1955) studied the phospholipids and found a higher proportion of sphingomyelin to lecithin in the beta fraction than in the alpha fraction. Gillies et al., (1956) determined the fatty acid composition of the low density lipoproteins and found that beta lipoprotein contained large amounts of linoleic in addition to stearic, palmitic and oleic acids. Chylomicrons on the other hand contained stearic, palmitic, oleic and palmitoleic acids but no linoleic acid. These differences are probably due to the fact that cholesterol is esterified chiefly with linoleic acid and is of

course present in largest amounts in beta-lipoprotein, whereas the chylomicron fatty acids derive mainly from triglyceride.

Physical characteristics of the lipoproteins

The high density lipoproteins have molecular weights ranging from 165,000 to 435,000 (Shore, 1956). The low density lipoproteins have much higher molecular weights, the lowest being about 2,100,000, hydrated (Oncley, 1956) and this may increase to as high as 250 million for the chylomicrons (Shore, 1956). Oncley et al., (1947) have shown that beta-lipoprotein is 60 per cent water by weight and that this water has an important structural role in the molecule since, if removed, much of the lipid splits off. From their measurements they concluded that the molecule was a sphere with a diameter of 185 Å. They suggested that since the molecule had so many of the properties of a protein, the polypeptide chains must be arranged on the surface with the lipid inside. They concluded that the alpha-lipoprotein is ellipsoid in shape with a long axis of 300 Å and a short axis of 50 Å.

With this account of the composition and structure of the serum lipoproteins we now turn to our investigation of some of the factors which influence the concentration and distribution of the serum lipids.

CHAPTER 3

SERUM LIPIDS IN PREGNANCY AND THE PUERPERIUM

Although it is known that the various lipid fractions of blood may be altered by the administration of substances such as heparin, (Hahn, 1943; Boyle et al., 1952; Nikkila, 1952), and oestrogens, (Eilert, 1949; Barr, 1953), a full account of physiological variations is lacking. During pregnancy it is possible to study the serum lipids under the influence of profound, though physiological, hormonal and metabolic disturbances.

Earlier studies have produced results which are not entirely in agreement. Boyd (1934) stated that there was a rise in serum lipids, phospholipid during the first trimester and cholesterol later. He also noted an increase in neutral fat. Dieckmann and Wegner (1934) concluded that serum cholesterol increased later in pregnancy because there was either faulty elimination by the liver or retention for lactation. They quote Denecke (1924) who found that after delivery cholesterol was rapidly excreted in milk, bile and urine, and had returned to normal levels by the fourteenth day. Oliver and Boyd (1955) after a careful study of 12 normal primigravidae stated that between the 31st and 33rd weeks of pregnancy there was a highly significant rise in the plasma ester and total cholesterol, the cholesterol:phospholipid ratio (C/P ratio) and the beta-lipoprotein cholesterol. By the 20th week postpartum these values had decreased considerably, but were still higher than the levels at the 12th week of pregnancy.

It seemed relevant to repeat these studies in a large, homogeneous group of women, to extend them to include observations on the beta, prebeta, and alpha lipoprotein complexes and to examine any possible relationship between these observations and such factors as maternal weight gain, baby's birth weight, breast feeding or any others which might arise.

Material and Methods

This investigation includes 48 primigravidae between the ages of 17 and 29, the mean age being 22 years. They were enrolled at times varying from the 11th to the 33rd week and all were in good general health with normal blood pressure, urine, chest X-ray and electrocardiogram. In 6 cases pregnancy developed abnormally and the findings are considered separately.

Each woman was seen in the afternoon of her monthly visit to the ante-natal clinic (and, in some cases, more frequently nearer term); on admission in labour, if possible; 6-7 days after delivery; and at the routine post-natal visit 5-7 weeks later. Difficulties inherent in the organisation of a series of this size, and the occasional absence of a case from clinic attendance, account for small numerical deficiencies at each stage.

On each occasion a sample of venous blood was taken for estimation of haemoglobin, packed cell volume, serum cholesterol and for electrophoresis. Total serum cholesterol was measured by the micro-method of King (1951) and electrophoresis, preparation and evaluation of the lipoprotein patterns as described elsewhere (Chapter 2). Wasserman and Kahn reactions were tested as part of the routine ante-natal care, as was blood grouping.

TABLE IV. Mean total serum cholesterol values and beta:alpha lipoprotein ratios of 42 normal primigravidae during pregnancy and the puerperium.

	Week	13	17	21	25	29	33	37	39	Labour	5-7 days post-partum	6-8 weeks post-partum
Serum Cholesterol mg. %	No. of Subjects	11	22	24	29	34	39	29	23	22	39	38
	Mean	191	206	218	229	252	243	250	246	256	239	228
	S.D.	28	46	45	39	41	58	45	55	47	53	43
Lipoprotein Ratio	No. of Subjects	10	19	21	23	30	37	27	23	21	37	37
	Mean	1.14	1.20	1.26	1.44	1.62	1.72	1.85	1.43	1.70	1.59	1.37
	S.D.	0.19	0.28	0.33	0.38	0.45	0.41	0.44	0.26	0.47	0.38	0.43

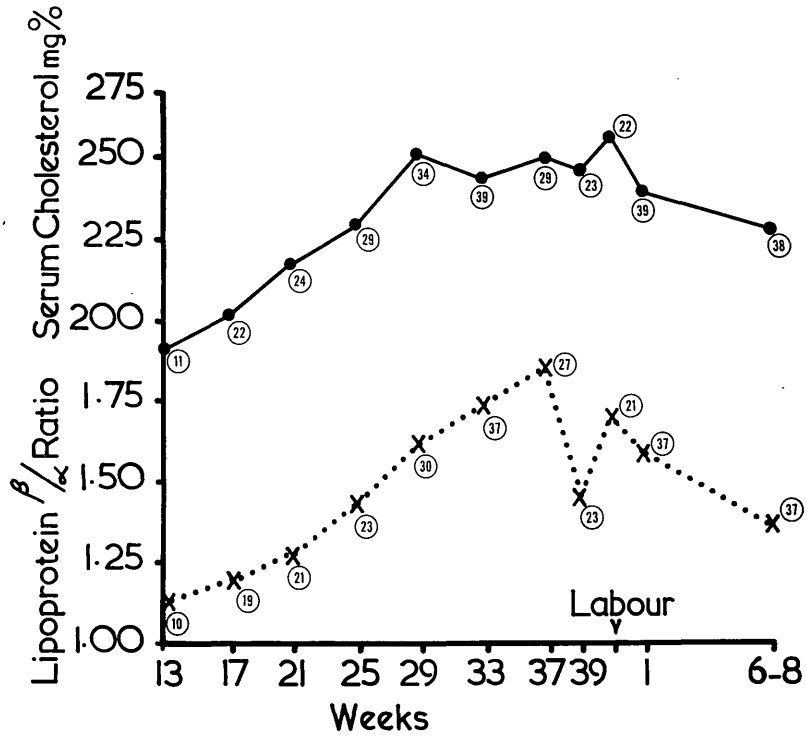


Fig. 2. Graph of results in Table IV. The circled figures give the number of subjects in each sample.

RESULTS

The principle adopted by Oliver and Boyd (1955) in fixing the zero point of each pregnancy is followed here. Parturition is taken as the first day of the 40th week and prepartum stages calculated retrospectively. The results are grouped in periods of four weeks, the week named being the first of each period. The sequential serum cholesterol and lipoprotein results for each subject are given in Appendices I and II.

Total Serum Cholesterol (Table IV and Fig. 2)

Total serum cholesterol rose from a mean of 191 mg. per cent (S.D. 28) at the 13th week to a mean of 252 mg. per cent (S.D. 41) at the 29th week, and remained at about this level until term. It fell after parturition and by the 6th to 8th week had reached a mean of 228 mg. per cent (S.D. 43). This was still much higher than the mean at the 13th week of pregnancy.

The same 22 women were seen both at the 17th and 29th weeks of pregnancy. The mean cholesterol values of this group were 206 mg. per cent and 252 mg. per cent respectively. The mean difference of 46 mg. is significant ($P < 0.01$). The form of the cholesterol curve (Fig. 2) suggests that by the 13th week, the effective starting point of this investigation, the serum cholesterol had already begun to rise.

Lipoprotein Ratio (Table IV, Fig 2)

The mean beta:alpha lipoprotein ratio rose from 1.14 (S.D. 0.19) at the 13th week to 1.85 (S.D. 0.44) at the 37th week. This difference is significant ($P < 0.01$). The ratio fell to 1.43 (S.D. 0.26) in the week before term.

However only 12 of those subjects seen at the 37th week were seen again at the 39th and the mean lipoprotein ratios at those times were 1.76 and 1.51 respectively. This difference is unlikely to be significant ($0.1 > P > 0.05$). The slight rise from 1.47 at the 39th week to 1.55 at labour in another sequential group of 12 is not significant ($P > 0.5$). By 6-8 weeks post-partum the mean ratio had fallen to 1.37 (S.D. 0.43). This figure is considerably higher than at the 13th week of pregnancy.

The form of the lipoprotein curve (Fig. 2) gives less indication that a change in beta:alpha lipoprotein ratio has occurred before the 13th week and suggests that it continues to rise for some time after the serum cholesterol has attained its maximum. This could be attributed to increases in either the phospholipid or fatty acids of beta-lipoprotein during the weeks preceding labour.

Pre-beta lipoprotein

It proved difficult to devise a wholly consistent method for the assessment of this lipoprotein moiety, and the scheme eventually adopted is no better than a rough working guide. Thus, if no pre-beta lipoprotein was seen in any electrophoresis strip in a subject series the grading was 0; where pre-beta lipoprotein appeared with some frequency and one or more of the bands could be graded ++ the entire series was given that grading. Intermediate patterns were accorded a + grading. On this basis, of the 42 normal subjects 8 were classed as 0, 24 as + and 10 as ++. The subjects in the 2 groups of 8 and 10 were compared in respect of the following values: a) lowest total serum cholesterol recorded; b) highest serum cholesterol recorded,

TABLE V. Mean values of a) lowest serum cholesterol, b) highest serum cholesterol, c) weekly weight gain, and d) babies' birth weights of 2 groups of primigravidae selected on the degree of pre-beta lipoproteinaemia.

GROUPS	(a) Lowest serum cholesterol (mg.%)	(b) Highest serum cholesterol (mg.%)	(c) Mother's weekly weight gain (lbs.)	(d) Birth weights (lbs.)
No pre-beta lipoprotein (8 cases)	181.5	281.5	1.05	7.12
Range	139-242	212-358	0.33-1.60	6.25-8.45
2+pre-beta lipoprotein (10 cases)	203.9	313.7	1.30	7.19
Range	157-253	267-394	0.75-2.00	5.95-8.25
	t = 1.84 0.1 >p>0.05	t = 1.31 p > 0.10	t = 1.30 p > 0.10	

TABLE VI. Cholesterol and lipoprotein values at the 33rd. week of pregnancy and 6-8 weeks post-partum of 5 women whose average weekly weight gain during pregnancy exceeded 1.4 lb.

Case No.	Average weight gain lb/week	33rd. week		6-8 Weeks Post-partum	
		Cholesterol mg. %	Lipoprotein ratio	Cholesterol mg. %	Lipoprotein ratio
1	1.85	260	2.62	266	2.21
8	1.63	225	1.87	262	1.76
26	1.42	343	2.75	219	1.59
35	1.42	256	2.16	248	1.98
46	1.80	231	2.00	242	1.47
Mean	1.62	263	2.28	247	1.80
Series Mean	1.10	243	1.72	228	1.37

c) mother's average weekly weight gain, and d) baby's birth weight. The results are summarised in Table V. Statistical analyses of the two sets of figures do not demonstrate a significant difference between the groups but nevertheless there seems to be a trend of significance insofar as three of the values, namely the highest and lowest serum cholesterol readings and the mother's average weekly weight gain are all highest in the group characterised by a pre-beta lipoprotein band which persists at high levels throughout pregnancy.

Weight change (See Appendix I)

The average weekly weight gain during pregnancy was 1.1 lb. (0.5 kg.). The average weight loss from the maximum recorded during pregnancy to the weight at 6-8 weeks post-partum was 22.4 lb. (10.2 Kg.).

Table VI shows the cholesterol and lipoprotein value at the 33rd week and in the phase 6-8 weeks post-partum of 5 women whose average weekly gain in weight exceeded 1.4 lb. (0.64 Kg.). At both times their average cholesterol values exceeded the series mean by 8 per cent, (263 and 247 as against 243 and 228) and their average lipoprotein ratios exceeded it by 31 per cent (2.28 and 1.80 as against 1.72 and 1.37). The 33rd week cholesterol and lipoprotein values of those women were tested against the corresponding values of 5 of the women whose average weight gains were grouped nearest to the mean. This small sample had an average cholesterol level of 261 mg. per cent and there was obviously no difference between the groups, but the average lipoprotein ratio was 1.82, the difference between them being marginally significant ($t = 2.42$; $0.05 > p > 0.02$). Four of the 5 women with increased weight gain

had slight ankle swelling towards term but none was considered by the obstetrician managing the case to be "toxaemic". The birth weights of the 5 babies were normal.

The corresponding figures for those whose weekly gain in weight was less than 0.8 lbs. (0.36 Kg.) show nothing of note. No connection was discovered between lipid trends and weight loss after parturition.

Birth Weight (See Appendix II)

The babies' weights ranged from 5 lbs. 9 oz. (2.5 Kg.) to 8 lbs. 15 oz. (4.0 Kg.). There was no obvious relationship between maternal lipid values and the birth weight of the child.

The effect of feeding

Only 6 of the women were still breast feeding their babies at the time of the final post-natal visit; 19 had transferred to bottle feeding before leaving hospital and the remainder some time thereafter. All of the 19 and most of the others who had gone on to bottle feeding had been given stilboestrol. Because of this, and also because of the disparity in the group sizes, and the scatter of the times at which the feeding transition occurred, no valid conclusions could be drawn regarding the effect of infant feeding or the serum lipid values.

Pregnancy developed abnormally in 6 of the subjects and the main details of these cases are given in the following brief accounts.

Case 10 (J.C.) Normal until term. Sudden onset of eclampsia during labour, which was three weeks later than the expected date. Spontaneous delivery of a live, female child weighing 9 lbs. 2 oz. (4.15 Kg.), the heaviest of the series.

Uneventful puerperium. Mother and child in good health at the post-natal visit.

The serum lipids conformed to the general pattern, apart from a steep rise in the lipoprotein ratio from 1.73 at the 39th week to 2.56 during labour. There was no associated rise in serum cholesterol. Pre-beta lipoprotein grading 0.

Case 13 (H.S.) Rather nervous woman, but otherwise normal until she was admitted at the 37th week with mild pre-eclampsia. Discharged improved after one week. Spontaneous delivery of a macerated, male foetus, weighing 6 lbs. 14 oz. (3.1 Kg.). No obvious cause apart from pre-eclampsia. Normal puerperium.

The lipoprotein ratio remained fairly constant near the 13th week level, throughout pregnancy and the puerperium. Pre-beta lipoprotein grading +.

Case 20 (J. McC.) Normal throughout pregnancy. Spontaneous delivery of a limp, asphyxiated male child weighing 5 lbs. 12 oz. (2.6 Kg.) which died soon after birth. Autopsy revealed a large diaphragmatic hernia and patent foramen ovale.

The serum cholesterol rose from 217 mg. per cent at the 20th week to 355 mg. per cent at the 38th week and the lipoprotein ratio reached the very high values of 3.27 and 3.34 at the 35th and 38th weeks, respectively. Pre-beta lipoprotein grading ++.

Case 45 (J.G.) Normal until near term. Caesarean section performed because of severe pre-eclampsia. Live female child weighing 6 lbs. 9 oz. (3.0 Kg.).

There was no significant variation of the lipids from the general pattern. Pre-beta lipoprotein grading 0 .

Case 24 (C.C.) Mild thyrotoxicosis suspected on clinical grounds at the 25th week. Labour induced by artificial rupture of the membranes because of mild pre-eclampsia, 14 days after the expected date of delivery. Live male child weighing 8 lbs. 11 oz. (3.9 Kg.). Remission of thyrotoxic symptoms after delivery.

The lipoprotein ratio ranged closely about a mean value of 0.83 until term. One week after delivery it had risen to 1.82 and was 1.81 seven weeks later. The serum cholesterol, which ranged closely about 158 mg. per cent until term, was 182 mg. per cent one week post-partum and 306 mg. per cent seven weeks later. Pre-beta lipoprotein grading 0.

The average weekly weight gain in this case was 0.55 lbs. (0.25 Kg.), the second lowest in the entire series.

Case 49 (A.M.) Moderate enlargement of thyroid noted. "Nervous" but not convincingly thyrotoxic. Given small doses of phenobarbitone. Spontaneous, normal delivery of a live male child weighing 8 lbs. 15 oz. (4.0 Kg.). At the post-natal visit she was more composed. There was no change in the size of the thyroid.

Serum cholesterol rose from 225 mg. per cent at 16 weeks to 389 mg. per cent at term, but fell normally thereafter. The lipoprotein ratio was slightly above average throughout. Pre-beta lipoprotein grading : ++.

DISCUSSION

This study confirms that significant changes occur in the serum lipids during normal pregnancy and mainly in those lipids which comprise the beta-lipoprotein complex. For although the paper electrophoretic method of studying

the lipoproteins is only semi-quantitative it is clear from an overall assessment of the stained paper strips that the increase in the beta:alpha ratio is due to an increase in beta lipoprotein and not to a decrease in alpha lipoprotein, which seems to remain fairly constant throughout. The experimental conditions of the work reported in the next chapter allowed some degree of quantitative comparison of changes in alpha lipoprotein but these will not be anticipated.

Beta-lipoprotein may be increased by increases in its cholesterol, phospholipid or fatty acid components. Since the increase in serum cholesterol seems to reach its maximum about the 29th week of pregnancy while the whole beta-lipoprotein fraction goes on increasing up to the 37th week, it is reasonable to infer that either the phospholipid or fatty acid components, singly or together are increasing up to this point.

Oliver and Boyd (1955) give an excellent account of the endocrine factors which may affect serum lipids. These are so complex that any attempt to use them to account for the lipid changes reported here can be no more than speculative oversimplification. Among other things they draw attention to the paradox that the large physiological increases in circulating oestrogens which occur in pregnancy (Preedy and Aitken 1957) are associated with an increase in the blood lipids, while administered oestrogen acts conversely (Eilert, 1949). But there is no proof that the augmented oestrogen level is the only lipogenic factor. The possible effect of altered secretions of the pituitary and adrenal hormones cannot be discounted. On the other hand the abnormal lipid pattern of case 24, the woman with thyrotoxicosis, suggests that however

potent these other endocrine secretions may be as lipogenic factors, they can be effectively over-ruled by increased thyroxine production.

Cairns and Constantinides (1954) and Sundberg (1955) have shown that the mast cell content of female arteries during the years of reproductive activity is greater than in males of the same age group. Oestrogens may to some extent determine this difference and herein lies another theoretical approach to an explanation of the lipid changes, for mast cells contain heparin (Holmgren and Wilander, 1937; Oliver et al., 1947 Ehrich et al., 1949) and heparin can reduce the levels of circulating beta-lipoprotein (Nikkila, 1952). If the relationship between blood oestrogen levels, mast cells and heparin production was a direct and simple one, rising blood oestrogen levels should increase heparin production and so lower the beta: alpha lipoprotein ratio; the reverse, in fact, of the changes observed in this study. It is possible however that the very high blood oestrogen levels of pregnancy exhaust the mast cells, depress their activity, or reduce the number in the tissues, thus lowering heparin production and so contributing to the lipoprotein changes demonstrated. The slow return to normal lipoprotein values during the puerperium may be due to delayed recovery of mast cell function.

There is some experimental support for this idea in the work of Westin (1955), Westin and Odelblad (1956) and Bergstrom et al., (1958) who have shown that the administration of oestrogens to oophorectomised mice significantly lowers the mast cell content of uterus and vagina. It is also

supported by the studies of Baglioni and Lorenzoni (1955) on the Walker cell tumour. They showed that whereas small doses of oestrogen were followed by an increase in the number of mast cells at the periphery and in the capsule of the tumour, larger doses led to a fall in the number of mast cells in the same regions. The possible role of the tissue mast cell in atherosclerosis and lipaemia is examined experimentally later in this thesis.

The small group with maximum weight gain invites comment but again this can be little more than speculative. If the weight gain is not solely a manifestation of mild toxaemia, it may reflect increased growth hormone activity, which may also cause the high lipoprotein ratio. But in this case we might have expected big babies. The birth weights of the 5 babies were, however, normal. And Oliver and Boyd (1954) were unable to produce a rise in blood cholesterol by the intravenous injection of growth hormone, the activity of which had previously been demonstrated by injection into rats.

Lastly it must be observed that the pre-beta lipoprotein band first reported by Dangerfield and Smith (1955) and later shown by Besterman (1957) to be an almost invariable finding in the serum of patients following myocardial infarction, occurs more often than not in the serum of normal primigravidae.

CHAPTER 4

THE EFFECT OF EPI-OESTRADIOL BENZOATE ON THE SERUM LIPIDS OF CHOLESTEROL FED CHICKENS

While the previous study was in progress the opportunity arose to investigate the uncomplicated effect of a semi-synthetic oestrogen on the serum lipids of the chicken. This substance, epi-oestradiol benzoate, was one of a series of substances being tested for their anti-atherogenic effect, by a group of workers led by Mr. R. S. F. Campbell of the Department of Veterinary Pathology, Glasgow University, and I am grateful to him and the group for the chance to associate with them in a small part of their long-term investigations. The complete account of this particular experiment is reported in Campbell et al. (1959). Only that part of it concerned mainly with the biochemical aspects is reported here.

METHODS

Sixty Golden Legbar - Light Sussex cockerels, one month old, were allocated to 5 groups of 12 birds and housed in individual cages. Weight was distributed comparably in each group. Each group was subjected to an experimental regime of 16 weeks' duration. The birds were weighed at weekly intervals. One of the groups used in the complete study has no relevance for this report. The groups included were as follows:-

(B) Fed a lipogenic diet of commercial chick mash supplemented by 1 per cent cholesterol for 16 weeks.

TABLE VII. Monthly average weights of each group of chickens (in gms.)

Weeks	B	C	D	E	F
0	307	310	305	307	310
4	642	662	641	628	638
8	1187	1138	1243	1288	1262
12	1580	1565	1527	1731	1755
16	1777	1851	1861	1973	2001

(C) Diet as (B) for 16 weeks. After the 8th week given epi-oestradiol, 1 mg. intramuscularly daily.

(D) Diet as (B) for 16 weeks. After the 8th week given epi-oestradiol, 5 mg. intramuscularly daily.

(E) Commercial mash only for 16 weeks.

(F) Commercial mash for 16 weeks. After the 8th week given epi-oestradiol, 1 mg. intramuscularly daily.

Blood for lipid analyses was withdrawn from an alar vein at the start of the experiment and at 4-weekly intervals thereafter. Total serum cholesterol was estimated by a modification of the Sperry-Schoenheimer technique (1934) and lipid phosphorus by the method of King (1951). Total phospholipid was estimated as 25 times the lipid phosphorus value and the cholesterol - phospholipid ratio (C/P ratio) calculated from these figures. Lipoproteins were analysed by paper electrophoresis as described in Chapter 2. Complete sequential lipoprotein analyses were carried out only in groups B, D, E and F. Group C was included after the 8th week.

All birds were sacrificed at the 16th week for detailed histopathological examination of the heart and main vessels and the condition of comb, wattles and testes was noted.

RESULTS

Weight

The monthly weight averages are shown in Table VII. During the first 8 weeks there is no difference between the birds fed normal and cholesterol enriched mash. During the next 8 weeks however the cholesterol fed birds

TABLE VIII. Mean monthly lipid values of all experimental groups. For reasons which are explained in the text, the beta:alpha lipoprotein ratios of group D, at the end of the 3rd. and 4th. months, were not calculable, but were obviously immense.

GROUP	WEEKS	CHOLESTEROL mg. %	PHOSPHOLIPID mg. %	C/P RATIO	BETA:ALPHA RATIO
B	0	209	237	0.88	0.61
	4	1028	380	2.71	2.96
	8	931	327	2.84	3.06
	12	426	203	2.11	2.18
	16	299	155	1.93	1.67
C	0	188	223	0.85	-
	4	773	337	2.29	-
	8	781	313	2.50	-
	12	440	295	1.49	2.26
	16	289	203	1.42	1.90
D	0	193	230	0.84	0.63
	4	650	288	2.24	3.11
	8	576	270	2.31	2.53
	12	2513	3970	0.63	-
	16	3032	4300	0.70	-
E	0	205	237	0.90	0.61
	4	145	242	0.60	0.59
	8	178	247	0.72	0.73
	12	185	215	0.86	0.91
	16	164	182	0.90	0.69
F	0	180	237	0.76	0.59
	4	158	257	0.61	0.94
	8	196	285	0.69	0.77
	12	175	265	0.66	1.70
	16	177	275	0.65	1.83

weigh on average 172 g. less than the others. It proved impracticable to keep accurate records of food intake but the lighter birds seemed to eat less. The cholesterol fed birds receiving oestrogen (C and D) were heavier than those receiving cholesterol alone.

Oestrogenic Effects

All birds receiving 5 mg.epi-oestradiol per day (D) showed marked hypoplasia of combs, wattles and testes. Seven birds in group C and 3 in group F showed similar changes. Three of the birds in group B showed partial inhibition of sex characters, but none showed changes as severe as in the oestrogenised animals.

Lipids

The mean monthly values of the serum cholesterol, phospholipid, C/P ratio and beta: alpha lipoprotein ratio are given in Table VIII. It can be seen from the table that the various lipid values in each group were satisfactorily comparable at the start of the experiment.

For simplicity of reporting the biochemical results will be described first, and the electrophoresis results next. The relationship between the two will be educed in the discussion.

Cholesterol, Phospholipid, and C/P ratio

Group E In this, the normal control group, there was no noteworthy change in either of the 3 levels during the experiment, apart from a slight fall in the cholesterol level with a corresponding fall in the C/P ratio at the end

of the first month and some lowering of the phospholipid level at the end of the 4th month.

Group F The administration of 1 mg. of epi-oestradiol per day to birds receiving normal diet was followed by a slight though doubtfully significant fall in the C/P ratio compared with group E. This seemed to be due to minimally divergent tendencies in the phospholipid levels of the two groups, those in group F rising slightly, and those in group E falling.

Group B The inclusion of 1 per cent cholesterol into the normal diet produced a steep rise in the cholesterol levels but a less profound rise in the phospholipid levels so that the C/P ratio rose to more than three times its normal value during the first and second months of abnormal feeding. Thereafter, presumably because of enhanced cholesterol elimination there was a spontaneous return to more normal cholesterol levels, in spite of the continuation of cholesterol in the diet. There was also some reduction in the phospholipid levels during the 4th month, so that although the serum cholesterol was about one and a half times its starting value the C/P ratio was more than twice the initial figure.

Group C During the pre-oestrogen phase of the cholesterol diet the serum cholesterol levels in this group did not rise to quite the same high figure as in group B. Nevertheless they were high enough to give C/P ratios which once again were about three times normal values. During the period of oestrogen administration (1 mg./day) the serum cholesterol fell to levels similar to those in group B, but the phospholipid levels fell to a lesser degree

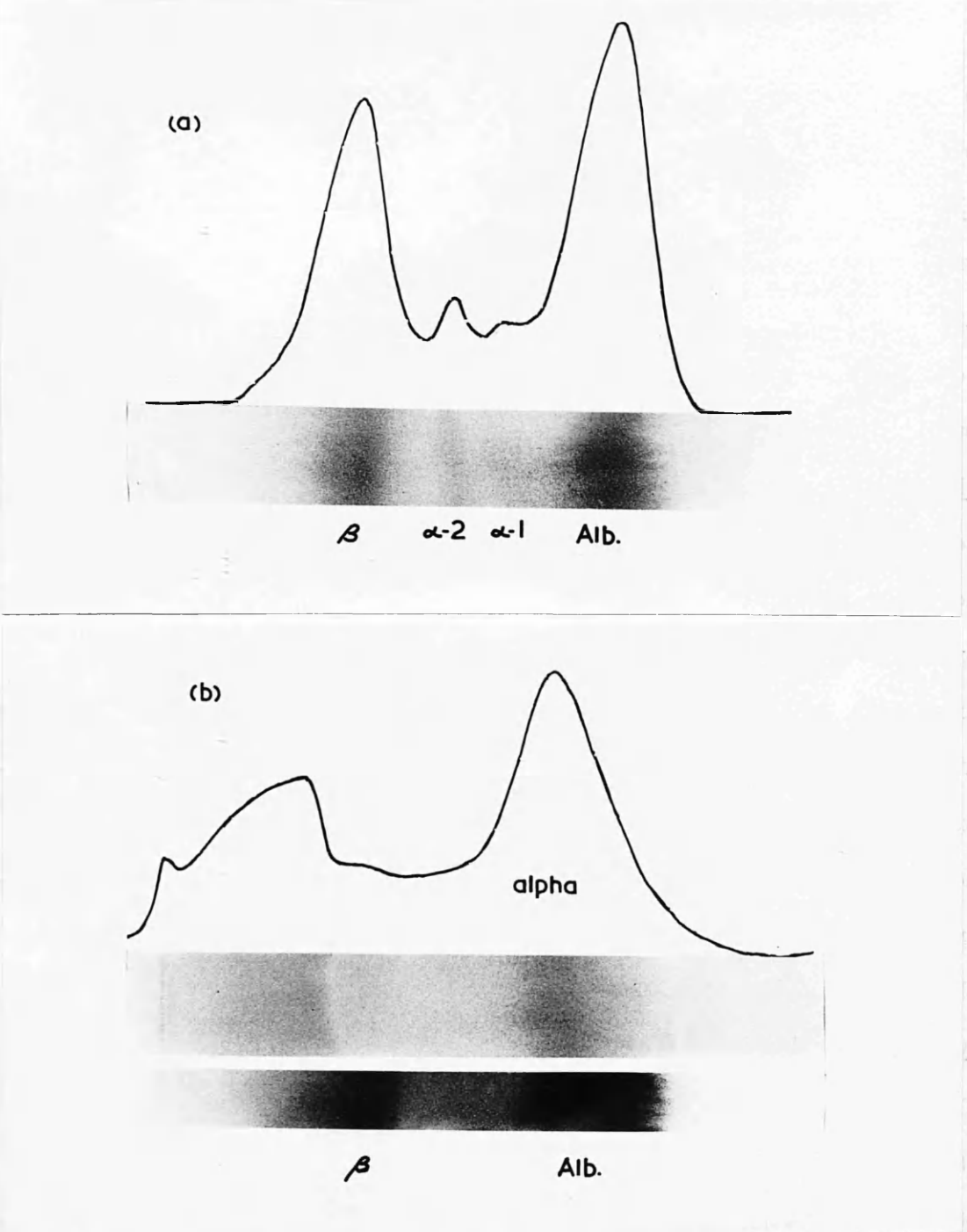


Fig. 3. Protein and lipoprotein patterns of normal chicken serum.

so that the C/P ratio was somewhat lower. (1.42 compared to 1.93). It is doubtful if these figures are significant in themselves, but it is interesting that the same tendency is apparent in the two groups E and F, and B and C, namely that this particular dose of oestrogen (1 mg./day) while having no effect on the serum cholesterol seems to have a slight effect in maintaining the serum phospholipid and so tends to lower the C/P ratio.

Group D It is clear from an examination of the results in groups C and D that the higher dose of oestrogen has evoked a radically different response which is qualitative rather than quantitative. There is an enormous rise in serum cholesterol and phospholipid, associated with great milkiness of the serum. The increase in phospholipid is of a greater degree than the increase in cholesterol, so much so that in spite of the gross hyperlipaemia the C/P ratio is actually slightly lower than the comparable normal of group E (0.70 as against 0.90).

Lipoproteins

Group E Fig. 3 illustrates the normal protein and lipoprotein patterns of the chicken and is representative of the patterns in the control group, throughout the four months. While these cannot be regarded as identical with corresponding human patterns there is sufficient similarity to allow some equivalence in nomenclature, with this major difference - the young chicken has nothing which corresponds to human gamma globulin. The protein bands therefore comprise only β - globulin, α 2-globulin, α1-globulin and albumin.

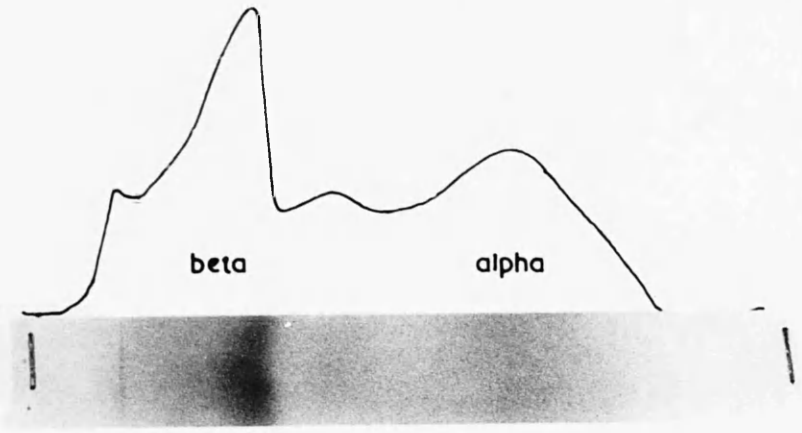


Fig. 4. Lipoprotein pattern of chicken E6 showing a well- developed beta-lipoprotein band.

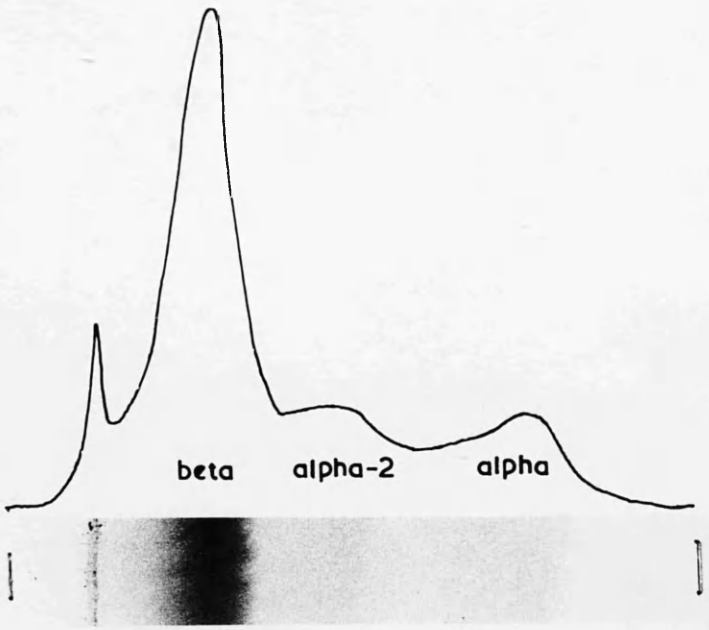


Fig. 5. Lipoprotein pattern after 1 month of oestrogen administration (bird F4). There is a prominent beta-lipoprotein band and the alpha band has regressed. An alpha-2 lipoprotein is clearly detected by scanning.

While the typical normal chicken lipoprotein pattern contains an alpha-lipoprotein band which corresponds to human alpha-lipoprotein, both in amount and in its association with the albumin - α1 globulin complex, there is no exactly comparable beta-lipoprotein. The stainable lipid which is present in a position intermediate between the starting point and beta-globulin is probably that part of the beta-lipoprotein complex which consists mainly of low density lipoproteins. However, there is some spontaneous variation and Fig. 4 shows the lipoprotein pattern in chicken E 6 where there is a well developed beta-lipoprotein complex and a higher β / α lipoprotein ratio.

Group F During the first 2 months the lipoprotein patterns were similar to those in Group E. Fig. 5 shows the striking change in the pattern after 1 month of oestrogen (bird F4). The difference is essentially due to the emergence of a prominent beta-lipoprotein band, although the degree of increase in the beta:alpha lipoprotein ratio may also be due in part to a reduction of the alpha lipoprotein. Now since there has been no rise in the cholesterol levels of this group and only a marginal increase in phospholipid it is reasonable to infer that the denser beta-lipoprotein band is perhaps due to some increase in the triglyceride or fatty acid component of the complex, and it is worth comment that some of the sera in this group were slightly turbid or opaque. This tendency reached its peak in group D where all the sera were a dense milky white.

Group B. On the cholesterol diet the lipoprotein pattern was grossly abnormal by the end of a month. There were three aspects to this change. Firstly an increase in the main beta lipoprotein band itself, an increase in the band

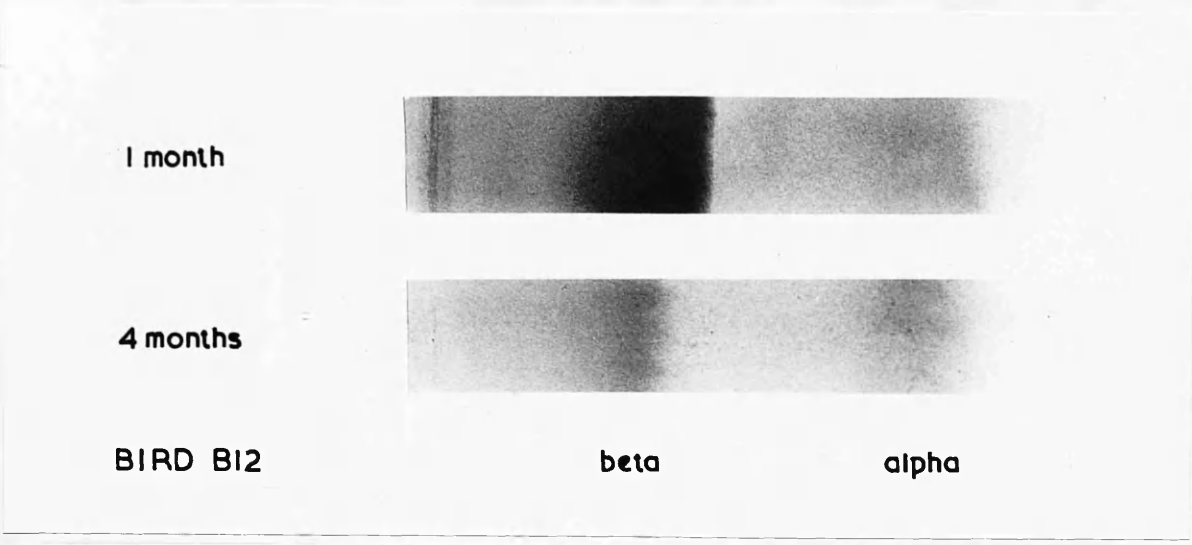


Fig. 6. Lipoprotein pattern after 1 month of cholesterol feeding and 3 months later, to show the spontaneous regression of beta-lipoprotein which has occurred during the continuous administration of cholesterol.

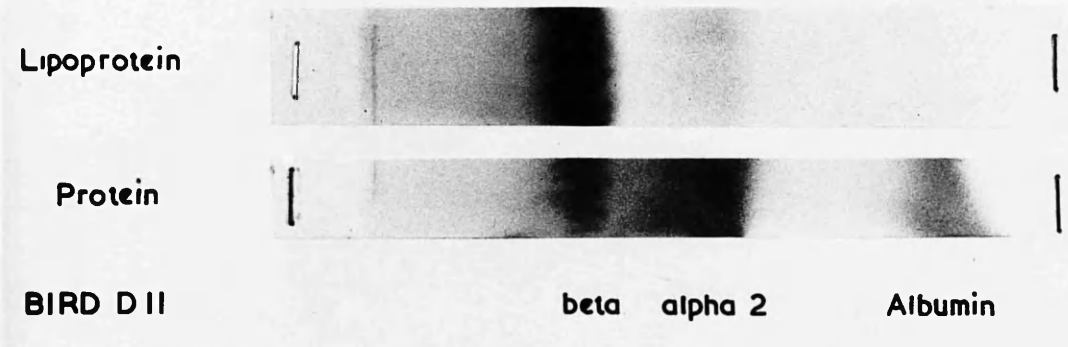
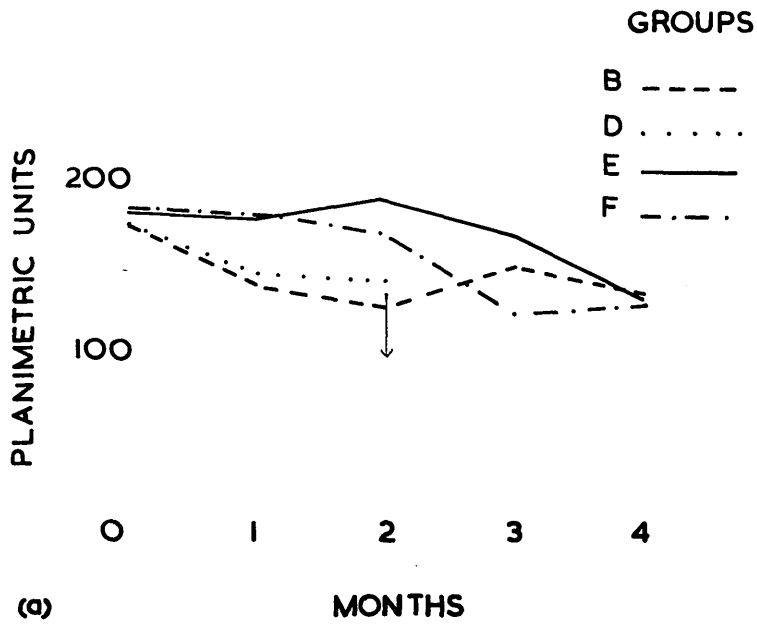


Fig. 7. Serum protein and lipoprotein patterns (bird D11) showing the gross increase in beta-lipoprotein, the disappearance of alpha-lipoprotein and the emergence of an alpha 2-lipoprotein. The proteins are also altered. Alpha 2-globulin predominates while albumin and beta-globulin are much diminished.

between the starting point and beta-lipoprotein (the neutral fat, low density lipoprotein area) and possibly also a slight decrease in alpha lipoprotein (Fig. 6). But during the third and fourth months the beta:alpha ratio fell, due mainly to a reduction in beta lipoprotein.

Group C The lipoprotein patterns in this group were not studied during the first two months. But during the second part of the experiment they were, and differed in no important respect from the corresponding lipoprotein patterns of group B.

Group D This was the group given 5 mg. of epi-oestradiol benzoate daily while on the 1% cholesterol enriched diet. The serum became grossly lipaemic and seemed to be considerably more viscous than in any of the other groups. It was technically impossible to electrophorese the usual 0.1 ml. of serum and only 0.02 ml. was analysed. With this smaller amount of serum no alpha lipoprotein was visible and it is impossible to say whether this was due to the smaller quantity of serum applied or whether it represented a real disappearance of the lipoprotein. But attempts to run slightly larger amounts of serum seemed to indicate that there was a real and probably profound reduction of the amount of alpha lipoprotein present. Beta lipoprotein stained intensely and a new band appeared running ahead of it and quite separate from it. This band corresponds with alpha$_2$-globulin which is also much increased. Fig. 7 shows the striking alteration in the lipoprotein and protein electrophoresis patterns compared with the normal in Fig. 3. This was the only group in which the serum proteins were significantly affected and it can be seen from Fig. 7 that alpha$_2$-globulin has increased to being the largest fraction



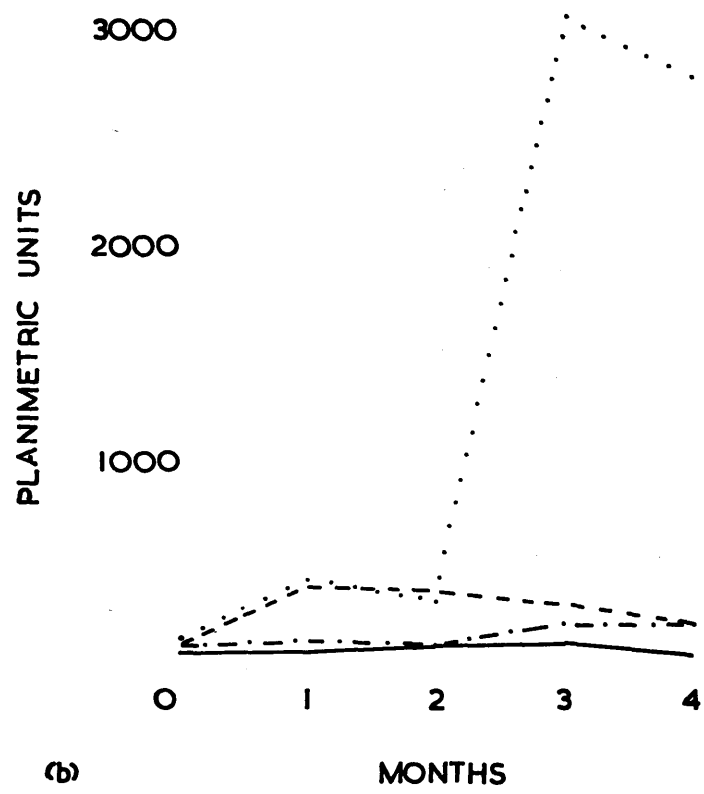


Fig. 8. Graph illustrating changes in the beta- and alpha-lipoprotein planimetric areas for the different experimental groups. (a) alpha-lipoprotein (b) beta-lipoprotein.

and albumin has diminished to being the smallest, with beta globulin intermediate.

For the reason given above no beta: alpha lipoprotein ratios were calculable in this group at the end of the 3rd and 4th months, but they were obviously immense.

Because the lipid stain is never exactly at the same strength from one staining procedure to another, and because of the intrinsic limitations of the method itself accurate quantitative comparison of similar lipoprotein complexes at different time intervals is not possible. However the various monthly batches of electrophoresis strips were stained from the common stock of Sudan Black B in use at that time, under the same laboratory conditions and given the same cleaning rinses with 50 per cent ethanol. Fig. 8 therefore is at least a rough indication of changes in the alpha and beta lipoprotein complexes relative to one another at the different monthly stages of the experiment. This suggests two tendencies in the alpha lipoprotein complex; firstly that it is reduced by the administration of the oestrogen to normal birds (group F) and secondly that it falls as beta lipoprotein rises (group B). Its virtual disappearance from group D may be attributable to the combination of these effects.

DISCUSSION

The main background to this study was an attempt to reverse the atherosclerosis which is easily induced in chickens by a cholesterol enriched diet (Dauber and Katz, 1942). But my personal interest in this work and the aspects

which I shall discuss here were those concerned with the oestrogen control of the serum lipids, particularly the lipoprotein complex and a comparison with the changes in pregnancy, and following the therapeutic administration of oestrogens to human adults.

It must be realised at the outset that such a comparison is full of pitfalls. Firstly in pregnancy we are dealing with an increase in a physiologically produced group of oestrogens while in this study and in most other human and animal studies the oestrogens under test are synthetic or semi-synthetic. Secondly, while in the experimental animal rigorous control of potentially effective agents is possible, in pregnancy the intrinsic interplay of mutually augmentary and antagonistic factors is unpredictable. Thirdly, it is virtually impossible to achieve comparable circulating blood oestrogen levels. The truth of this can be understood from the following facts. The average circulating oestrogen levels in the last week of pregnancy, about 15 μg/100 ml. are 40-50 times greater than the circulating oestrogen levels of the normal sexually active woman about the 25th day of the menstrual cycle (Preedy and Aitken, 1957). On the rough basis of a circulating blood volume of 5 litres the total amount of circulating oestrogen in the pregnant woman just before term, the time at which she is considered to be most highly oestrogenised, is about 750 μg. Doses of oestrogen used in human trials have ranged from 10 μg. ethinyl oestradiol/day (Marmorston et al., 1958) and 1 mg./day (Steiner et al., 1955, Oliver and Boyd, 1956) up to 20 mg./day of a mixture of conjugated oestrogens such as Premarin (Robinson et al., 1956) with

apparently similar effects on the serum lipids. In the experimental animal doses have ranged from 17 µg./day of oestradiol benzoate in rats (Priest et al. 1957) to 5mg. daily of epi-oestradiol benzoate to chickens, as in this study.

The results of this experiment conform in the main to that of other workers in the animal field. Priest et al. (1957) distinguish between the effects of low and high doses of oestrogen in rats. While both produce an increase in lipaemia the low dose tends to produce a greater increase in cholesterol and higher doses a greater increase in phospholipid. In their extensive experience of oestrogens in chicken atherosclerosis Katz and Stamler (1953) also find that oestrogens increase the serum lipids, but a relative increase in phospholipid is an almost invariable feature. The remarkable thing about the chicken studies of Katz and his co-workers is that in spite of the increase in circulating lipid which is usually associated with the administration of oestrogen the incidence of atherosclerotic lesions can be reduced and even reversed (Pick et al., 1952 a, b,). The reason given for this is that the increased phospholipid maintains the stability in solution of the excess cholesterol.

The physio-chemical theory of this explanation has been postulated by Dervichian (1949), who writes "The natural lipoproteins contain ionic lipids (i.e. phospholipids and fatty acids) and non ionic lipids (cholesterol esters, cholesterol, glycerides)... It appears that non-ionic lipids cannot associate by themselves with proteins while they can associate with the other ionic lipids." The stability of the non-ionic lipids therefore seems to depend not only on the presence in the blood of adequate amounts of the ionic phospholipids, a concept

widely appreciated by clinicians and biochemists, but also on the presence of ionic fatty acids, a less widely appreciated chemical concept which may prove to be the functionally important role of fatty acids in the treatment of human atherosclerosis, if this is successful.

The possible suppression of alpha lipoprotein and the emergence of an alpha-2-lipoprotein in the D group of animals in this study seem to represent a qualitative difference rather than a quantitative difference in the lipoprotein patterns. Does the large dose of oestrogen inhibit in some way the enzymatic conversion of the beta-lipoprotein to alpha-lipoprotein by lipoprotein lipase, which is thought to be mediated by heparin? If heparin is reduced in amount is it because the mast cell content of the tissues is lowered? Two observations are of some interest in this respect. Firstly, blood withdrawn from the normal chicken clots with great rapidity and secondly, mast cells are very scanty in the tissues of normal chickens and are found to be completely absent from the myocardium (Campbell and Watson, 1957). These observations led to the work on mast cells which is reported later. But at the moment they make it difficult to answer the questions just asked, because if the normal chicken is lacking in mast cells, and consequently in mast cell heparin, what is there for the administered oestrogen to suppress? And in any case why is the action of administered oestrogen on human lipids quite the reverse, in so far as it lowers the total serum cholesterol level and at the same time increases the fraction of cholesterol in the alpha lipoprotein (Eilert, 1949 and many others). The only effect of administered oestrogens which seems to be common to human and animal studies is that of increasing the circulating phospholipid to a greater or lesser degree and at the same time reducing the cholesterol: phospholipid ratio.

The large dose of oestrogen used in this study has also produced a major alteration in the balance of serum proteins. How far this is a primary effect and how far a result of the altered requirement of protein for lipid carriage must be left unanswered. Moore (1948) noted a comparable change in cocks given oestradiol propionate, but no reciprocal change in hens given testosterone propionate. In an earlier paper (Moore, 1945) he shows that not only may serum protein patterns be characteristic of the species but in some cases characteristic also of the strain (e.g. the rat) and in others of the sex (e.g. the chicken).

Whatever the effect of oestrogens on the serum lipids and however beneficial from the purely biochemical point of view, there is as yet no convincing claim that they are of value in lowering the morbidity from atherosclerosis, while their feminising effects generally render them unacceptable for long term therapy.

CHAPTER 5

THE EFFECT OF CERTAIN MARINE AND VEGETABLE OILS ON THE SERUM LIPIDS OF THE CHICKEN

As evidence accumulated for the effectiveness of certain oils as hypocholesterolaemic agents in humans, workers sought for a common property in different oils which would explain their action. Kinsell and Sinclair (1957) argued that the effective factor was linoleic acid and that atherosclerosis and hypercholesterolaemia were manifestations of a deficiency of essential fatty acids. This simple and attractive hypothesis is difficult to sustain however when the following evidence is considered. Firstly significant lowering of serum cholesterol, phospholipid and beta-lipoprotein occurs in humans following the ingestion of sardine oil (Keys et al., 1957), whale oil (Malmros and Wigand, 1957), and pilchard oil (Bronte-Stewart et al., 1956), oils which are poor in "essential" fatty acids although rich in other unsaturated fatty acids, (Thomasson, 1953). Secondly serum lipid levels have been depressed by olive oil, rapeseed oil and linseed oil (Ahrens et al., 1957; Malmros and Wigand, 1957) which consist of mono- and tri-unsaturated fatty acids and non-essential linolenic acid. And so the emphasis has swung away from the presence of essential fatty acids as the important lipid lowering factor, to the idea that the total mean unsaturation of the fat is the critical requirement.

This is an account of a small pilot study carried out at the Veterinary School in 1957 to assess the relative effectiveness of seal, pilchard and corn oils as hypocholesterolaemic agents. The iodine values of these oils

(taken from Bronte-Stewart, 1958, as compiled from various authors) are 130-170, 160-183 and 103-128 respectively. The iodine value of a fat is regarded as an index of its degree of unsaturation. In addition to the study of these oils, and in view of a paper by Jones et al., (1956) which seemed to show that maize germ was a more potent hypocholesterolaemic agent than the expressed oil, we included maize germ, wheat germ and extracted maize germ in the dietary regimes.

MATERIALS AND METHODS

Thirty Golden Legbar - Light Sussex Cockerels, one month old, were allocated to 6 groups of 5 birds and housed in individual cages. Weight was distributed comparably in each group, and equal amounts of food given. Each group was fed on standard chicken mash containing 1 per cent cholesterol and supplemented as follows:

(G) 10 per cent seal oil.

(H) 10 per cent pilchard oil.

(I) 10 per cent corn oil.

(J) 10 per cent whole maize germ.

(K) 10 per cent extracted maize germ.

(L) 10 per cent whole wheat germ.

This experiment was carried out at the same time as that reported in Chapter 4 and 2 of the groups in that study were used as controls. These were group E, which was fed normal mash only and group B, which was given the mash supplemented with 1 per cent cholesterol.

TABLE IX. (a) Cholesterol levels (mg.%) and (b) lipoprotein ratios of cholesterol fed chickens whose diets were supplemented with seal oil (G), pilchard oil (H), corn oil (I), maize germ (J), extracted maize germ (K) and wheat germ (L). The comparable figures for normal birds (group E, chapter 4) were 178 mg.% and 0.73, and for cholesterol fed birds (group B) 931 mg. % and 3.06.

	G		H		I		J		K		L	
BIRD NO.	a	b	a	b	a	b	a	b	a	b	a	b
1	400	3.01	319	4.50	757	4.92	504	2.52	1097	3.56	362	1.55
2	333	2.00	703	3.14	411	1.76	394	1.30	943	3.78	238	1.50
3	433	1.28	638	6.64	1035	4.04	305	1.17	2810	8.00	316	0.50
4	447	1.77	281	2.31	765	2.84	366	1.56	1680	6.15	300	1.45
5	304	2.63	366	2.50	940	4.53	369	0.98	819	4.45	564	1.22
AVERAGE	383	2.14	461	3.82	781	3.62	388	1.51	1470	5.19	356	1.24

After 8 weeks of the dietary regimes blood was withdrawn from an alar vein and cholesterol and lipoprotein estimations carried out. The birds were killed at this point for examination of vascular pathology. This aspect of the study is not strictly relevant to the main theme of this thesis and is not reported here.

RESULTS

The cholesterol and lipoprotein values are shown in Table IX and a representative lipoprotein pattern from one bird in each group in Fig. IX. The smallness of the group precludes worthwhile statistical analysis of the figures but nevertheless certain facts seem obvious enough to be valid.

If the mean cholesterol values of groups G, H, J and L are compared with those of group B it seems clear that the addition of seal and pilchard oils and maize and wheat germ to a cholesterol enriched diet retards the expected rise in the serum cholesterol. Such an effect is not so obviously obtained with corn oil (group I). The most striking result is the greatly enhanced hypercholesterolaemia associated with the administration of the extracted maize germ (group K).

There is a rough but certainly not direct relationship between the cholesterol and lipoprotein values in individual sera. This is obvious from a comparison of groups G and H where the mean cholesterol values are not dissimilar (383 and 461 mg. per cent respectively) while the mean beta: alpha lipoprotein ratios are much further apart 2.14 and 3.82 respectively). The almost normal appearance of the lipoprotein patterns in group L in spite of the raised cholesterol levels is worth comment. In fact there may be some

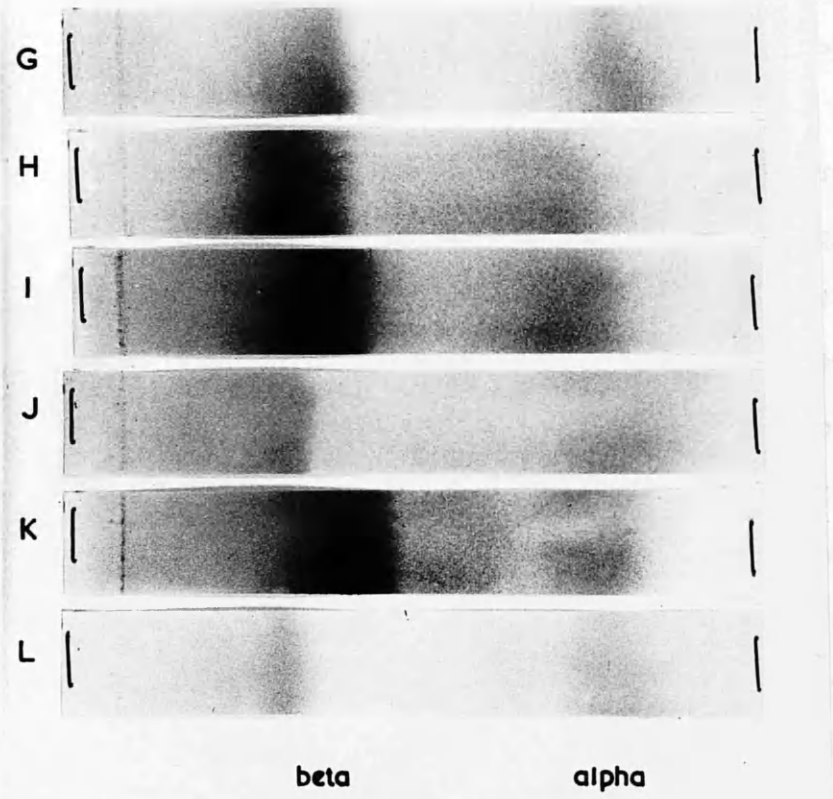


Fig. 9. Representative lipoprotein patterns from each group of chickens on oil and cereal germ supplemented diets. Note the appearance of alpha 2-lipoprotein in group K.

increase in the alpha lipoprotein of this group. It is also worth noting that of all the groups only K shows a distinct alpha-2 lipoprotein, the lipoprotein band which emerged in the highly oestrogenised and grossly hypercholesterolaemic chickens of group D in Chapter 4.

DISCUSSION

Despite the small number of birds used this study seems to confirm that the serum cholesterol is influenced by the nature of the dietary fat involved. There is no convincing evidence here of the effectiveness of corn oil in lowering the hypercholesterolaemia of the cholesterol-fed chick, but the highly unsaturated seal and pilchard oils seem to be active as cholesterol lowering agents. These facts are in keeping with the belief that the degree of unsaturation of a fat is to some extent a measure of its effectiveness as a cholesterol lowering agent.

This experiment does not reveal how these substances act, whether by increasing the excretion of cholesterol into the gut, inhibiting its absorption depressing its synthesis or promoting its sequestration in other body tissues.

The disparity in the lipoprotein values of the seal and pilchard oil groups (G and H) considered with the reasonably comparable cholesterol values provides tenuous evidence that these oils may have different capacities for cholesterol esterification. The larger amount of beta-lipoprotein in group H could be due to a larger amount of fatty acid esterified with the lipoprotein's cholesterol. It must be stressed that the mass of a lipoprotein will be increased by an increase in the mass of any one of its lipid components. It is not known what increments occur in the Sudanophilia of a lipoprotein when equal

amounts of cholesterol, phospholipid and fatty acid are incorporated in it. There is no reason to assume that they are similar. While this question of the nature and degree of cholesterol esterification by different dietary oils may be critical its solution requires the appropriate analytical techniques, the silicic acid column and gas-liquid chromatography being particularly suitable.

The two cereals tested proved particularly effective not only in limiting the degree of hypercholesterolaemia but also in limiting the development of the beta-lipoprotein fraction. This experiment confirms the work of Jones et al., (1956) for the hypolipaemic effect of the whole maize germ is clearly greater than the extracted corn oil alone. Whether this enhanced activity is due to the presence of sitosterols, tocopherols, carotenoids or other non-saponifiable materials can only be a matter for conjecture. Certainly beta-sitosterol which is present in maize germ has a cholesterol lowering effect (Beveridge et al., 1957).

The appearance of alpha-2 lipoprotein in group K, the group with the highest cholesterol levels is an interesting phenomenon. It will be recalled that this occurrence was observed in cholesterol-fed chickens given 5 mg. doses of epi-oestradiol benzoate (group D, chapter 4). The one apparent similarity between these groups is the very high cholesterol level produced by the experimental regimes. One wonders if the alpha-2 lipoprotein is a sort of "spill-over" product due to saturation of the beta-lipoprotein with cholesterol, or alternatively if it represents an intermediate stage in the incomplete metabolic transfer of fatty acids from beta to alpha-1 lipoprotein.

The most surprising result of this study however is the apparent hypercholesterolaemic effect of the extracted germ. This suggests that there may be a powerful hypercholesterolaemia promoting substance in the maize germ which is normally blocked by the extracted constituents. It also prompts the suspicion that breakfast cereals which are now commonplace in the Western diet may contribute to the endemic hypercholesterolaemia of Western civilisation. This idea is currently being followed up.

CHAPTER 6

AN ASSESSMENT OF CORN OIL IN THE MANAGEMENT OF PATIENTS AFTER CARDIAC INFARCTION

The success of long-term anticoagulant administration in the after-treatment of patients who have suffered cardiac infarction (M.R.C., 1959) complicates alternative approaches to this therapeutic problem. Many physicians already feel committed, on ethical grounds, to a treatment which has been shown to diminish the recurrence and mortality rates in such patients, and are justifiably reluctant to experiment with other forms of treatment, whose value is not yet proved, if this means withholding anticoagulant therapy. Nevertheless the administration of anticoagulants involves a new hazard for the patient, that of haemorrhage; requires strict control of dosage, which if the treatment is to become generally available will mean a national laboratory service; and does not, so far as is known, affect the underlying pathological condition of atherosclerosis. This investigation was started before the M.R.C. report had been published. But in any case there is considerable justification for the assessment of a treatment which operates naturally through dietary means, requires no essential laboratory control and which may correct or improve the basic pathology.

Statistical advice was taken as to how many patients would be required to render significant a fall in the 2-yearly mortality rate from 20 to 5 per cent, after a myocardial infarction. It was considered that only a reduction of this order, in the 2-year mortality, would afford unequivocal justification for the

projected treatment. However two groups of 70 patients each would have been necessary, one on treatment and one an untreated control group. Such a large number was neither available nor manageable if even the simplest systematic biochemical study was to be undertaken.

The outcome therefore was a less ambitious trial which had these aims:

1. Is some kind of long-term dietary programme feasible, and if so, what?
2. Does it have a sustained or merely transient effect on the serum lipids?
3. Are its clinical results encouraging? For there is as yet no proof that the ingestion of unsaturated fats has a beneficial effect on arterial disease (Morrison, 1955).

THERAPEUTIC PLAN

At the outset it was decided not to arrange an inflexible therapeutic scheme, but to be guided by experience and modify where necessary. At this time there was much disagreement among authors about the most effective dietary methods for the manipulation of serum lipids. Armstrong et al. (1957) and Williams and Thomas (1957) claimed respectively that the addition of 57 gms. of corn oil or 100 gms. of sunflower seed oil to an ordinary diet reduced the serum cholesterol, while Shapiro et al. (1957) found that 70 gms. of corn oil per day added to a normal diet increased the serum cholesterol. There was evidence that a low fat diet alone would lower serum cholesterol (Keys et al., 1955; Wolmot and Swank, 1952) but that the addition of corn oil to

such diets greatly augmented this effect. (Beveridge et al., 1955; Ahrens et al., 1957; Keys et al. 1957).

Eventually three dietary schedules were tried before the third one, still in force, was accepted as satisfactory. A bigger number of dietary variations could have been devised, but one had to avoid the speculative multiplication of schedules, which, if distributed among the limited number of patients available, could not have been evaluated adequately.

Corn oil (Maize Oil B.P. - linoleic acid content 56 per cent) was chosen as the supplementary dietary oil. Messrs. Boots Ltd. undertook to supply and distribute it to all patients included in the trial and to guarantee its fatty acid composition. There was doubt at first if patients would find the pure oil palatable and it was made up as a 50 per cent emulsion. The emulsion formula was Acacia 10%, Na Saccharin 0.003%, Prep. Vanilla 0.025%, Delax Flavour 0.06%, Benzoic Acid 0.1% and Chloroform 0.2% and water to 100%. The corn oil emulsion (C.O.E.) proved quite unacceptable and was replaced by the plain oil (C.O.). The three schedules tested in sequence were: -

In Phase I : 8 ozs. C.O.E. per day (i.e. 4 ozs. of corn oil) plus normal diet.

In Phase II : 2 ozs. C.O. per day plus normal diet.

In Phase III : 2 ozs. C.O. per day supplementing a low animal fat diet. Two diets were prepared, one of 1630 calories (155 gms. C, 65 gms. P, 80 gms. F) and the other of 2080 calories (245 gms. C, 75 gms. P, and 85 gms. F). When 2 ozs. of corn oil is added to these diets the daily caloric intakes

TABLE X. Low animal fat diet sheet issued to patients taking 2 ozs. of corn oil per day.

Low Animal Fat Diet. 1630 calories.
155C, 65P, 80 F.

Foods to avoid:-

Animal fats, including butter, dripping, lard, cream, fat meat, egg yolk, whole-milk cheese.

Foods which may contain animal fat, such as ice-cream, cakes made with eggs, pastry.

Use daily:

Skimmed milk - $\frac{3}{4}$ pint.
Vegetarian margarine - $\frac{3}{4}$ oz.
"Marmite" - 1 teaspoonful - as drink or sandwich.
Wholemeal bread, rolls, biscuits - as indicated.

Sample Menu

Breakfast

1 cup cereal, eg. porridge, cornflakes.
Bread, $1\frac{1}{2}$ thin slices or/2 oatcakes or/ 3 Ryvitas or/ 1 wheaten roll.
Milk, margarine from allowance.
Tea. No sugar.

Mid a.m. Tea with milk.
1 plain biscuit, eg. digestive.

Dinner

1 cup fat-free soup.
Good helping lean meat, fish, chicken, tripe.
2 small potatoes.
Vegetable - good helping fruit or/ jelly or/ milk pudding using milk from allowance.

Tea

Lean meat with salad or/ fish or/ tripe or/ chicken or/ skim milk (cottage) cheese.
Fruit if liked. Vegetables if liked.
1 thin slice wholemeal bread or/ 2 Ryvitas or/ 2 Vitaweats.
Milk and margarine from allowance.
Tea with milk.

Supper

Remainder of milk allowance
1 plain biscuit.

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become 2045 and 2595 calories respectively and the oil comprises 41.5 per cent of the total fat in the former and 40.0 per cent of the total fat in the latter. This small percentage difference is plainly of no practical significance. A diet was allocated according to the height, build and occupation of the patient and in this way some degree of metabolic parity was achieved. It seemed more realistic to do this than to give all subjects the same diet when individual needs were obviously quite diverse. The 1630 calorie diet sheet is reproduced in Table X.

Each man was given a 2 oz. medicine glass and told to take the oil as and when it suited him. Some took the full dose first thing each morning, some divided it out over the day, others did otherwise. All were encouraged to give an honest and frank opinion of the 'treatment'. Each man understood that if he wished he could withdraw from the study at any time, but that while still participating in it he was expected to adhere to his treatment schedule meticulously. The men, as a group, were co-operative and conscientious and I feel sure that the general adherence to the diet was as good as one could expect to achieve, and probably much better than the response of patients in general to routine dietary prescribing. Some dietary relaxation was permitted at Christmas and New Year and during summer holidays. One patient (J.D. No.3) abandoned a Continental holiday since its main purpose was to have been culinary pleasure.

THE SUBJECTS

All subjects were men between the ages of 40 and 65 admitted with, and surviving, a first attack of acute myocardial infarction. The diagnosis of infarction rested on a typical history and unequivocal electrocardiographic evidence of myocardial infarction. In one case only (S.B. No. 10) the diagnosis was accepted, although the electrocardiogram showed nothing more definite than acute ischaemia, because following the acute clinical attack the E.S.R. and leucocyte count rose and the blood pressure fell. Men with diabetes or renal disease or whose blood pressures exceeded $\frac{160}{100}$ after 4 weeks in hospital were not included. If the blood pressure rose to higher levels subsequently the subjects were not discarded from the trial. The severity of the acute illness did not exclude a patient. For example one man (A.L. No. 6) developed paroxysmal ventricular tachycardia during the second week of his illness which was reversed by intravenous procaineamide. This post-infarct arrhythmia usually carries a bad prognosis, but 20 months after it this man is still doing a full and hard day's work.

24 patients have been studied for at least 8 months and most of them for much longer. 9 are in Group I, 5 in Group II and 10 in Group III. One patient in Group I (R.R. No. 8) was eventually put on anticoagulants because of worsening angina which might have denoted impending reinfarction. None of the others has received anticoagulants apart from the treatment of the acute illness.

INVESTIGATIONS

Before being discharged from the ward each patient's height and weight were recorded. He was seen thereafter at monthly intervals for the first few months

and after a time every 2 months. He attended at the same time of the morning and on the same day of the week. He was asked specifically about angina, dyspnoea, ankle swelling or indigestion. Blood pressure and weight were recorded and blood was withdrawn for cholesterol and lipoprotein estimations. Electrocardiograms were taken at intervals or when indicated.

Standard weights were calculated from height and age according to British Tables (Levine, 1923). The serum cholesterol, in this, and all subsequent studies reported here, was estimated using Henly's (1957) modification of the method of Zlatkis et al. (1953). This method is much simpler than that used hitherto and much more reproducible. All cholesterol estimations were done in duplicate and the mean value recorded. When readings differed by more than 10 mg. per cent the estimation was repeated. Lipoprotein analysis was carried out as in Chapter 2.

ORGANISATIONAL PROBLEMS

8 ozs. of Corn Oil Emulsion per day is almost 3 pints per week. From the beginning Messrs. Boots undertook the regular delivery of supplies at monthly intervals. Later when the dose was reduced to 2 ozs. of plain oil per day the problem of bulk was not so critical, but by this time the delivery system was well established and was kept in operation. This may have been a factor in maintaining a 100 per cent participation rate.

The problem of selection of a suitable base line for pretreatment lipid levels will be discussed presently. It might have been advisable to begin treatment one to three months after the patient's discharge from hospital, but

there were advantages in establishing the regime before he left hospital, especially when the low animal fat diet was introduced.

The problem of whether or not to have an untreated control group also arose. Such a group would have been essential if conclusions about morbidity and mortality were to be drawn. But as mentioned earlier the number available would have been too small for this purpose, and considering the stated and limited aims of this study a control group was unnecessary. Each subject acted as a control for his own serum lipid readings and the practical issue of the feasibility of the treatment was plainly a personal problem. In any case it would have been difficult to justify the prescription of 8 ozs. of a bulky inert material simulating corn oil emulsion while at a later stage some other oily substance would have been required to simulate plain corn oil, and in this connection no oil could be regarded as inert.

ESTABLISHING A CONTROL LEVEL

Considerable and spontaneous variations may occur in the serum cholesterol of normal individuals under normal conditions. (Gordon and Brock, 1958; Thompson et al., 1959). But the conditions which ensue in an acute case of myocardial infarction are extremely complex and there is no reason why lipid variations should be more predictable. The patient admitted to hospital following an infarction is usually given anticoagulants and powerful analgesics, forbidden smoking, allowed only a small diet at first, and put at fairly complete rest. In some cases he may be given noradrenaline. The serum cholesterol falls after myocardial infarction (Biorck et al., 1957) and other forms of acute physical stress such as surgery (Kyle et al., 1952) and pneumonia (McQuarrie and Stoesser, 1932).

The changes observed by the last named author occurred when the diet was kept constant. Smokers have significantly though not grossly higher cholesterol levels than non-smokers (Gofman et al., 1955; Bronte-Stewart, 1956) and withdrawal of tobacco may reverse this difference. The information on caloric restriction is conflicting. Walker et al. (1953) claimed that small diets reduced the serum cholesterol by a mean value of 17 mg. per 100 ml. in a group of 39 subjects. On the other hand Moore et al., (1954) found that weight loss was frequently associated with an increase in cholesterol levels and some of the subjects in Walker's group also showed a rise in cholesterol levels although their weights fell.

The normal anticoagulant regime employed for patients ultimately admitted to this study was a combination of heparin and phenindione for the first 24 hours and thereafter phenindione alone. Heparin has a striking though relatively short lived effect on the lipoproteins. (Nikkila, 1952) but none on the total serum cholesterol (Soffer and Murray, 1954; Kuo et al, 1956; Herbert et al., 1959). The last group of authors also studied the effect of phenindione and the coumarin compounds Acenocoumarin and Ethyl Biscoumacetate on the total serum cholesterol. They found that the coumarins produced a temporary elevation of serum cholesterol but that phenindione had no effect.

It is obvious from this short account of factors pertinent to the patient with acute myocardial infarction that a hypothetical synthesis of the information would be valueless if not actually misleading. An independent study was

TABLE XI. Data for 8 male patients during the 4 weeks period following an acute myocardial infarction.

a = serum cholesterol in mg.%; b = beta:alpha lipoprotein ratio; c = pre-beta:beta lipoprotein ratio

	DAY 1			DAY 2			DAY 3			DAY 6			DAY 9			DAY 12			DAY 15			DAY 22			DAY 29		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
PATIENTS GIVEN HEPARIN AND PHENINDIONE																											
F.D.	324			254			255	1.92	0.33	232	3.61	0.26	229	2.36	-	285	2.47	-	256	1.81	0.24	267	2.31	0.25	263	1.83	0.20
J.H.	232	2.59	0.16	273	1.47	0.22	215	1.97	0.22	209	1.82	0.25	196	1.65	0.16	250	2.73	0.26	215	2.14	0.22	280	2.12	0.23	249	2.64	0.26
T.M.	180	2.50	0.20	173	2.13	0.26	174	2.32	0.26	182	3.04	0.13	201	3.36	0.26				184	2.76	0.14	215	2.50	0.10	219	1.78	0.24
W.M.	243	2.28	0.23	230	2.45	0.37	214	2.43	0.28	246	2.19	0.25	246	2.42	0.39	241	3.60	0.16	303	2.86	0.21	291	3.04	0.22	291	3.38	0.20
T.S.	210			194	2.09	0.10				145	1.07	0.16	121			101	1.24	-	166	2.28	0.28	172	1.84	0.14	207	1.74	0.16
AVERAGE	237.8	2.46	0.20	224.8	2.04	0.26	214.5	2.16	0.27	202.8	2.35	0.21	198.6	2.45	0.20	219.3	2.51	0.11	224.8	2.37	0.22	245.0	2.36	0.19	245.8	2.00	0.17
NO ANTICOAGULANTS																											
W.B.	242	3.05	0.15	259	2.52	0.22	251	2.84	0.14	258	2.36	0.22	204	1.82	0.35	207	1.94	0.12				208	1.62	0.32	242	1.85	0.23
A.C.	279	1.23	0.16	247	1.14	0.22	270	1.60	0.18	245	2.24	0.17	229	2.70	0.24	234	1.71	0.21	207	1.32	0.20	237	2.00	0.17	256	2.42	0.25
A.G.	233			215	1.65	0.22	177	1.77	0.12	174	2.08	0.27	165	1.72	0.15	172			187	1.75	0.13	186	2.10	0.26	197	1.63	0.21
AVERAGE	251.3	2.14	0.16	240.3	1.77	0.22	232.7	2.07	0.15	225.7	2.23	0.26	199.3	2.08	0.25	204.3	1.85	0.17				210.3	1.91	0.25	231.7	1.97	0.23

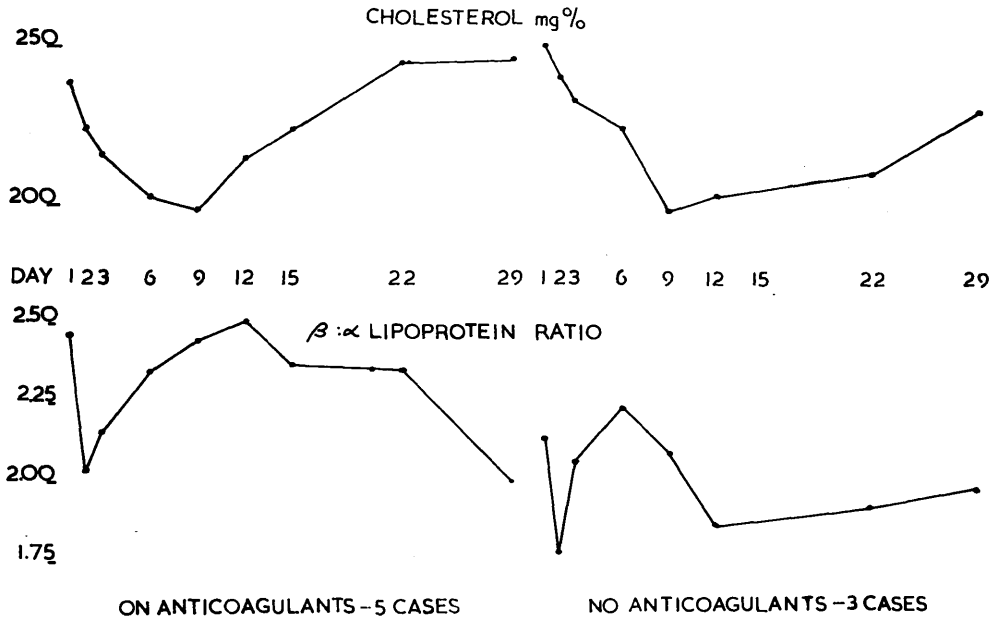


Fig. 10. Serum lipids of 8 patients after acute myocardial infarction.

thus carried out on a small group of patients to determine when, after a cardiac infarction, serum lipid levels became stable enough to be acceptable as baseline or control values. This study was carried out in two stages which are now reported.

STAGE I

8 patients were studied, 5 of them uncomplicated cases of acute myocardial infarction treated with anticoagulants (group A) and 3 treated without (group B) - in each case because of a clear history of gastro-intestinal disease or operation. None was so shocked as to require noradrenaline but each conformed to the diagnostic criteria detailed earlier.

Blood was withdrawn for cholesterol and lipoprotein estimations on the day of admission (Day 1) and on the following days thereafter - 2, 3, 6, 9, 12, 15, 22, 29. As far as could be assessed from the clinical history each patient had been admitted within a few hours of the onset of infarction. Uncomplicated cases were usually discharged about the 29th day of their illness and it seemed inadmissible to delay this normal routine. In the event however the results obtained show that a more prolonged in-patient study would have made little or no difference to the value of the information obtained.

In Table XI the cholesterol and lipoprotein data are given in detail. The few blank spaces indicate technical or organisational errors. The average cholesterol and beta:alpha lipoprotein ratios are charted in Fig. 10. Obviously the serum cholesterol level does fall after a myocardial infarction, reaching its lowest levels about the 9th day in both groups, and returns towards the starting values thereafter. The maximum average fall is not great however, being 83.5 per

cent and 79.5 per cent of the starting values in Groups A and B respectively, although individual cases show wide variation. In T.S. the fall from 210 to 101 mg. per cent in 9 days with a return to 207 mg. by the 29th day shows considerable cholesterol lability. The results for T.M. show no fall in cholesterol levels after the infarction, but the slightly higher figures obtained at the 22nd and 29th days may indicate that a slight fall had occurred by the time the first blood sample was withdrawn.

In group A the average 22nd and 29th day cholesterol levels are slightly higher than the initial levels, but the differences are not significant. In group B the 22nd and 29th day levels have not quite returned to the starting levels. The numbers are too small to say whether this is a real difference between the two groups but it is conceivable that patients with an alimentary complaint and receiving aluminium hydroxide, as did two of the group B trio, might have a slightly different cholesterol response pattern.

The pattern of the beta:alpha lipoprotein ratio is interesting. Whether patients are receiving anticoagulants or not it falls abruptly on the 2nd day. In each group there is a rebound towards the day 1 level, and this may occur more rapidly in the anticoagulant group than in the other. In the individual case, however, the lipoprotein ratio is usually unpredictable and marked swings in value may occur, unrelated to changes in the serum cholesterol level.

Pre-beta lipoprotein was present in all subjects, in 6 cases on every occasion. In P.D. it was not detected in 2 samples and in S.S. it was absent from 1 sample. While the considerable alterations which occur in the pre-beta:

beta lipoprotein ratio are probably to some extent real, technical causes play a part. This lipoprotein value also seems to bear no relationship to the serum cholesterol levels, a conclusion also reached by Besterman (1957). The average ratio for a group of 73 'normal' men, who had neither symptoms nor E.C.G. evidence of cardiac ischaemia, was 0.05 (See Chapter 13). The corresponding figures for the subjects studied here are greatly in excess of this, on average about four times as great.

Besterman (1958) encountered major variations in the fate of prebeta lipoprotein in the large series of infarction patients which he studied. In 15 cases whose sera were studied on the first day of infarction prebeta lipoprotein was present in 8 and absent from 7. In 36 per cent of cases in which prebeta lipoprotein was present intravenous heparin caused it to disappear; in the other 64 per cent it did not. In this study heparin was not being given intravenously apart from the first dose and blood was usually withdrawn a few hours after its administration so that a heparin-lipoprotein effect is unlikely to have been encountered.

STAGE I CONCLUSIONS

1. The pattern of lipid change during the 29 days following a myocardial infarction seems to be roughly similar whether patients receive anticoagulants or not.
2. Serum cholesterol falls until about the 9th day, then begins to rise and reaches the starting levels about the 22nd to 29th days. The values on these days however may still underestimate the 'true' or pre-infarction levels.
3. There seems to be an abrupt fall in the beta: alpha lipoprotein ratio on the 2nd day irrespective of whether anticoagulants are given or not.

TABLE XII. Serum cholesterol levels of 6 male patients on the 22nd. and 29th. days following myocardial infarction and 4, 12 and 20 weeks after discharge from hospital.

Case	Day 22	Day 29	Week 4	Week 12	Week 20
T.B.	195	162	217	199	181
J.C.	198	200	195	218	214
A.H.	221	228	272	184	224
M.H.	260	252	310	261	230
W.McG.	311	313	309	316	296
A.G.	289	323	299	248	296
	245.7	246.4	267.0	237.7	240.7

A rebound to starting values occurs during the next few days, but thereafter individual trends are unpredictable.

4. In any investigation on the long-term control of serum lipids, especially cholesterol, after a myocardial infarction, base line values should not be estimated earlier than the 22nd day of the illness.

STAGE II

This small group study was undertaken to test the validity of the 22nd and 29th day cholesterol levels as control values. Six patients admitted with myocardial infarction had serum cholesterol readings on the 22nd and 29th days of their illness. They were discharged on no special treatment and seen again 4, 12 and 20 weeks later, at the same time of the morning on the same day of the week. The cholesterol levels are shown in Table XII. There was a mean rise of 23 mg. per cent during the first 4 weeks out of hospital but this is clearly not significant ($p > 0.10$) since it incorporates a rise in 3 of the values and a fall in the others. At the 12th and 20th weeks the mean and individual figures are near the two control figures. During this test period 4 of the subjects showed little variation in their cholesterol levels while 2 (A.H. and M.H) had values ranging from 184 to 272 and 230 to 310 mg. per cent respectively. This small sample reflects the findings of Gordon and Brock (1958) and Thompson et al. (1959).

Without giving reasons Oliver and Boyd (1953) chose not to accept as representative of established infarction cholesterol levels estimated within 5 weeks of the acute attack. The extra week they allow may give a truer standard but from the results presented here the difference is probably no more than marginal.

TABLE XIII. Data for 9 male subjects given corn oil emulsion and ordinary diet, then corn oil and ordinary diet and finally corn oil and low animal fat diet.
a = beta:alpha lipoprotein ratio; b = pre beta:beta ratio; c = serum cholesterol in mg.%; d = weight in lbs.

		Control		8 ozs.C.O. Emulsion + Normal Diet						2 ozs. Corn Oil + Normal Diet						2 ozs. Corn Oil + Low Animal Fat Diet											
		Average of 2 results		Week 4		Week 8		Week 12		Week 4		Week 8		Week 12		Week 4		Week 8		Week 16		Week 24		Week 32		Week 40	
Case	Age	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
1 H.B.	52					1.97	-	2.04	0.12	Fresh infarction on 27.11.58.						Withdrawn.											
2 J.C.	59	1.13	-	2.40	0.16	Sudden death at home.																					
3 J.D.	47	2.14	0.12	2.41	0.12	2.81	0.14	2.32	0.09	1.77	0.12	2.16	0.14	1.85	0.13	1.47	0.13	2.02	-	2.80	0.19	1.83	0.22	2.32	0.15	1.21	0.12
4 J.G.	64	2.04	0.10	1.77	0.19			1.94	0.13	2.60	0.11	2.50	0.14	2.34	0.16	1.15	-	1.74	0.28	3.30	0.13	1.14	0.16			2.34	0.12
5 J.K.	62	1.84	0.18	1.98	-	1.28	0.10	2.71	0.12	1.50	-	1.59	-	Withdrew because of dyspepsia. Ba. Meal: D.U.													
6 A.L.	54	1.80	-	2.25	-	1.45	-			1.61	0.08	1.58	-	2.32	-	1.93	0.15	2.70	0.19	1.85	0.13	1.54	0.15			1.28	-
7 J.L.	57	2.08	-			2.68	0.12	2.10	-	1.33	-	2.59	0.12	2.24	0.14	2.39	-			2.67	0.32	2.49	0.10	2.22	0.27	3.08	0.23
8 R.R.	46	2.12	-			4.06	0.15	3.56	0.12	2.60	-	2.42	0.15	2.58	0.21	2.65	0.15	3.74	0.24	2.84	0.28	3.73	0.22	3.64	0.12	2.88	0.22
9 H.W.	54	2.00	0.28	1.99	0.10	Autopsy confirmation of fresh infarction on 2.11.58.																					
Averages	55.0	1.89	0.09	2.13	0.10	2.38	0.09	2.45	0.10	1.91	0.05	2.14	0.09	2.27	0.13	1.92	0.09			2.69	0.21	2.15	0.17			2.16	0.14
Case	Standard weight	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
1 H.B.	139	260	156	252	161	272	166	289	167																		
2 J.C.	155	221	154	237	157																						
3 J.D.	147	271	161	301	164	373	168	407	173	285	161	361	156	364	151	353	148	350	145	288	139	214	135	209	135	196	136
4 J.G.	135	214	140	263	149	282	153	275	154	266	151	256	153	308	154	341	153	272	157	225	151	230	155	204	156	239	154
5 J.K.	165	256	153	354	159	399	165	372	164	322	160	377	154														
6 A.L.	179	230	181	242	184	244	191	257	192	262	190	219	182	286	174	259	172	220	170	208	167	177	167	190	168	188	167
7 J.L.	179	291	196	301	200	300	203	363	201	290	202	248	195	400	194	456	187	328	174	263	174	278	173	261	173	269	174
8 R.R.	143	252	182	266	182	314	184	342	184	251	180	280	181	364	180	278	181	265	180	248	183	235	188	239	187	244	184
9 H.W.	143	223	132	325	132	344	133																				
Averages	153.7	246.4	161.7	271.2	165.3	316.0	170.4	329.3	175.0	279.3	174.0	290.1	170.1														
3,4,6, 7 & 8 only	156.6	251.6	172.0	274.6	175.8	302.6	179.8	328.8	180.8	270.8	176.8	272.8	171.4	344.4	170.6	337.4	168.2	287.0	165.2	246.4	162.8	226.8	163.6	220.6	163.8	227.2	163.0

STAGE II CONCLUSIONS

Taking into account the considerable individual variations which can occur, serum cholesterol levels on the 22nd and 29th days after an acute myocardial infarction are suitable base line values for subsequent observations. In the tables which follow in this chapter the individual control cholesterol level recorded is the average of the 22nd and 29th day levels.

THE DEFINITIVE TRIAL

The 24 patients whose results are reported here fall naturally into 3 groups because of the manner in which the trial evolved.

GROUP I

The therapeutic scheme for this group transpired as follows: -

Phase I for 3 months

Phase II for 3 months

Phase III for up to 10 months and still continuing.

For the nature of the different phases see the introductory pages of this chapter.

This group consisted of 9 men, the first to be introduced to the trial, and in the light of experience with them the treatment plan was modified.

RESULTS

The group lipid and weight data are detailed in Table XIII. The average cholesterol levels and weights of 5 subjects, who have participated throughout the entire period of the trial hitherto, are shown in Fig. 11 along with the personal record of one of them (No. 3, J.D.).

Phase I

During this part of the trial 2 of the patients died, one (H.W.) of a

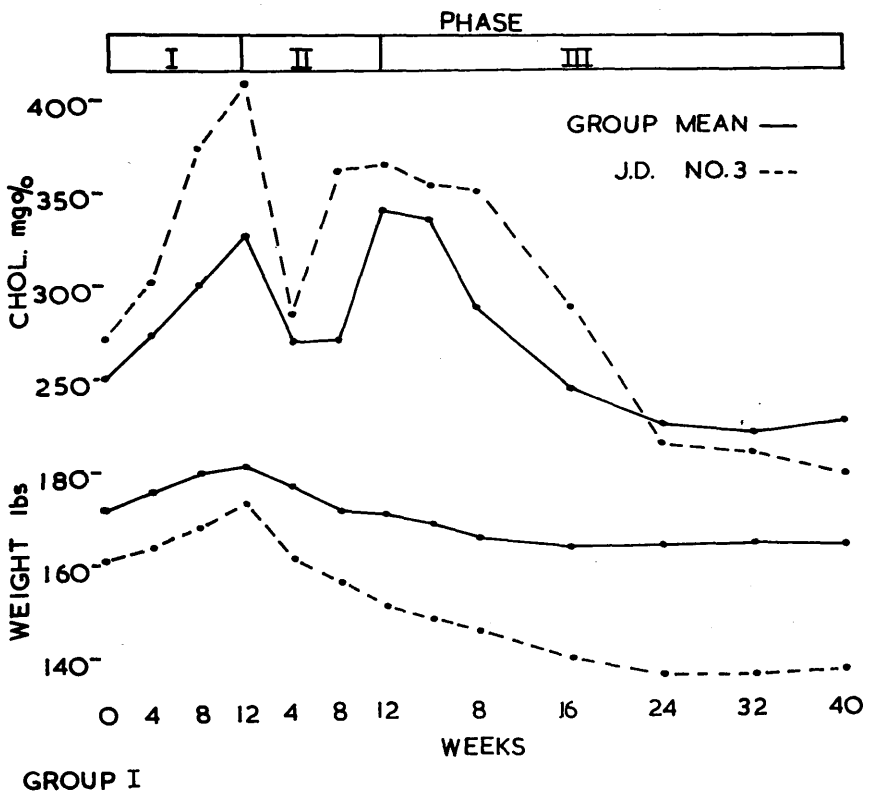


Fig. 11. Graph derived from Table XIII illustrating the average cholesterol and weight trends of Group I.

fresh infarction demonstrated at autopsy and the other (J.C.) probably from the same cause, for he died suddenly at home 5 weeks after discharge from hospital. A third patient (H.B.) had a fresh infarction and was readmitted to another medical unit. He was withdrawn from the trial. A fourth patient complained of troublesome dyspepsia not only during this phase of the investigation when it proved to be a common symptom, but also during the second phase when the emulsion was withdrawn and plain oil substituted. Following a barium meal, which demonstrated a duodenal ulcer, this patient (J.K.) was withdrawn during phase II, but was later re-enrolled in the trial of trichlorothyronine reported in another chapter. The remaining 5 patients persisted with the corn oil emulsion, were transferred to the second phase therapy and then after 3 months of this entered the third and extant phase and are still participating in it.

All complained of troublesome dyspepsia, which comprised flatulence, heartburn and a sense of epigastric distension, varying from mild to severe. All 6 who completed phase I complained of dyspnoea, again varying in severity and 4 of them had slight effort angina, but in a fifth (R.R.) it was severe. None of the E.C.G.s showed deterioration however and in fact in 5 of the cases there was evidence of improvement. All gained weight, in 5 of the men the gain ranging from 11 to 14 lbs. and almost certainly this accounted in part if not wholly for their unsatisfactory clinical state.

Serum cholesterol rose in each individual and after 8 weeks the mean rise of 66.4 mg. per cent for the 8 subjects observed is significant ($t = 3.58$; $p < 0.01$). The average cholesterol level rose again in the third 4-week period.

The mean beta: alpha lipoprotein ratio showed an upward trend during this phase, in keeping with the role of beta-lipoprotein as the main cholesterol receptor, but individual results were quite variable. No real change in the prebeta-lipid band seemed to occur.

It was soon obvious that this regime was a therapeutic failure and could not be persisted with. Although the deaths and reinfarction could not definitely be attributed to the treatment schedule the possibility could not be ignored nor could the undesirable sequelae of indigestion, weight gain, dyspnoea and possibly angina. At this stage it was not clear if these undesired effects were due to the emulsifying agent or to the large dose of oil, which by supplying an extra 1026 calories per day would certainly cause weight gain. This problem was investigated in an animal experiment which is reported in Chapter 8. Anticipating the results of this it is probably correct to infer that the emulsion base had no effect on the weight gain but was the main cause of the alimentary discomfort.

It was anticipated that the men would spontaneously adjust their diets to accommodate the increased caloric intake. Two of them however made no attempt to do this, while 4 did, 3 reducing the carbohydrate mainly and one curtailing the fat.

Phase II

The transfer from corn oil emulsion to plain corn oil in a smaller dose proved acceptable to all except J.K. who was shown to have a duodenal ulcer, as mentioned earlier. Patients no longer complained of dyspepsia. Weight began to fall and in 4 weeks the mean reduction was 4 lbs. In 4 cases dyspnoea

disappeared or improved, in 1 it was unchanged and in 1 (R.R.) it worsened. Of the 5 who had experienced angina 2 no longer did, 2 were unchanged and in 1 (R.R.) it worsened. Four of the E.C. G.s showed continuing slight improvement, usually manifest as lessening inversion of T waves, but in R.R. the E.C.G. was unchanged and showed evidence of moderately severe antero-lateral ischaemia.

During the first 4 weeks of Phase II the cholesterol levels fell and the mean reduction of 58.4 mg. per cent in 6 cases is significant ($t = 3.07$; $p < 0.05$). Thereafter although the mean weights fell from 176.8 to 170.6 lbs. the cholesterol levels began to rise again and the mean increase of 73.6 mg. per cent in the 5 men seen at the end of the 4th and 12th weeks of this phase, is significant ($t = 4.13$ $p < 0.02$). Again the beta:alpha lipoprotein ratio tended to mirror the cholesterol trends but individual results varied.

It was by now clear that plain corn oil with an unmodified diet, even in the presence offalling weight (though not necessarily in the presence of genuine weight reduction) would not reduce serum cholesterol levels, an observation supported by the information from the 5 subjects in Group II. After 3 months of Phase II therefore Phase III was introduced.

Phase III

As stated earlier one of two low animal fat diets was allocated to the subjects taking into account their height, actual weight relative to their ideal weight, and their occupation. The diets plus 2 ozs. of corn oil gave total daily caloric yields of 2045 and 2595 calories respectively. In one or two cases the diets were changed from bigger to smaller when the patient was not losing weight

satisfactorily, or in the other direction when he complained of hunger. The diet although rather monotonous was tolerated by all patients, both in this and in the other 2 groups.

On the new regime weights continued to fall, giving a mean reduction of about 7 lbs. during the first 16 weeks and running fairly steadily thereafter. Three of the men (Nos. 3, 6, and 7) ultimately achieved weights below their standard weight but R.R. (No. 8) changed not at all and remained about 40 lbs. above his standard weight throughout the trial. This man caused considerable anxiety. His angina responded only indifferently to glyceryl trinitrate. He complained occasionally of dyspeptic symptoms which suggested hiatus hernia, but after two negative barium meals at intervals of 9 months it was considered both safe and advisable to put him on anticoagulants and he has been so treated for 3 months. Three of the other 4 subjects eventually stopped having exertional dyspnoea and the fourth had it on severe exertion only. Two became angina free and 2 occasionally had chest pain on severe exertion. Apart from R.R. the E.C.G.s continued to show minor improvements and in one (J.D.) it became completely normal. This man's progress must represent the ideal result which can be achieved by this regime (See Fig. 11).

The mean cholesterol levels for the 5 men still left in Phase III fell gradually but steadily, reaching a mean value of 226.8 mg. per cent after 24 weeks, and this is significantly less than the mean value of 344.4 mg. per cent at the end of Phase II ($t = 10.95$; $p < 0.01$). It should be noted that the fall was not sudden. By the 32nd week of Phase III the mean cholesterol level of 220.6 mg. per cent is significantly less than the mean control level of 251.6 mg.

per cent for the same 5 subjects. ($t = 3.26$; $p < 0.05$).

Of these 5 men one (J.G.) is retired but does odd-jobbing at bricklaying and constructional work; 3 are working a full day and one of these, (A.L. No. 6) the man who had ventricular tachycardia, has a heavy job in a tyre manufactory; the 5th (R.R.) although restricted and at times apparently crippled by severe angina maintains a supervisory interest in his own business.

CONCLUSION FROM GROUP I

1. Corn oil emulsion is unpleasant to take.
2. 4 ozs. of corn oil added to normal diet leads to a rise in weight and serum cholesterol, with aggravation of symptoms such as angina and dyspnoea.
3. The transfer to 2 ozs. of plain oil with normal diet eliminates the "emulsion dyspepsia", and gives a temporary fall in cholesterol, which is soon reversed, however, in spite of continuing weight reduction.
4. A corn oil, low animal fat diet produced a gradual fall in cholesterol levels, which has been maintained hitherto, to a point significantly below the starting control values.
5. Although weight and cholesterol levels have both fallen in certain instances there is no direct relationship between them and in Phase II of this Group study serum cholesterol levels were rising while weights were falling.

TABLE XIV. Data for 5 male subjects given corn oil with normal diet for 3 months and thereafter corn oil with a low animal fat diet.
a = beta: alpha lipoprotein ratio; b = pre-beta:beta ratio; c = serum cholesterol in mg.%; d = weight in lbs.

		Control		2 Ozs. Corn Oil + Normal Diet						2 Ozs. Corn Oil + Low Animal Fat Diet													
		Average of 2 results		Week 4		Week 8		Week 12		Week 4		Week 8		Week 12		Week 16		Week 24		Week 32		Week 40	
Case	Age	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
10. S.B.	58	2.01	0.16	2.43	0.14			2.31	0.21	2.61	0.12	2.50	0.15	1.73	0.16			1.69	0.23	2.02	0.26	2.24	0.25
11. J.B.	53	1.86	0.05	2.26	-	1.91	-	2.08	0.18			1.93	-	2.79	0.10	2.22	0.26	1.67	0.10	1.47	0.12	2.10	0.17
12. W.C.	55	1.83	0.06	1.86	0.11	2.22	0.08	2.07	0.11					1.43	0.22	2.52	0.16			2.32	0.26	1.42	0.22
13. J.H.	44	2.83	0.17	3.45	0.20	3.12	0.17	3.26	0.15	2.91	0.14	3.15	0.11							2.26	0.12	1.98	0.17
14. F.McG.	55	1.45	-	1.75	0.14	1.27	0.12	2.38	0.16	1.28	-	1.80	0.33	1.46	0.35			3.02	0.29	2.72	0.26	2.39	0.17
Averages	53.0	2.00	0.09	2.35	0.12	2.13	0.09	2.42	0.16	2.27	0.09	2.37	0.15	1.85	0.21			2.13	0.21	2.16	0.20	2.03	0.20
Case	Standard weight	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
10. S.B.	155	341	159	227	159	271	161	334	158	325	159	316	158	300	160	307	159	270	161	224	160	245	161
11. J.B.	155	287	160	260	160	285	159	360	160	318	160	376	159	308	161	302	161	318	163	274	164	270	165
12. W.C.	139	182	156	213	156	299	158	348	155			264	150	263	149	266	148	266	148	198	151	227	148
13. J.H.	139	288	161	289	161	337	164	400	163	320	156	375	158	320	151	303	150	339	154	303	156	284	148
14. F.McG.	127	238	132	227	135	224	135	308	134	312	135	286	138	236	137	214	133	254	134	222	138	216	139
Averages	143.0	267.2	153.6	243.2	154.2	283.2	155.4	350.0	154.0	318.8	152.5	323.4	152.6	285.4	151.6	278.4	150.2	289.4	152.0	244.2	153.8	250.4	152.2

GROUP II

The therapeutic scheme for this group of 5 patients worked out as follows:

Phase II for 3 months

Phase III for up to 10 months and still continuing.

This group of men entered the trial at the point when corn oil emulsion was being abandoned.

RESULTS

The lipid and weight data are detailed in Table XIV and the mean values charted in Fig. 12.

Phase II

No patient complained of dyspepsia. Four had slight exertional dyspnoea and of these 2 had angina of effort. One man (W. C.) had neither angina nor dyspnoea. Four showed T wave improvement in the electrocardiogram.

The important lipid change during this phase was a rise in serum cholesterol from a mean of 267.2 to 350.0 mg. per cent after 12 weeks, but again the initial response to the corn oil was a slight fall in cholesterol after the first 4 weeks. The beta:alpha lipoprotein ratio rose from 2.00 to 2.42. The mean difference of 0.42 is hardly significant ($t = 2.17$; $0.10 > p > 0.05$). There was no real change in weight during this phase.

Phase III

During this period all improved. Three were eventually symptom free. One man, however, (F.McG.) though improved insofar as angina and dyspnoea were concerned, developed severe claudication of the left leg. Angiography revealed an early block of the distal third of the left femoral artery and he

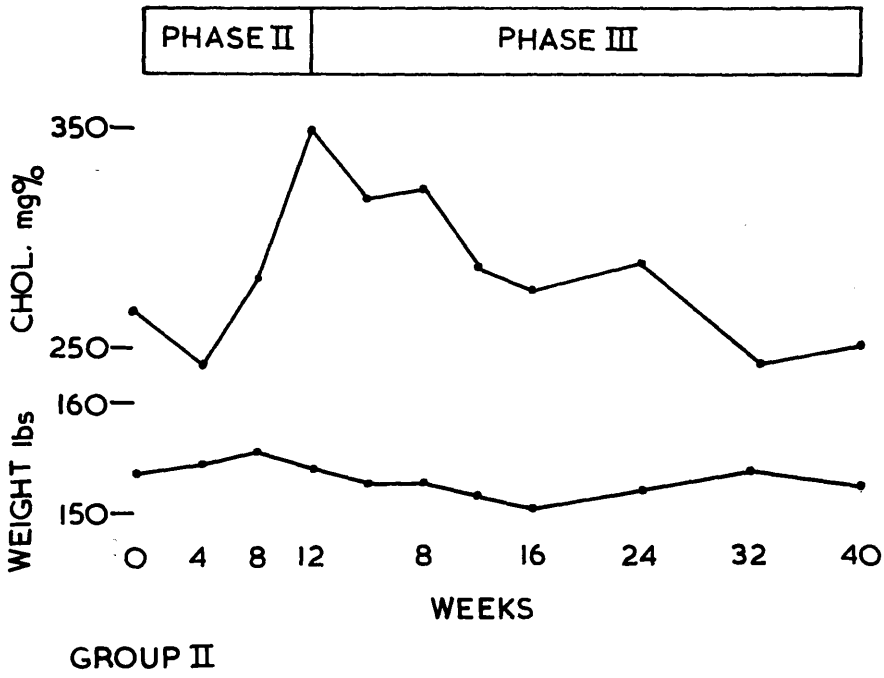


Fig. 12. Graph derived from Table XIV illustrating the average cholesterol and weight trends of Group II.

was greatly helped by phenol block of the left lumbar sympathetic ganglia. This man, a labourer, is one of the 2 not working. The other (J.H.) is a painter and lacks confidence for high work. The other 3 are working full time. All E.C.G.s have shown improvement and that of S.B. is now almost normal.

Cholesterol levels fell steadily and gradually reaching their lowest values at the 32nd and 40th weeks. The fall of 99.6 mg. from the highest mean figure of Phase II is significant ($t = 14.00$; $p < 0.001$) but the mean fall of 16.8 mg. from the initial control level is not significant ($t = 2.04$; $p > 0.1$). It is clear from a study of Table XIV that with one exception (S.B.), in whom there seems to have been a real lowering of an initially elevated cholesterol, no definite reduction below the control level has been achieved in the individual case.

As a group this sample did not lose weight and were about 10 lbs. heavier than their mean ideal weight. Two men put on weight (J.B. 5 lbs. and F.McG. 7 lbs.) without their cholesterols rising while 2 lost weight (W.C. 8 lbs. and J.H. 13 lbs.) without their cholesterols falling. S.B. achieved his 96 mg. reduction in serum cholesterol although gaining 2 lbs.

The beta:alpha and pre-beta beta lipoprotein ratios showed considerable fluctuation, although the mean increase of 0.11 in the pre-beta:beta ratio at the 32nd and 40th weeks, compared with the mean control of 0.09, is significant ($t = 3.41$; $p < 0.05$).

CONCLUSIONS FROM GROUP II

1. 2 ozs. of corn oil added to a normal diet may produce a slight fall in serum cholesterol at first but this is quickly offset by a striking rise,

TABLE XV. Data for 10 male subjects given corn oil and a low animal fat diet.
a = beta:alpha lipoprotein ratio; b = pre-beta ratio; c = serum cholesterol in mg.%; d = weight in lbs.

		Control		2 Ozs. Corn Oil + Low Animal Fat Diet													
		Average of 2 results		Week 4		Week 8		Week 12		Week 16		Week 24		Week 32		Week 40	
Case	Age	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
15. A.A.	64	2.35	0.20	1.44	-	2.23	0.26	1.63	0.22	2.69	0.14			1.69	0.12	2.43	0.21
16. J.D.	43	3.44	-	2.39	0.19	3.23	0.33	3.64	0.20	3.88	0.18			3.02	0.19	3.20	0.32
17. A.G.	59	2.41	-	2.55	-	2.30	-	1.95	0.20	2.06	0.21			1.91	0.11	1.47	0.19
18. H.G.	61	2.87	0.11	1.06	0.12	1.91	0.26	1.37	0.13					1.14	0.18		
19. T.H.	55	1.51	0.14	1.84	-	1.50	0.19	1.64	0.18	1.37	0.28			1.15	0.09	1.30	0.16
20. J.L.	49	3.22	0.25	2.48	0.18	1.57	0.11	1.58	0.27	2.10	0.28						
21. W.McG.	60	2.02	0.15	4.91	0.23	2.76	0.19	1.50	0.19					DIED			
22. J.M.	53	1.80	-	2.50	0.23			3.14	0.23	3.51	0.32			2.49	0.20	1.90	0.23
23. J.R.	51	2.14	0.15	4.35	-	3.33	0.06	1.41	0.14	3.57	0.29			2.02	0.16	1.23	0.27
24. J.R.	44	1.72	-	2.15	-	2.87	0.21	1.83	0.12	2.12	0.14			2.96	0.24	1.97	0.27
Averages	53.9	2.35	0.10	2.57	0.10	2.52	0.18	1.97	0.19	2.67	0.22			2.05	0.16	1.94	0.24
Case	Standard weight	c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
15. A.A.	155	289	154	250	154	266	156	214	155	222	158	196	162	150	161	157	158
16. J.D.	146	323	143	421	148	342	149	338	149	313	149	288	144	284	146	270	146
17. A.G.	165	272	192	244	192	290	182	242	181	258	186	250	183	210	182	182	177
18. H.G.	169	238	152	171	178	193	173	190	177	191	174			170	173		
19. T.H.	147	229	134	262	127	242	127	200	127	174	125	206	129	179	131	166	129
20. J.L.	147	289	150	250	151	252	153	252	152	262	148						
21. W.McG.	155	308	142	332	133	272	132	264	130	343	132	291	134	DIED			
22. J.M.	147	238	126	266	133	238	127	220	127	192	124	207	126	186	126	210	125
23. J.R.	147	313	141	335	140	332	142	334	141	263	141	300	143	252	149	301	145
24. J.R.	174	271	154	284	154	318	154	256	155	239	156	233	158	230	156	266	152
Averages	155.2	277.5	151.8	281.5	151.0	274.5	150.0	251.0	149.4	244.6	149.4	245.7	147.4	211.4	153.0	223.1	147.4
Averages for 7 subjects treated for 40 weeks.		a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
		2.20	0.07	2.49	0.06	2.58	0.18	2.18	0.19	2.74	0.22			2.18	0.14	1.94	0.25
		c	d	c	d	c	d	c	d	c	d	c	d	c	d	c	d
	154.4	277.0	149.1	294.6	149.7	289.7	148.1	258.6	147.9	237.3	148.7	240.7	149.3	217.3	150.1	223.1	147.4
										t= 4.83	p < 0.01	t= 3.75	p <0.01	t= 6.65	p <0.001	t= 3.20	p <0.02

unaccompanied however by clinical or electrocardiographic deterioration.

2. The substitution of a low animal fat diet reverses the cholesterol rise but as far as this small group is concerned the ultimate serum cholesterol levels are not significantly less than the control values. Possible reasons for this are discussed later.

3. The pre-beta:beta ratio is significantly raised by the Phase III regime. No definite pattern of response for the beta:alpha ratio is evident.

GROUP III

This group of 10 men was treated with the corn oil, low fat diet regime from the beginning, and 7 of them have been observed for 40 weeks.

RESULTS

The lipid and weight data are detailed in Table XV. The mean cholesterol and weight values for the 7 subjects seen for the entire period of 40 weeks are charted in Fig. 13.

By the end of the study period 5 of the 10 men were working, 3 were enjoying a fairly active retirement, 1 was unemployed though employable if he could find light work and one had died presumably of a fresh infarction, during the 23rd week of treatment. Seven are without angina or dyspnoea, one has no dyspnoea but trivial angina and one has a slight degree of each.

The man who died (W.McG.) was never really well. He had persistent angina and dyspnoea and developed claudication of the right leg which was shown to be due to a right femoral occlusion and was greatly improved by a phenol block. He had been observed for 5 months before it was decided to include him in the therapeutic trial and his cholesterol levels during this time are shown in

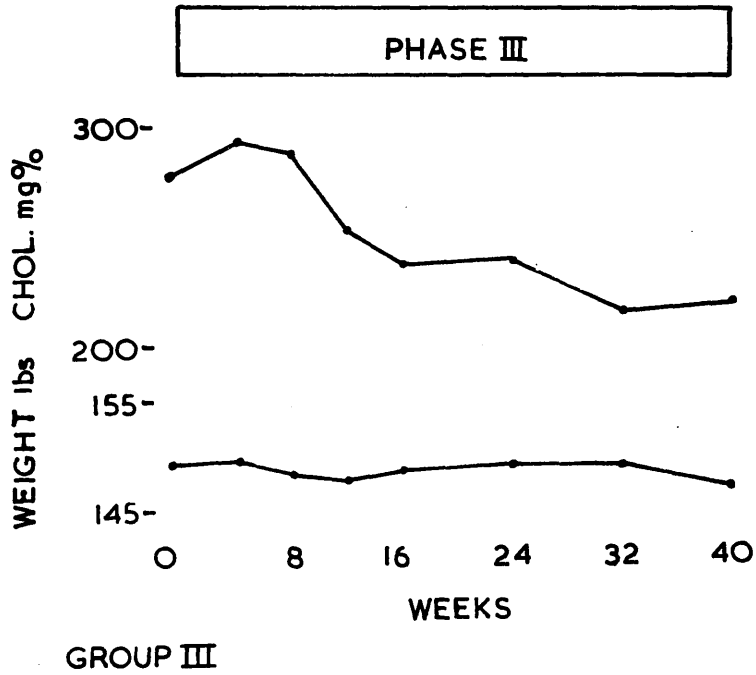


Fig. 13. Graph derived from Table XV illustrating the average cholesterol and weight trends of 7 subjects from Group III.

Table XII. The control reading given for him in Table XII is the average of these 5 readings. Clearly no useful reduction in cholesterol level was achieved in his case.

J.M. (No. 22) was very ill during the acute illness and was given intravenous noradrenaline, but not anticoagulants because of a history of radiologically established duodenal ulcer. He developed a severe "frozen shoulder" on the right side which was not improved by intra and periarticular steroids, but did respond to short wave diathermy, massage and exercises.

Apart from T.H. whose E.C.G. is virtually unchanged all of the others have shown greater or lesser degrees of improvement in their electrocardiograms.

Definite evidence of falling cholesterol levels is delayed until the 12th week but by this time the mean fall of 26.5 mg. per cent in the whole group of 10 subjects is significant ($t = 3.05$ $p < 0.02$). When the cholesterol levels for the 7 men seen up to the end of the 40 week period are compared with their control mean of 277.0 mg. per cent the average reductions of 39.7, 36.3, 59.7 and 53.9 mg. at the 16th, 24th, 32nd and 40th weeks respectively are each significant. The appropriate t and p values are given in Table XV.

A.A. (No. 15) and A.G. (No. 17) had particularly good reductions in their cholesterol levels. A.A. gained weight and A.G. lost weight. A.G. was another patient who was observed for 5 months before introducing him to the trial (see Table XII) and the cholesterol reduction achieved in his case is real and considerable. Two of the men have not obtained cholesterol reduction (J.R. 23 and J.R. 24).

The beta:alpha lipoprotein ratio was again unpredictable but the pre-beta band increased in intensity and the average increase in the pre-beta:beta ratio of 0.17 for the 7 cases seen up to week 40 is significant. ($t = 3.68$; $p < 0.02$).

CONCLUSIONS FROM GROUP III

1. A low animal fat diet augmented by 2 ozs. of corn oil will in certain cases produce and probably maintain lower blood cholesterol levels.
2. The nature of weight change bears no obvious relationship to the response.
3. The pre-beta lipoprotein band is increased in intensity but no definite alteration occurs in the beta:alpha ratio.

GENERAL DISCUSSION

The first question which this study asked can, I think, be answered positively. A low animal fat diet supplemented by a relatively unsaturated vegetable oil, such as corn oil, is acceptable to the average patient and can be persevered with. If this kind of diet is ultimately proven to make a positive contribution to health there is in fact no real difference between its status of acceptability and a diabetic routine. Corn oil although not a culinary treat is not unpleasant, indeed is almost tasteless and lacks the smell and cloying texture which contribute to the unpalatability of oils such as castor oil. There is certainly no need to garnish it and an attempt to do this here was singularly unrewarding. A daily dose of 2 ozs. (57 gms.) would seem to be just about right. More would require further restriction of dietary fat with a corresponding increase in dietary monotony and less would perhaps be ineffective.

One cannot say with certainty that each man adhered to the regime with undeviating accuracy. What is important however is that this group of men

adhered to it as well as any group of men can probably be expected to do in practice, and it is the actual achievement rather than the potential attainment which will determine the limits of effectiveness. The spur to co-operation is no greater than the patient's own desire for good health. Undoubtedly this attitude can be greatly reinforced where there is a wife who also is anxious on this score.

There seems little doubt that the definitive dietary plan does lower the serum cholesterol, though not in all subjects. The easy answer to this difficulty is that such patients were not taking the prescribed diet. I am not convinced that this explanation is the true one and will return to the matter later. As far as permanence of effect is concerned there is no evidence that this cannot be maintained. Two of the men in Group I (J.D. and A.L.) have been taking corn oil for at least 15 months and have not yet developed "tolerance" to its effect. Their serum cholesterols, at the time of writing are respectively 28 and 18 per cent lower than the starting levels and there is no sign that they are about to rise, or that in the case of J.D. it has stopped falling. In Group II we have one man (S.B.) and in Group III four (A.A., J.D., A.G., and T.H.) who have achieved substantial reductions in serum cholesterol and show no sign of escaping from control after 10 months of treatment.

There is no a priori reason why individuals should develop tolerance to this regime. Whereas oestrogen and thyroid therapy for lipid reduction in otherwise normal people produce unphysiological sequelae, a dietary manipulation of this kind is physiological and there is nothing intrinsically unnatural for the body to adjust itself to or against. But we are still left with the problem

that not all subjects have improved. This may of course be due to the degree of participation. But there may be other possibilities. In 1937 Sperry observed that each person has a "characteristic" serum cholesterol level. It is conceivable that after years of dietary adaptation this "characteristic level" becomes set at a higher level and is maintained, by homeostatic mechanisms, against dietary manipulations.

It is sometimes a condition for inclusion in a trial of hypercholesterolaemic substances that initial cholesterol levels be above a certain selected minimum. This was the case in Chapter 7 where the effects of the thyroid analogues are reported. But in this study every patient had had a myocardial infarction, and if the introductory chapter is to be interpreted in its widest sense, these subjects had some lipid derangement. If this is related to the serum cholesterol in some way then one assumption must be that these individuals' serum cholesterols, whatever their level, were abnormal for them. In this respect it is of interest that certain patients with base-line cholesterol levels which would generally be regarded as within normal limits achieved considerable reduction in these levels; for example A.L. No. 6 (230 to 188), H. G. No. 18 (238 to 170), and T.H. No. 19 (229 - 166 mg. per cent).

The fact that some men do better than others may be because of some personal factor or factors which go together to make a more responsive "type". The numbers of variables which could be investigated in this respect is vast and beyond the scope of this work as at present organised. But one clue at least may be contained in the figures of Table XVI. Here two selected groupings of the men are made with special reference to weight. We have seen already that increase or decrease in weight by itself is not the critical factor determining the

TABLE XVI. A comparison of weight change in 2 groups in whom the mean cholesterol response was substantially different.

Weight in lbs.; cholesterol in mg. per cent.

Group	Subject	Standard weight	Initial weight	Final weight	Initial cholesterol	Final cholesterol	Difference %
Cases having at least a 16% fall in cholesterol	3. J.D.	147	161	136	271	196	-27.7
	6. A.L.	179	181	167	230	188	-18.3
	10. S.B	155	159	161	341	245	-28.2
	15. A.A.	155	154	158	289	157	-45.7
	16. J.D.	146	143	146	323	270	-16.4
	17. A.G.	165	192	177	272	182	-33.1
	18. H.G.	169	182	173	238	170	-29.6
	19. T.H.	147	134	129	229	166	-27.5
	Average	157.9	164.3	155.9	274.1	196.8	-28.2
Cases having a rise in cholesterol or a fall not greater than 6%	4. J.G.	135	140	154	214	239	+11.4
	8. E.R.	143	182	184	252	244	- 3.2
	11. J.B.	155	160	165	287	270	- 5.9
	12. W.C.	139	156	148	182	227	+24.7
	13. J.H.	139	161	148	288	284	- 1.4
	Average	142.2	159.8	159.8	244.6	252.8	+ 5.2

cholesterol response. The groupings made in Table XVI are, first, one in which each subject's cholesterol reduction was 16 per cent or more of the control level and, second, one in which the individual cholesterol levels either rose or fell by less than 6 per cent of the control reading. These groups are compared in respect of their standard weights, starting weights, final weights and final cholesterol levels. The good cholesterol response group with a mean reduction of 28.2 per cent in cholesterol levels is much better off regarding weight. Although a heavier group in terms of its mean standard weight it was within 6.4 lbs. of it at first and reduced to 2 lbs. below it latterly. Not so the poor response group which had a mean increase in cholesterol levels of 5.2 per cent. For though a lighter group assessed by its mean standard weight, it was 17.6 lbs. heavier than this at first and lost no weight in the course of the study.

The mean difference of 56.0 mg. per cent between the final cholesterol levels of these groups is significant ($t = 2.75$; $p < 0.02$).

Here then is a possibility, supported by evidence in Chapter 13 that an individual's serum lipid levels depend to some extent on his "capacity" to remain within or return to the normal weight range for his height and age. This "capacity" may be a complex property compounded of hormonal, enzymatic, intellectual and other functions. But it is something which may be real and analyseable. However it is important not to overindulge in random speculations like this which can always be confronted with facts which do not fit, for both J.R.s (23 and 24) in this study have weights lower than their standard weights and yet have shown virtually no reduction in their serum cholesterol levels.

The sera of all subjects revealed a pre-beta-lipoprotein band at some stage of the study. The mean pre-beta:beta ratio was higher in all groups at the end of the study than the mean control value. When 17 subjects, for whom there is a record of this ratio both at the beginning and end of the study, are compared, the mean increase in the pre-beta:beta ratio of 0.13 or 186 per cent is significant ($t = 5.19$; $p < 0.001$). Whether this is attributable to the normal evolution of the pre-beta lipoprotein after infarction or the prolonged ingestion of corn oil cannot be said. Pre-beta lipoprotein contains fatty acids and whether or not these have been augmented or modified by the prolonged ingestion of linoleic acid must await an answer by advanced chromatographic or isotopic techniques. In fact an assessment of the value of the unsaturated oils on the basis of their ability to lower the serum cholesterol level may be misleading. What may really matter is an advantageous change in the nature of cholesterol esterification with or without lowering of its total level.

CLINICAL ASPECTS

Finally, what can be said for the regime as a preserver of life and maintainer of health? The 2 deaths and 1 reinfarction during Phase I were far from encouraging. But one must admit that this part of the trial was ill-conceived and may have been positively harmful. At first I contemplated a rough comparison with the reinfarction and mortality rates of the M.R.C. anticoagulant trial control group. But this would have been misleading for a number of reasons and was not pursued. What is given then is a bare statement of the clinical status of the subjects at this point.

Twenty men who have had a myocardial infarction have now taken a low animal fat diet supplemented with corn oil for 32 to 40 weeks. Some of these of course have sampled earlier experimental diets. Twelve are working (60%) 3 are unemployed (15%) but could be working if appropriate jobs were available, 4 are retired (20%) and 1 is dead (5%) presumably from a fresh infarction. Otherwise there have been no reinfarctions. Fourteen are free of angina and 15 have no dyspnoea. An assessment of these symptoms is always relative however and depends on the degree of effort expended. One is recovering from a "frozen shoulder" and 2 have suffered from intermittent claudication which was relieved by phenol block, one of these being the deceased patient.

The final treatment adopted is feasible, acceptable and physiological. Its fundamental value in reducing cardiac morbidity and mortality can only be assessed in a large and fully controlled trial, probably at the national level. The ultimate aim may indeed be to persuade Western Civilisation to alter its dietary in a way which embodies the principles contained in a regime such as this.

GENERAL CONCLUSIONS

1. The addition of corn oil to the diet without some restriction of dietary fat will lead to an elevation of the serum cholesterol, and if enough is given also cause an increase in weight, which is undesirable and may actually be harmful in patients who have suffered a myocardial infarction.
2. A corn oil, low animal fat diet is feasible as a long term measure, and in certain individuals, but not necessarily all, it will lower and maintain at such lowered levels, the serum cholesterol, whether the initial value be high or "normal".

3. This effect will perhaps be most readily achieved in patients whose weights are either near, or can be reduced to, their standard or ideal weight.

4. In the course of this treatment the pre-beta-lipoprotein band is greatly increased. This may reflect some fundamental change in the esterification of the cholesterol contained in this fraction.

5. A clinical impression that this regime contributes to the clinical betterment of patients after a myocardial infarction can only be confirmed by a study of larger and controlled series.

CHAPTER 7

THE EFFECT OF DEXTRO-DI-IODOTHYRONINE AND TRICHLORTHYRONINE ON THE SERUM LIPIDS OF PATIENTS WITH MYOCARDIAL ISCHAEMIA

It is well established that thyroid extract lowers the serum lipids in hypothyroid and euthyroid subjects, and at the same time increases the basal metabolic rate (Levy and Levy, 1931; Turner and Steiner, 1939; Gildea et al., 1939). Serum cholesterol and beta-lipoprotein are usually high in myxoedema and low in thyrotoxicosis. In both conditions they alter towards normal when appropriate treatment is introduced. If only the lipid lowering and metabolism raising properties of thyroid hormone could be divorced this substance would achieve an important role in the control of blood lipids. Attempts to do this have already been made unsuccessfully.

Tri-iodothyroacetic acid (TRIAC), an acetic acid analogue of tri-iodothyronine first synthesised by Pitt-Rivers (1953), also lowers the serum cholesterol. Early work seemed to indicate that this could occur without a rise in the B.M.R. (Lerman and Pitt-Rivers, 1955; Trotter, 1956). But Oliver and Boyd (1957) could not maintain the cholesterol lowering effect even by increasing the dose of TRIAC. Three of the 12 subjects studied developed angina and their exercise tolerance was decreased. In one of these 3 the B.M.R. rose, but in the other 2 it did not. They suggest, as do Barker and Lewis (1956), that TRIAC may increase the oxygen requirements of myocardium without causing an observable increase in the B.M.R. The latter authors have shown that TRIAC given to hypothyroid and euthyroid rats increases the oxygen uptake of heart tissue to a greater degree than liver, skeletal muscle, and kidney. Oliver and Boyd concluded that TRIAC

would probably not prove suitable for the long term control of hypercholesterolaemia in patients with coronary disease and such a therapy has certainly not become established.

Nevertheless the search for "safe" lipid-lowering thyroid analogues has continued and in mid-1959 two new substances became available for testing. Both were the result of research in the laboratories of Glaxo Ltd. and as a result of extensive laboratory testing both were believed to be effective lipid lowering substances and devoid of undesirable side effects. They were at this point untried in human subjects. The substances were the dextro isomer of di-iodothyronine (DT2) and a particularly interesting compound 3, 5-3' trichlor-D-thyronine (TCT), the chlorine equivalent of tri-iodo-thyronine. In the light of previous experience with such substances it was decided to proceed with caution.

METHODS

Six patients, 3 men and 3 women, all with clinical and electrocardiographic evidence of myocardial ischaemia or infarction, have been studied. Two conditions for inclusion were a serum cholesterol of not less than 280 mg. per cent and no evidence of hyperthyroidism. In 3 cases this was determined by B.M.R. estimations and/or ^{131}I studies; clinical assessment of the other 3 was considered adequate. Base line cholesterol levels were determined during a control period in which dummy tablets were prescribed. The standard dose of DT2 was 50 mg. three times a day, but the dose of TCT varied from 5 to 20 mg. three times a day.

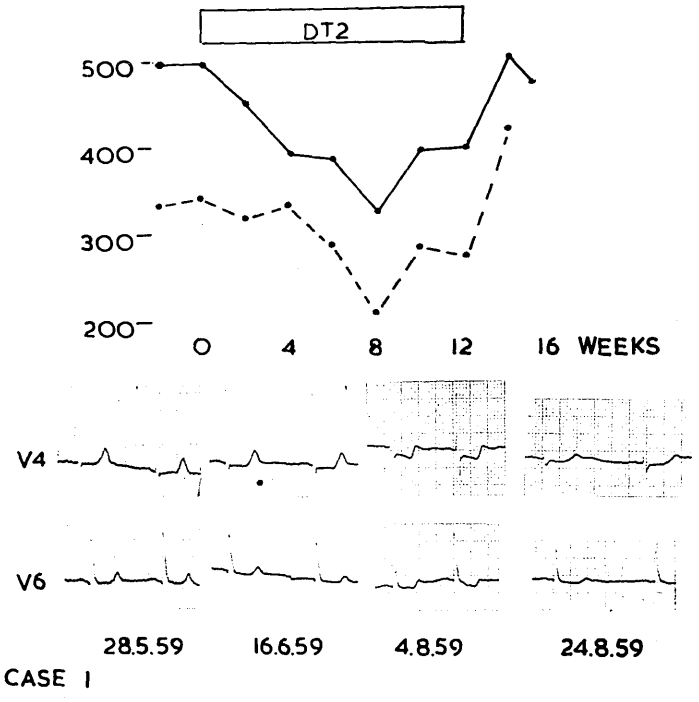


Fig. 14. Lipid and E.C.G. changes in Mrs. J. L. (case 1) during treatment with DT2.
—— Cholesterol (mg%) - - - beta:alpha lipoprotein ratio.

Patients were seen at fornightly intervals, weighed and interrogated, specific details being sought about angina, dyspnoea and palpitation. Blood was withdrawn for cholesterol and lipoprotein estimation and E.C.G.s were recorded on most but not all of these attendances. In some cases B.M.R. and/or ^{131}I studies were carried out during the trial.

RESULTS

Each subject is reported separately.

Case 1 Mrs. J. L. Aged 66 (Fig. 14)

This patient had suffered recurrent effort angina for years. There was a history of rheumatic fever at 16 years, and she had aortic incompetence. B.P. $^{190}/_{90}$. W.R. and Kahn negative. A slight hypochromic anaemia was present the haemoglobin being 76%. E.C.G. showed minimal changes of postero-lateral myocardial ischaemia, which improved while in hospital. She was an excitable woman and the lower of 2 "unsedated" B.M.R. readings was 28% above standard. But radioiodine studies on 5.5.59 were normal, the 24 hour gland uptake being 40% and 48 hour protein bound iodine (PBI) being 0.04% of the dose/litre.

DT2 was started on 13.5.59. Serum cholesterol levels fell thereafter, and the beta:alpha lipoprotein ratio responded likewise. The serum cholesterol fell from 498 mg. to 327 mg. per cent in 8 weeks, and then began to rise. Again it was followed by the lipoprotein ratio. About this point her angina became more troublesome, and by 4.8.59, that is after 12 weeks on DT2, the E.C.G. had worsened considerably. Treatment was stopped and the patient readmitted to hospital. B.M.R. was now 17% above standard. The serum cholesterol and lipoprotein ratio rose quickly to pretreatment levels, but the E.C.G. showed

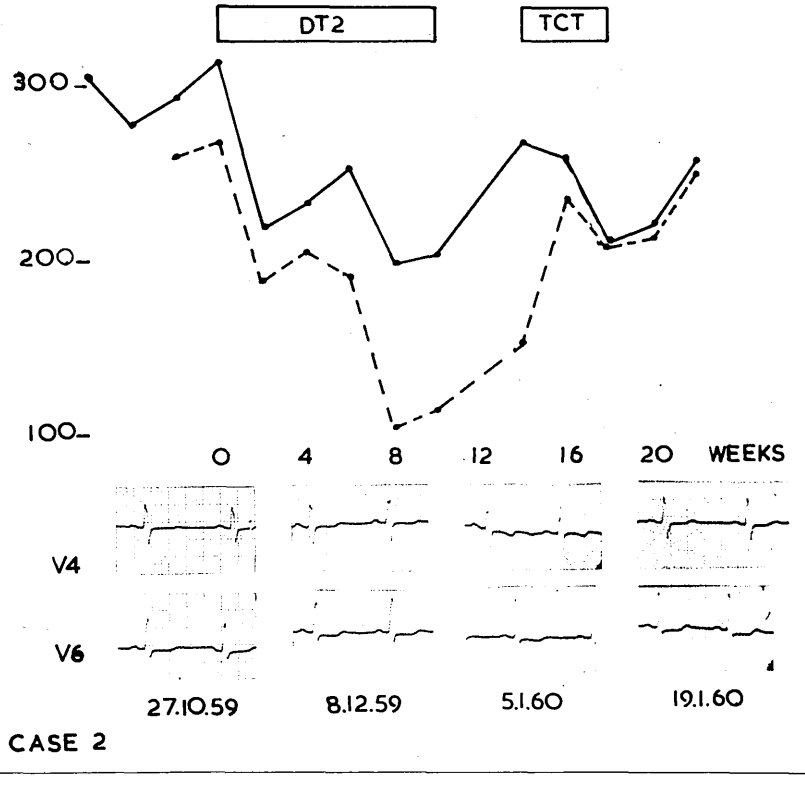


Fig. 15. Lipid and E.C.G. changes in Mrs. J. G. (case 2) during treatment with DT2 and TCT.
—— Cholesterol (mg%) - - - beta:alpha lipoprotein ratio.

considerable improvement. She was discharged on 5.9.59 much improved and has remained in, for her, a reasonable condition until now.

Radioiodine studies were repeated in this patient on two further occasions. On 26.5.59, 6 weeks after starting on DT2, the gland uptake was 12% of the dose, and the 48 hour P.B.I. 0.02% of the dose per litre. These figures indicate impaired utilisation of iodine. On 10.8.59 the 24 hours gland uptake was only 4% of the dose and the 48 hour P.B.I. 0.01% of the dose per litre. These figures were interpreted as revealing considerable iodide block probably due to the daily intake of drug iodine.

The results in this patient show that DT2 does lower the serum cholesterol and beta:alpha lipoprotein ratio without apparently increasing the B.M.R. Nevertheless during its administration myocardial ischaemia may worsen, or supervene. All of these effects were reversed when the drug was withdrawn. It seems possible that on a constant dose spontaneous "escape" from the lipid lowering effect can occur.

Case 2 Mrs. J. G. Aged 63 (Fig. 15)

This woman, with a 5 year history of effort angina and dyspnoea, was admitted as a suspected acute myocardial infarction. This was not confirmed however and the final diagnosis was that of acute myocardial ischaemia. B.P. $^{180}/_{95}$. Poor heart sounds. Hb. 106%. After being discharged she was observed for 4 weeks. She continued to have moderate effort angina readily relieved by glyceryl trinitrate. When DT2 was introduced the serum cholesterol fell rapidly from a mean control level of 298 to 198 mg. per cent and the beta:alpha lipoprotein ratio from 2.64 to 1.03. But during this time her angina probably

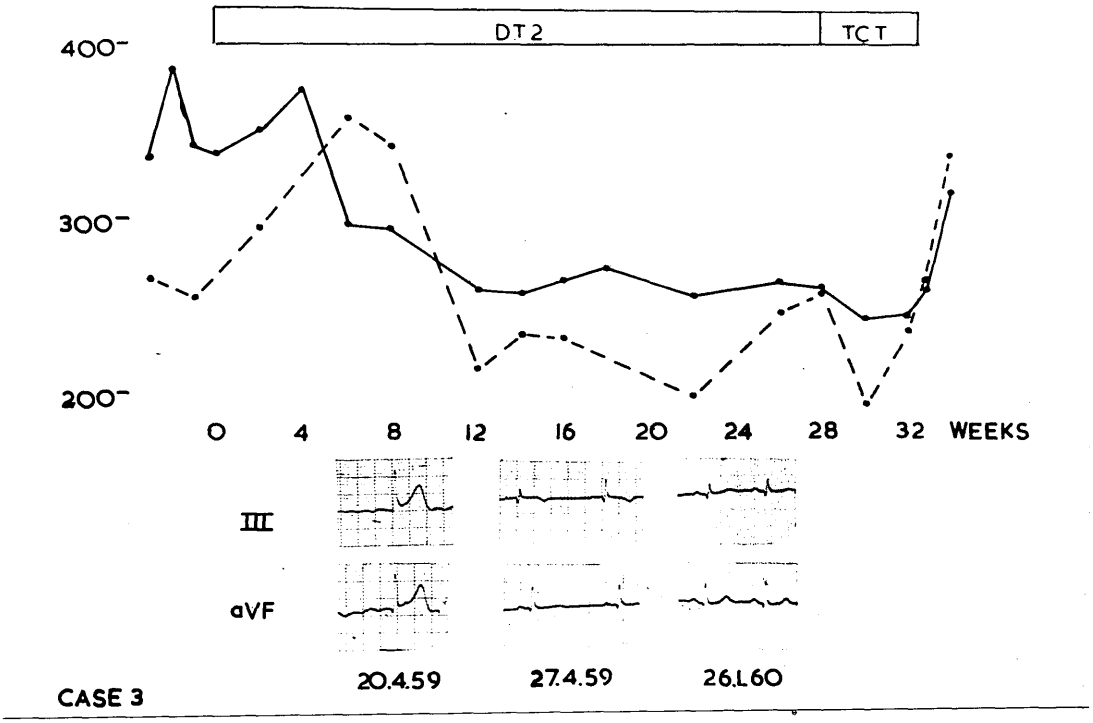


Fig. 16. Lipid and E.C.G. changes in Miss M. B. (case 3) during treatment with DT2 and TCT.
—— Cholesterol (mg%) - - - beta:alpha lipoprotein ratio.

worsened and she certainly had to take glyceryl trinitrate more frequently. DT2 was stopped therefore after 10 weeks and the cholesterol and lipoprotein levels rose sharply and the angina lessened.

After 4 weeks T.C.T. 5 mg. t.i.d. was started and the cholesterol fell from 267 to 210 mg. per cent, but the lipoprotein ratio did not return to the previous very low levels. For the first 2 weeks of this regime she was moderately well, but by the 4th week she was complaining of severe angina and the E.C.G. showed increased T wave changes of lateral ischaemia. Treatment was again stopped and once more the angina and E.C.G. improved and the serum cholesterol rose.

The results in this subject again show that DT2 has a potent and rapid effect in lowering the serum cholesterol and beta:alpha lipoprotein ratio and that a low dose of TCT (15 mg./day) shows the cholesterol effect to some extent. However the patient's angina was not improved and may have been aggravated. During the administration of TCT the E.C.G. deteriorated.

Case 3 Miss M. B. Aged 49 (Fig. 16)

This patient was admitted with an acute posterior myocardial infarction and treated with anticoagulants. She made an uneventful recovery and was discharged after 4 weeks in hospital. Hb. 86% B.M.R. 5% below standard. Radioiodine studies showed normal thyroid function with a 24-hour gland uptake of 40% of the dose and negligible total plasma iodine blood levels after 48 hours.

DT2 was started after 4 weeks outpatient observation. There was a delay of 4 weeks before the serum cholesterol began to respond, but thereafter it fell steadily from a mean control level of 355 to 258 mg. per cent and main-

tained this level without appearing to "escape" from it. The beta:alpha lipoprotein ratio also showed a delayed fall. For 28 weeks she remained well and without angina, the E.C.G. improving gradually. But during the week ending 26.1.60 she experienced moderately severe retrosternal pain. There was no deterioration of the E.C.G. Nevertheless DT2 was stopped at this point and TCT (45 mg./day) started without there being a break in therapy. There was a further slight fall in cholesterol of 18 mg. per cent. But during the 5th week on TCT she was readmitted to hospital following the acute onset of crushing retrosternal pain which radiated to the arms and which she protested was worse than the pain of her original infarction. She was not unduly collapsed however, and the E.C.G. remained unchanged, but by the 7th day of admission the E.S.R. had risen from 10 to 28 mm. and the leucocyte count from 9.0 to 13.4 thousand per cm.

TCT was of course discontinued immediately and the serum cholesterol rose from 244 to 314 mg. per cent in a week. Radioiodine studies were carried out within a few days of readmission when it was clear that she had not suffered a definite reinfarction. The 24-hour gland uptake was now 24% of the dose, the 48-hour total plasma iodine level 0.04% of the dose per litre and the protein bound iodine negligible. These results show low normal uptake.

Again there was clear proof of the effectiveness of both DT2 and TCT as hypocholesterolaemic substances with no evidence of cholesterol escape from the former after 28 weeks of administration. But once more grave suspicion arose that both were responsible for the production of acute myocardial pain.

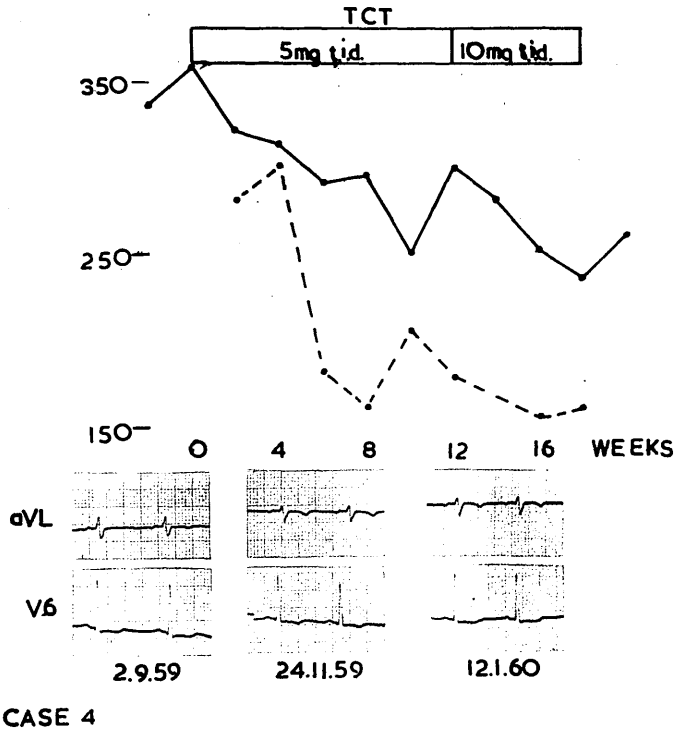


Fig. 17. Lipid and E.C.G. changes in Mr. J. L. (case 4) during treatment with TCT.
—— Cholesterol (mg%) - - - beta:alpha lipoprotein ratio.

Case 4 Mr. J. L. Aged 59 (Fig. 17)

This man may have had a myocardial infarction in 1955. He was treated at home then and there is no E.C.G. record of the illness. He was admitted to hospital on 3.11.58 as a suspected acute myocardial infarction but E.C.G. showed severe lateral ischaemia only and there were none of the clinical sequelae of infarction. He gave a history of dyspepsia and anticoagulants were not administered. Two days after admission he passed a tarry stool. The E.C.G. improved, blood disappeared from the stools and he was discharged on a bland diet. Barium meal examination showed scarring of duodenum without evidence of active ulceration.

During some months of outpatient observation he complained of moderately severe effort angina which required the frequent use of glyceryl trinitrate. Eventually TCT was given in a dose of 15 mg./day. The serum cholesterol fell rapidly and steadily from a mean control level of 347 to 249 mg. per cent, after 10 weeks. The beta:alpha lipoprotein ratio followed suit, falling from 3.00 to 1.62. During this time his angina lessened considerably and only a sense of chest tightness remained. At the 12th week the cholesterol had risen to 300 mg. per cent and the dose of TCT was increased to 30 mg./day. The cholesterol level fell steadily again, reaching a new low level of 236 mg. per cent, or 111 mg. lower than the initial control level. However at his routine visit on the 6th week after the dose of TCT had been increased he complained that for 3 days during the previous week he had suffered extreme chest pain, and dyspnoea and had to go home from work. The symptoms seemed to clear rapidly and spontaneously and he was much better at the time of his visit, but TCT was withdrawn

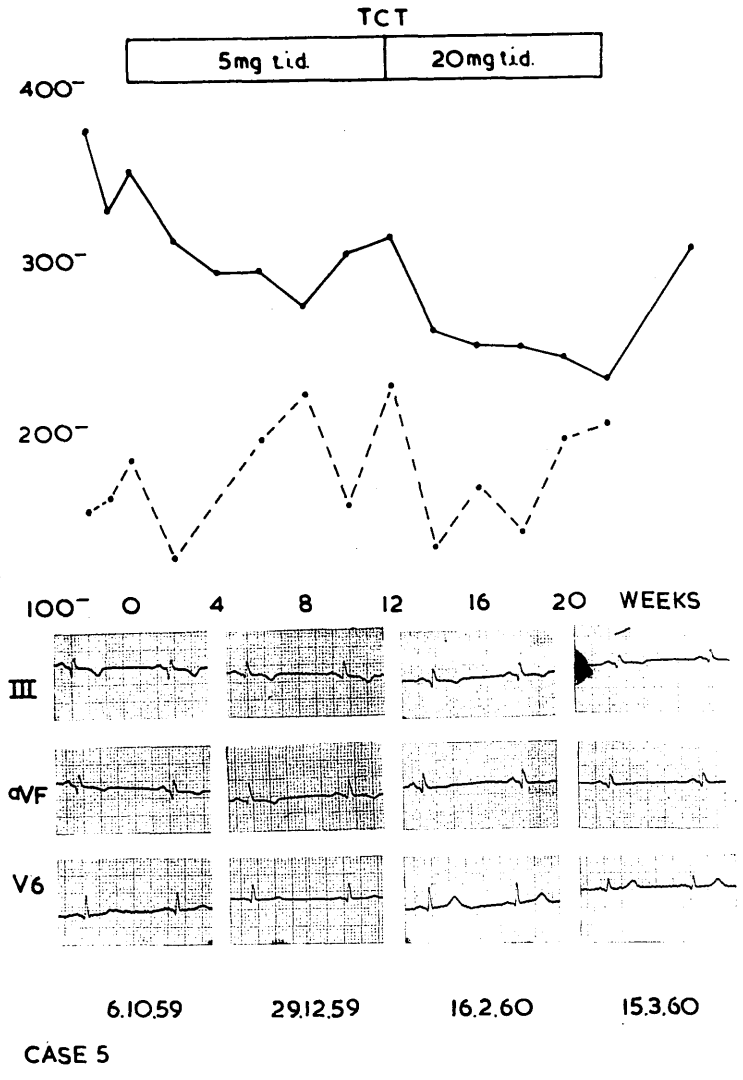


Fig. 18. Lipid and E.C.G. changes in Mr. J. K. (case 5) during treatment with TCT.
—— Cholesterol (mg%) - - - beta:alpha lipoprotein ratio.

and he was transferred to the low fat diet, corn oil regime. After 4 weeks of this his cholesterol had risen to 260 mg. per cent.

During the period of TCT administration the E.C.G. did not materially worsen. The slight alterations in T wave inversion in lead aVL (see Fig. 17) are probably postural. There is undoubtedly a degree of lateral myocardial ischaemia throughout, but the lateral leads of 12.1.60 taken a few days after his episode of severe pain show no significant deterioration.

The results in this patient suggest that TCT in as small a dose as 15 mg./day will produce considerable lowering of the serum cholesterol level but that "escape" seems likely to occur. A bigger dose will reverse this escape, but may precipitate angina.

Case 5 Mr. J. K. Aged 62 (Fig. 18)

This patient who had a posterior myocardial infarction in 1958 had previously participated in the corn oil trial, but had been withdrawn because of duodenal ulcer dyspepsia. After some months of occasional outpatient observation he was invited to take part in this study, and agreed.

At the time TCT was started he was feeling well and made no complaint of angina, dyspnoea or dyspepsia. He was retired from work. The initial dose was 15 mg./day. The serum cholesterol began to fall at once from a mean control level of 349 to 268 mg. per cent after 8 weeks. But it started to rise again and at the 10th and 12th weeks it was 298 and 307 mg. per cent respectively and once more it seemed that escape from the hypocholesterolaemic effect had occurred. No clear effect was produced on the beta: alpha lipoprotein ratio. During this period he remained in good health and the E.C.G. may have improved slightly, the Q waves in lead III becoming smaller.

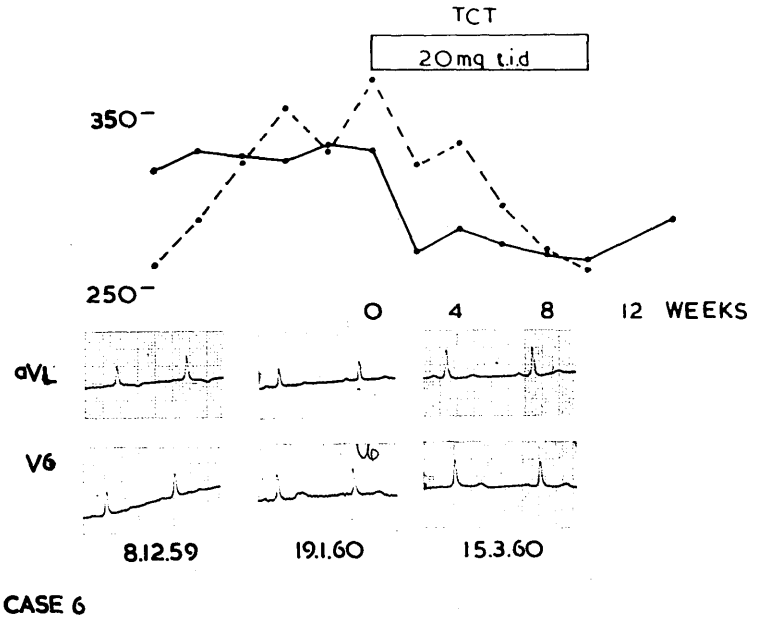


Fig. 19. Lipid and E.C.G. changes in Mr. W. T. (case 6) during treatment with TCT.
—— Cholesterol (mg%) - - - beta:alpha lipoprotein ratio.

After the 12th week the dose of TCT was increased to 60 mg./day and the cholesterol level fell once more, reaching 225 mg. per cent after 10 weeks. The patient was still symptom free at this point, but in view of the accumulating experience of the drug it was discontinued. The cholesterol level promptly rose.

Radioiodine studies were performed during the 9th week of the 60 mg. dose period, or in the 21st week after the drug was first started. The 24-hour gland uptake was 20% of the dose and the 48-hour blood levels were a total plasma iodine of 0.07% of dose/litre and protein bound iodine negligible. These results favour impaired thyroid function but their full significance will be discussed with the other results later.

Once more there is evidence of the profound hypocholesterolaemic effect of TCT, though at lower dose levels escape again occurred. No cardiac pain had been experienced by this patient up to the point at which the drug was withdrawn.

Case 6 Mr. W. T. Aged 52 (Fig. 19)

This man was admitted as an acute myocardial infarction but the final diagnosis was acute lateral myocardial ischaemia only. The admission symptoms settled quickly but persistence of numbness and pain in the left arm, certain neurological signs and neck X-ray revealed extensive cervical arthritis which was probably the result of a severe neck injury in 1942.

He was observed for two months after leaving hospital and then given TCT, 60 mg./day. The base line cholesterol level had been very constant about a mean of 325 mg. per cent. After 2 weeks of therapy it had fallen to 270 mg. per cent and remained near this level during the next 8 weeks showing no sign of a further

TABLE XVII. Weights (in lbs.) of 6 subjects before and after treatment with thyroid analogues.

Case	Weight before	Weight after
1	128	133
2	155	146
3	120	113
4	152	147
5	147	141
6	197	191

fall. The beta:alpha-lipoprotein ratio which had risen during the control period also fell. He remained symptom free throughout this time, but for the same reasons given in the previous case TCT was withdrawn and the cholesterol level rose.

Radioiodine studies were carried out during the 9th week of treatment. The 24-hour gland uptake was 30% of the dose and the 48-hour blood levels were a total plasma iodine of 0.02% of the dose/litre and the protein bound iodine was negligible. These results are within the normal range. The E.C.G. showed slight improvement in the course of therapy.

The fall in serum cholesterol was less marked in this patient than in the others but there seems little doubt that a real fall did occur.

The patients' weights before and at the end of treatment are given in Table XVII. Four of them (cases 2, 3, 4 and 5) lost on average almost 7 lbs. Case 1 gained 5 lbs, but most or all of this was probably due to oedema, and case 6 gained 2 lbs. Pre-beta lipoprotein was present in the serum of each subject but the pre-beta:beta ratio was not significantly affected by the treatment.

DISCUSSION

The thyroid analogues D-diiodothyronine and trichlor-D-thyronine are patently effective hypocholesterolaemic agents. They cannot, however, be regarded as innocuous, for of 6 patients studied 4 had episodes of severe anginal pain. TCT was withdrawn from the other two lest angina supervene. It is admittedly difficult to attribute angina to iatrogenic causes when patients are already prone to it. But if it occurs at all one of the objects of the therapy has been frustrated, and when it occurs during the administration of a type of drug which might cause it, the role of cause and effect cannot be

easily ignored.

Although electrocardiographic worsening occurred in 2 of the patients with angina (Nos. 1 and 2) no pathognomonic alterations were observed in 2 others (Nos. 3 and 4) seen shortly after the acute attack. Electrocardiographic evidence of acute myocardial ischaemia need not persist for long after an attack, but one might have expected some changes in keeping with the severity of the pain, as described by these patients. Perhaps there was something in the nature of the incident compatible with greater subjective intensity of pain in the presence of minor ischaemia. But the rise in E.S.R. and leucocyte count which followed the pain in Case 3 may indicate organic myocardial damage.

On such clinical grounds one must suspect that these substances, like others before them, affect cardiac metabolism. But the metabolic effect may be more general than this. The considerable weight loss in 4 patients requires comment, for all were taking their normal diet, none complained of an anorexia which might have impaired food intake, nor was their routine of physical activity altered in any way. Disregarding Case 1 in whom weight gain was probably due to oedema fluid, it is interesting that the least satisfactory cholesterol response was in Case 6, where body weight was virtually unchanged. It almost seems as if the hypocholesterolaemic response and weight reduction are interrelated effects of some primary activity, and that this is likely to be within the fundamental metabolic function of thyroid hormones generally.

The radioiodine studies are too few to permit generalisation. They suggest nevertheless that there is some impairment of thyroid function while TCT is being given. If TCT has thyroactivity, as seems likely, this effect may arise through depression of pituitary TSH. This may offer incidental scope for the substance in the treatment of exophthalmos. The low serum protein bound iodine levels are not necessarily abnormal, but equally they may indicate competition from TCT for a place in the plasma transport mechanism.

The phenomenon of cholesterol escape has been encountered with both analogues. This suggests that there is probably a critical minimum dose level. It is conceivable that this is the dose threshold above which thyroactivity effects begin to play their part.

In the main the beta:alpha lipoprotein ratio follows the pattern of cholesterol response, probably due to the concomitant depletion of beta lipoprotein cholesterol.

CONCLUSION

D-diiodothyronine and trichlor-D-thyronine are effective hypocholesterolaemic agents, but are not devoid of thyroactivity of a degree likely to increase the metabolic requirements of cardiac muscle and hence to produce ischaemic pain in patients with coronary artery disease.

CHAPTER 8

THE EFFECT OF CORN OIL, TRICHLOR THYRONINE AND AN EMULSION BASE ON THE SERUM CHOLESTEROL OF THE RAT, WITH A NOTE ON THE LIPOPROTEIN PATTERNS

Part I

In Chapter 6 it was shown that when adult males with coronary artery disease were given corn oil emulsified with acacia their serum cholesterol levels rose. It was of some interest and importance to discover whether this was due to the emulsifying agent and not solely a result of the rather large dose of oil given without dietary restriction of fat. But, in view of the poor clinical results which accompanied this part of the corn oil trial, it was obviously not ethical to undertake a human experiment in which serum cholesterol levels might rise or troublesome alimentary symptoms ensue. A small experiment with rats was therefore devised and at the same time the opportunity was taken to test the effectiveness of Trichlor thyronine (TCT) as a hypocholesterolaemic agent in the experimental animal. Although certain data from the manufacturer's laboratory were available, an independent laboratory assessment of such a potentially important new compound was obviously desirable.

METHODS

Forty-five one year old male albino rats were divided into 5 groups of 9 each. Unfortunately during the experiment 2 rats died from acute labyrinthitis and 2 of the groups were consequently one rat less. Weights were distributed comparably in each group. The animals were weighed at the start and again at the end of the experiment.

The lipogenic diet used in this experiment was standard rat bran containing 1 per cent cholesterol, 1 per cent sodium cholate, and 0.3 per cent methyl thiouracil. The smaller amounts of cholesterol and cholate, compared with the amounts used in the other experiments reported here, were used intentionally in the belief that any disparity of serum cholesterol levels induced by the test substances would be more obvious if the "control hypercholesterolaemia" was not excessively high. Nine rats from the same batch and of the same age were used as normal controls. This group of rats acted as a common control group for 3 separate experiments which were in progress at the same time and is referred to as Group P.

The diets were made up as follows:-

Group P - normal bran.

Group S - lipogenic diet.

Group T - lipogenic diet + 20% corn oil.

Group W - lipogenic diet + 40% corn oil emulsion.

Group X - lipogenic diet + 20% emulsion base.

Group Z - lipogenic diet + 0.02% trichlorothyronine.

Since the corn oil emulsion contained 50 per cent corn oil the amounts of oil given to groups T and W, and of emulsion given to groups W and X were identical. Each animal was given 20 gms. of diet per day and unlimited water. After 24 days of experimental feeding each animal was anaesthetised with ether, and blood withdrawn by cardiac puncture for cholesterol and lipoprotein studies. The animals were allowed to recover, except those of Group P which were killed for dissection, as described elsewhere. Cholesterol estimations were carried out using Henly's modification of the method of Zlatkis et al. (1953).

TABLE XVIII. Weights (in Gm.) of rats of groups S,T,W, X and Z before and after the experimental diets.

b = before a = after

	S		T		W		X		Z	
No.	b	a	b	a	b	a	b	a	b	a
1	265	240			285	280	305	315	325	340
2	315	310	300	315	295	295	290	280	320	295
3			300	325	270	285	300	310	275	255
4	280	275	325	360	300	300	345	335	280	270
5	325	315	285	290	350	350	285	275	300	270
6	320	310	325	325	360	355	350	360	310	275
7	340	330	345	340	280	285	310	310	290	285
8	300	290	330	320	285	290	295	295	345	320
9	280	275	275	280	345	345	350	360	330	310
Average	303	293	311	321	308	309	314	316	308	291

TABLE XIX. Cholesterol levels (mg.%) of rats in groups P,S,T,W,X and Z after 24 days on the experimental diets.

No.	P	S	T	W	X	Z
1	78	244		566	208	233
2	76	250	564	316	345	196
3	76		474	276	225	140
4	82	170	542	572	202	192
5	63	256	350	564	148	164
6	97	408	656	470	121	275
7	71	216	672	147	282	170
8	69	404	708	426	334	127
9	92	630	620	516	130	170
Average	78.2	322.3	573.3	417.0	221.7	185.2

The composition of the ordinary bran for rat feeding as supplied to the laboratory in parts by weight is whole meal flour, 45; Sussex ground oats, 40; white fish meal, 8; dried yeast, 1; skim milk, 3; cod liver oil, 1; sodium chloride, 1.

RESULTS

The weights of the rats at the beginning and end of the experiment are shown in Table XVIII and the cholesterol levels in Table XIX. Under routine experimental conditions it is quite impossible to know accurately how much of the measured diet an animal consumes, chiefly because of spillage and admixture with excrement in the cage. But daily observation of the animal's cage and its dietary zest allows a useful assessment of the relative intakes. On this basis groups P, S, T and X had roughly equivalent intakes and groups W and Z ate a little less than the others, but as much as one another. Groups T, W, and X gained slightly in weight while groups S and Z lost an average of 10 and 17 gms. respectively.

Cholesterol Levels

The modified lipogenic diet used in this experiment still produced a striking rise in the average rat serum cholesterol levels after 24 days of feeding (78.2 to 322.3 mg./100 ml.). The addition of corn oil to the diet increases the serum cholesterol level still further (to a mean of 573.3 mg./100 ml.) and the increase is significant. ($t = 3.76$; $P < 0.01$). But the addition of emulsified oil does not augment this increase and in fact the mean cholesterol level is considerably less than that achieved with the plain oil (417.0 compared with 573.3 mg./100 ml.) a difference which,however, is only significant at the 2 per

cent level ($t = 2.82$; $0.02 > P > 0.01$). Nevertheless the figures show convincingly that emulsification of the oil of itself is not a procedure likely to impart to it extra lipogenic properties.

The difference in the mean cholesterol levels of groups S and X (100.6 mg./100 ml.) although considerable is not significant ($t = 1.74$; $P > 0.10$). The likely reason for this can be suspected from a study of the distribution of the individual cholesterol values of group S, where the range is 170 to 630 mg./100 ml. This wide dispersal of serum cholesterol levels tends to occur with an oil free lipogenic diet and was observed in other experiments. Closer grouping of individual cholesterol levels is more certainly achieved by incorporating a small amount of edible oil in the dietary mix. However to return to the main point of this paragraph, there is no proof that the emulsifying agent has intrinsic hypocholesterolaemic properties.

Trichlorothyronine seems to have some hypocholesterolaemic potency in the rat although the mean difference of 137.1 mg. between groups S and Z is only significant at the 2 per cent level ($t = 2.56$; $0.02 > P > 0.01$) probably for the reason suggested in the preceding paragraph. Each rat in group Z was receiving 4 mg. of trichlorothyronine per day. The equivalent dose for a 70 Kg. human adult would be about 950 mg. per day, that is 20 to 60 times the dose tested in Chapter 7. However species susceptibility to drugs varies considerably and no conclusions should be drawn from this fact. Rats receiving the drug lost more weight than those in the control lipogenic group (S), they seemed to be more difficult to anaesthetise and to recover more rapidly from the anaesthetic.

These observations, which might indicate increased metabolism have not been confirmed by the parent laboratory, even although they used larger doses of T.C.T. (25 to 100 mg./Kg.) and more sensitive indices of thyroactivity such as antigoitre and oxygen consumption assays. (Dr. T. Binns. Personal communication).

CONCLUSIONS

(1) Corn oil augments the cholesterolaemia produced by a standard type of lipogenic diet suitable for rats. Although the amount of corn oil, namely 20 per cent of the diet, is considerable a similar result has been observed in other rat dietary experiments in which amounts of 5 and 10 per cent have been used. The reason for this has not been established but may be due to improved alimentary absorption of cholesterol or to increased palatability of the diet with increased total calorie intake.

(2) Corn oil emulsified with acacia is not more, and probably is less prone to augment dietary cholesterolaemia in rats than the plain oil. The emulsifying base itself is inert, however, insofar as serum cholesterol effects are concerned.

(3) The rising serum cholesterol levels encountered during the first stage of the human corn oil trial were probably due to increased dietary intake of fat and not to any specific cholesterol raising property of the emulsion.

(4) Trichlorothyronine (T.C.T.) is probably effective, in the dose employed, as a hypocholesterolaemic agent in rats. This study raises doubts that the effect is not entirely divorced from increased thyroactivity.

Part II

Not much has been written about serum lipoproteins in the rat. Filios et

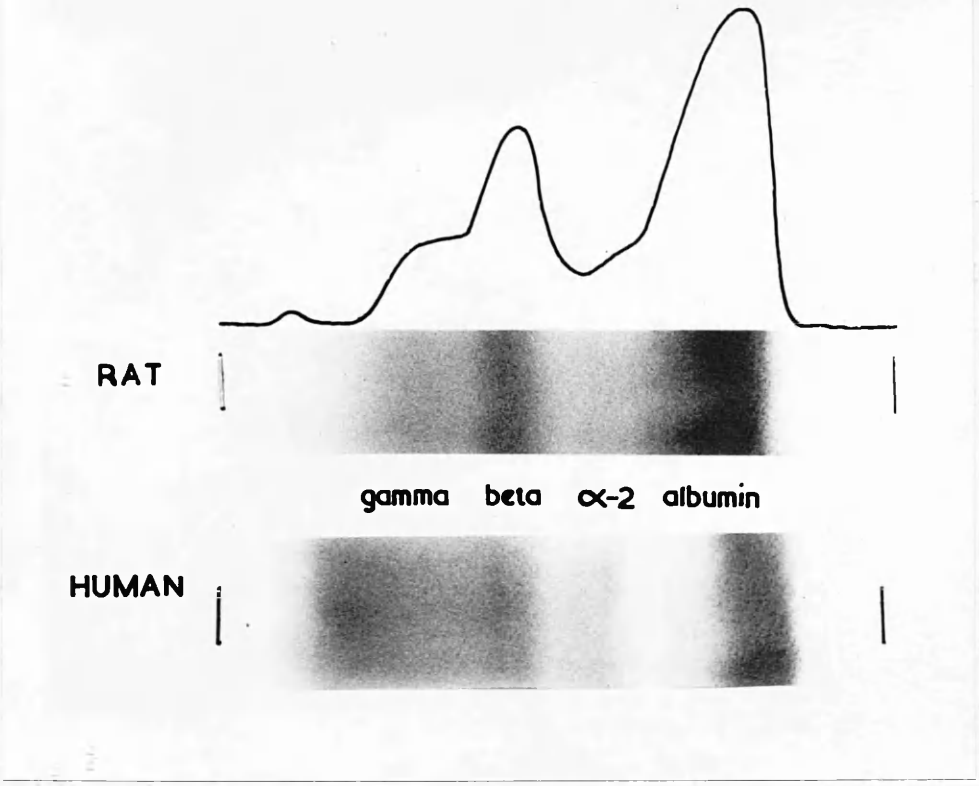


Fig. 20. Protein electrophoresis of normal rat serum. The human pattern is shown alongside.

al (1956) and Portman et al. (1956) mention that their experimental diets produced an increase in rat beta-lipoproteins but make no comment on the normal lipoprotein spectrum in this animal or whether the "beta-lipoproteins" of man and rat are structurally similar. Wilgram et al. (1957) observed an increase in the low density and alpha-lipoproteins of rats given choline supplemented cholesterol rich diets but the average maximum serum cholesterol level achieved in their study was only 128.1 mg. per cent, a degree of cholesterolaemia not usually accompanied by lipidosis or atherosclerosis in the rat.

In the course of the various rat experiments described in this thesis, lipoprotein electrophoresis was carried out systematically on all serum samples. An interesting and perhaps important phenomenon was observed during this routine work. This seems a suitable point at which to describe it and give a short account of normal and abnormal lipoproteins in the rat.

METHODS AND RESULTS

So that a clear reference standard can be established for the nomenclature of the lipoproteins a typical example of rat serum protein electrophoresis is shown in Fig. 20 There are 5 main bands - gamma, beta, alpha-2, and alpha-1 globulins and albumin. The relative amounts of each are slightly different from their percentage composition in human serum. Albumin and alpha-1 globulin run slightly behind the corresponding human bands but are denser. Beta-globulin lies in the same position as human beta-globulin, but gamma-globulin runs further than its human equivalent so that the gamma and beta-globulin bands in rat serum are not always clearly separate but frequently appear as differently

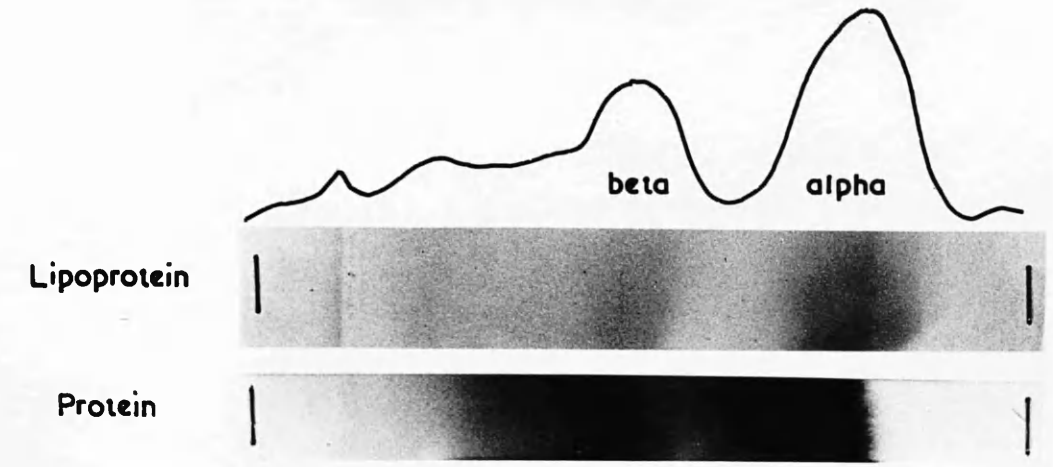


Fig. 21. Lipoprotein electrophoresis of normal rat serum. Alpha-lipoprotein includes a pre-albumin component.

Fig. 22. An alpha 2-lipoprotein emerges and increases in amount as the serum cholesterol rises. There is little stainable protein associated with it.

shaded components of one broad band. Rat alpha-2 globulin is a very faint band which is barely visible.

Fig. 21 shows the lipoprotein pattern of normal rat serum having a cholesterol content of 72 mg. per cent. The characteristic features are the paucity of lipid staining material present, most of which occurs as alpha-lipoprotein, and two other faint lipoprotein bands, (difficult to demonstrate in a reproduction though clear enough on the original paper strip), one associated with the beta-globulin band (a beta-lipoprotein) and the other running ahead of alpha-lipoprotein and just beyond the albumin front (a pre-albumin component). The next illustration (Fig. 22) shows what happens as the serum cholesterol rises. A new lipoprotein band emerges which increases in density with the increasing serum cholesterol concentration. This band is quite separate from beta-lipoprotein. It lies between the beta-globulin and albumin bands and is perhaps associated with alpha-2 globulin. A remarkable feature however is how little protein there seems to be in relation to it. In this respect it is similar to human pre-beta-lipoprotein. In some of the sera slight increases in the alpha-lipoprotein and pre-albumin band were noted, but were trivial compared with the abnormal emergent lipoprotein band, called alpha-2 lipoprotein.

The distribution of cholesterol between the different lipoprotein bands was estimated by a simple elution procedure, which the cholesterol method used in this chapter makes possible. A marker strip is cut from the electrophoresis paper and stained with Sudan Black B. The pieces of unstained paper corresponding to the lipoprotein bands thus identified are cut up with scissors and eluted overnight with the ferric chloride - acetic acid reagent. A portion of

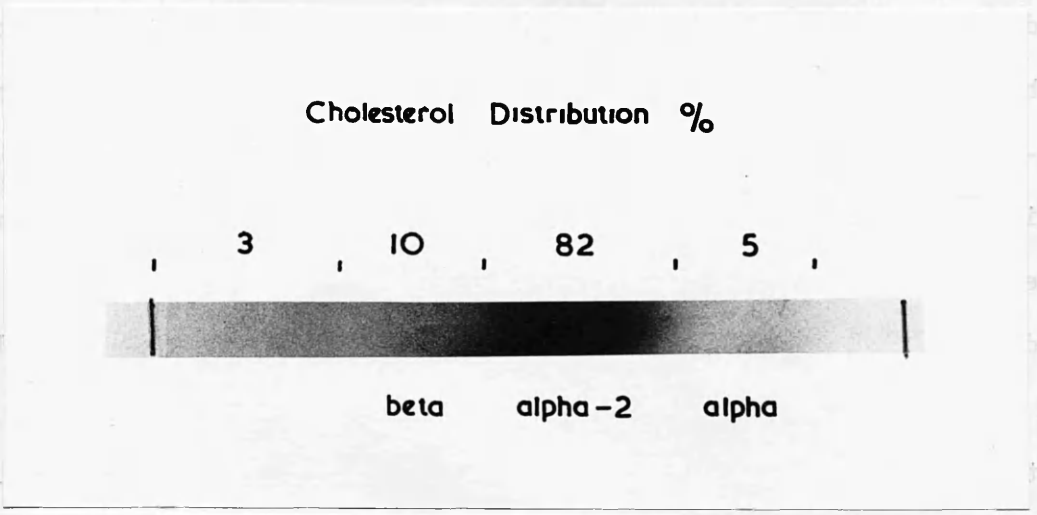


Fig. 23. Marker strip showing the percentage distribution of cholesterol among the lipoproteins in rat serum with a cholesterol level of 630 mg. per cent.

serum-free paper is used as a blank. Thereafter sulphuric acid is added to aliquots of the eluate, the colour allowed to develop and read as in the standard method. This gives a measure of the percentage distribution of cholesterol between the various bands. In one serum so studied, in which the cholesterol level was 630 mg. per cent, 5 per cent of the cholesterol was in alpha-lipoprotein, 82 per cent in the abnormal lipoprotein, 10 per cent in beta-lipoprotein and 3 per cent in the area between beta-lipoprotein and the starting line (Fig. 23). It is clear therefore that this new lipoprotein band contains all or most of the extra cholesterol held in the serum. In normal rat serum about 80 per cent of the cholesterol is carried in alpha-lipoprotein.

In the discussion on lipoprotein composition in Chapter 2 the relative instability of lipoproteins having a small protein component was mentioned. From the electrophoretic analysis described above it is clear that the new alpha-2 lipoprotein complex is deficient in protein and theoretically might be regarded as an "unstable lipoprotein". Spontaneous confirmation of this supposition occurred as follows.

After serum lipid analysis all sera were stored in a 4°C refrigerator for 2 or 3 weeks before discarding them. Not infrequently repeat lipoprotein electrophoresis would be carried out, for various reasons, up to 3 weeks after the initial run and invariably the patterns were similar to the originals. But after the rat sera had been in the refrigerator for about 5 days those with high cholesterol levels became turbid to the point of milkiness. When some of these were electrophoresed for a second time a fundamentally different lipoprotein pattern was obtained, as shown in Fig. 24. Alpha-2 lipoprotein had disappeared

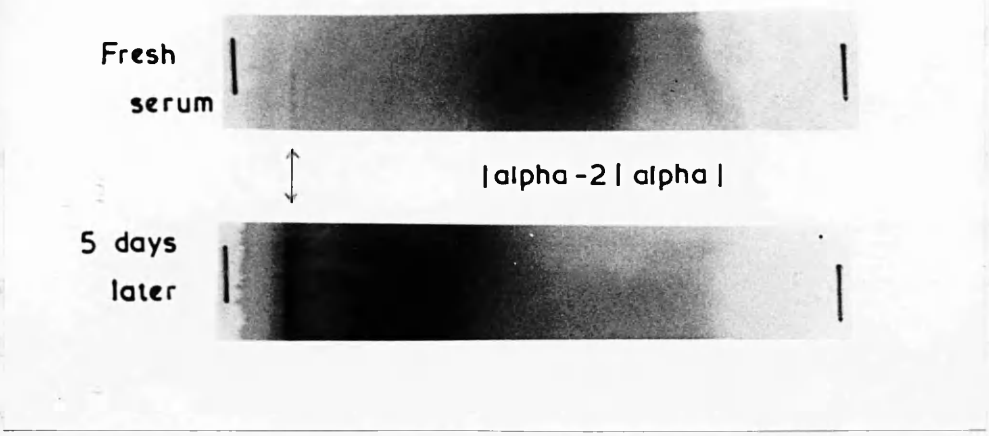


Fig. 24. Lipoprotein pattern of fresh hypercholesterolaemic rat serum and after 5 days refrigeration. The alpha 2-lipoprotein has disappeared and a new lipid staining band has appeared just ahead of the application line.

and instead a dense lipid band was present in front of the starting line. Elution analysis revealed that a radical redistribution of the cholesterol had taken place and that 85.5 per cent of it was now in the new lipid band, 11.5 per cent in beta-lipoprotein and 3 per cent in alpha-lipoprotein.

DISCUSSION

It seems reasonable to deduce from these experimental findings that when hypercholesterolaemia is produced in the rat an abnormal and highly unstable lipoprotein is formed which, on paper electrophoresis, runs to a position corresponding to alpha-2 globulin. After a few days storage at 4°C some physico-chemical change occurs involving this lipoprotein. The increase in serum turbidity suggests that the lipid has split away from its protein with consequent loss of solubility. Although the new electrophoretic position of the lipid is in the normal chylomicron or neutral fat zone it is clear from the amount of cholesterol contained in the band that it is not composed of typical chylomicron particles (see Table III).

A lipoprotein which is so unstable in vitro may also be unusually unstable in vivo. This could explain the ease with which tissue and vascular lipidosis is produced in the rat once a state of hypercholesterolaemia has been achieved. Wissler et al. (1954) attributed intimal lipidosis to the high cholesterol: phospholipid ratio which occurred in their rats given a lipogenic diet. This relative insufficiency of phospholipid could be an important contributory cause of the general lipoprotein instability postulated here. For Oncley et al. (1950) state that lipoprotein stability partly depends on the mosaic interlacing of

ionic phospholipid and protein molecules on the surface of the lipoprotein. If both protein and phospholipid are inadequate for the amount of lipid to be carried, the greatest possible degree of surface instability of the lipoprotein must presumably exist.

The important and fascinating question to which I can give no answer is why the increasing cholesterol pool does not associate with the beta-globulin which is abundant. For some reason this protein is apparently not available to the lipid. The protein-deficient state of human prebeta-lipoprotein may be a similar problem, the answer to which may contribute significantly to our understanding of at least one of the biochemical issues which seems to be involved in the serum changes associated with vascular lesions.

CHAPTER 9

MECHANISMS OF LIPID CONTROL

It is still not certain, in spite of the volume of work on the control of blood lipids, what is the primary lipid abnormality requiring correction, for the fundamental lipid derangement underlying the initiation and development of atherosclerosis has not been determined. Various lipid parameters are abnormal in subjects with this disease, but many of these are probably obligatory changes, developing because of change in one of the others. Thus if the primary fault is a rise in serum cholesterol this will imply a rise in the cholesterol/phospholipid ratio, an increase in the percentage of serum cholesterol in beta lipoprotein, (which seems to be the main carrier for cholesterol) and a rise in the ratio of free to esterified cholesterol (assuming there is some intrinsic limitation of esterification capacity). However, although these are secondary lipid derangements that is not to deny that they may be of primary importance as far as the pathogenicity of hypercholesterolaemia is concerned.

On the other hand the primary defect may be a deficiency of "clearing factor" and through this a delay in the post-prandial clearing of lipaemia and an accumulation in the serum of lipomicrons and low density lipoproteins (Korn, 1954). The true state of affairs may again be the reverse of this, namely a deficiency of clearing factor caused by its functional exhaustion in the presence of hyperlipaemia. A situation theoretically akin to this is the further reduction in pancreatic insulin which occurs in the untreated diabetic with uncontrolled hyperglycaemia. Since heparin is so intimately involved in the release and production of clearing factor some defect in the endogenous stores of heparin

might be suspected. The interesting, though inconclusive, observations of Cairns and Constantinides (1954) and Sundberg (1955) that tissue mast cells seem to be depleted in atherosclerotic vessels offer an attractive explanation for both the plasma and the tissue abnormality. But on the basis of work reported later one feels that this view cannot seriously be advocated. In any case although the intravenous administration of heparin undoubtedly leads to the appearance of a clearing factor in lipaemic plasma there is no proof that such a factor is evoked normally by lipaemia alone.

In the absence of precise knowledge about the aetiology of atherosclerosis, current therapy, despite the enormous amount of fundamental research which underlies it, is quite empirical. But this is no reason why therapeutic policies should be vague or irrational, and although our knowledge is limited it is important to appreciate what we are trying to do. Undoubtedly the commonest therapeutic aim is to reduce the serum cholesterol level. Hence the multiplicity of hypocholesterolaemic agents which are in a sense a pharmacological convenience enabling us, if they are effective, both to eat our cake and have it. The author's personal conviction is that the important evidence lies in those ethnic and epidemiological studies which seem to show that atherosclerosis is the end product of a dietary culture, and this is something which cannot readily be altered. For this reason it is probably essential that success in the pharmacological field be achieved if an acceptable method for lipid "normalisation" is to emerge.

Serum cholesterol can be depressed either by limiting its absorption from the gut or promoting its excretion in the bile. These mechanisms can be, and

in practice usually are, offset by the body's considerable capacity for cholesterol synthesis from non-lipid precursors. The biosynthesis of cholesterol is a complex process which requires an intimate knowledge of advanced biochemistry for its understanding. Many of the intricate threads in this process have been unravelled using isotopically labelled tracer substances. According to a detailed consideration of the subject by Cornforth and Popjak (1958) the key stages in cholesterol synthesis are the creation of the "acetate pool" and from it the synthesis of squalene via the intermediate synthesis of mevalonic acid. The enzymes acetyl-coenzyme A and aceto-acetate-Co A play a vital part in the early stages of this transition. The next stage is the progressive and possibly single stepwise upgrading of squalene through lanosterol to desmosterol and finally to cholesterol. It is clear therefore that there are many points at which cholesterol synthesis might be interrupted, but how far this can be done without endangering vital enzymatic processes, devitalizing liver parenchyma, (where most of this synthesis occurs) or simply encouraging the accumulation of cholesterol precursors which may be as atherogenic as cholesterol itself (Nichols et al., 1955) is not clear.

Because the acetate pool can be derived from non-lipid sources such as carbohydrate and protein, the dietary restriction of cholesterol or sitosterol depression of cholesterol absorption has only a limited and usually evanescent effect on the level of circulating cholesterol. Sitosterol, of course, also inhibits the absorption of bile cholesterol. Thyroid hormone and certain thyroid analogues, including two of the most recent studied here, are powerful cholesterol

lowering substances, but their site of action is uncertain. It is conceivable that they act directly on the liver cell, perhaps by increasing cellular oxygen consumption (Barker, 1956) and so affecting its capacity for cholesterol synthesis. There is a need for research into the nature of thyroid control of lipid metabolism. The serious problem with such substances, however, is whether their capacity for lowering cholesterol can be divorced from their effect on tissue metabolism. If the two effects are interdependent it must be considered unlikely that worthwhile progress can be made in this field.

The effect of oestrogens is complex. Oestrogens administered to human subjects cause a lowering of the serum cholesterol, and a similar effect is observed in rats, probably due to depression of hepatic synthesis (Boyd and McGuire, 1956). The effect in chickens and rabbits is the converse of this, and the serum cholesterol rises in pregnancy at a time when there is gross endogenous oestrogenisation, although admittedly there is a concomitant alteration in other hormones. From experience with one thyrotoxic primigravid woman in Chapter 3 it seems possible that the normal hypercholesterolaemia of pregnancy can be prevented by thyroid hormone. This suggests a strategic site for its hypocholesterolaemic effect. The long term administration of oestrogens to human subjects has certainly produced and maintained lowering of serum cholesterol levels, but there is no evidence that this has been accompanied by clinical betterment (Oliver and Boyd, 1959).

Various substances have been used for their effect on the hepatic biosynthesis of cholesterol, but all have caused serious toxic effects either in man

or in the experimental animal. Examples of some of these substances are phenyl- α -butyric acid which blocks the formation of acetyl - Co A from acetate (Steinberg and Fredrickson, 1955) and nicotinic acid which may compete for Co A in its detoxication to nicotinuric acid (Reddi and Kodicek, 1953).

The ability of the vegetable oils to depress serum cholesterol levels seems to depend on their degree of unsaturation, and hydrogenation abolishes their effectiveness. It has not been possible to identify the cholesterol lowering activity of an oil with specific fatty acids and some workers believe that the hypocholesterolaemic principle resides in the non-saponifiable fraction of the oil. Nath (1959), for example, attributes the effect to 1:2 - dienolglucose. The fact remains however that the effectiveness of an oil is to some extent related to its degree of unsaturation.

Whatever the active principle in the various oils the mode of action seems to be through the increased catabolic conversion of cholesterol to bile acids and hence to its excretion (Lewis, 1958). Saturated fats increase the serum cholesterol not by reducing biliary excretion, but by promoting the biosynthesis of cholesterol. A different mode of action and one perhaps more pertinent to the process of cholesterol deposition in arterial tissue is suggested by the work of Rutstein et al. (1958). They have shown that the in vitro deposition of cholesterol in human aorta is inhibited by linoleic acid and promoted by stearic acid.

The clearing action of heparin suggests that heparin or heparin-like substances might be of value in the control of blood lipids. But heparin to be effective has to be injected intravenously and of course it has anticoagulant

properties. Many synthetic sulphated polysaccharides originally produced as anticoagulants have antilipaemic activity, but all have proved to be toxic. (Astrup, 1953). Examples of such substances are dextran sulphate and "Manuronate", a sulphated alginic acid.

It has not been the aim of this Chapter to give an exhaustive account of factors which control blood lipids. Symposia have been held to consider isolated aspects of the problem. The object has been to crystallize the therapeutic intention and indicate in what regions it might expect to achieve success. The author, although admitting gaps in the evidence, is persuaded that dietary methods such as that tested here and advocated by other writers offer the safest, most rational and most fundamental means of controlling the blood lipids. Whether this will control atherosclerosis and thrombotic vascular disease is another matter.

PART II

CHAPTER 10

LIPAEMIA, HEPARIN AND BLOOD VISCOSITY

Mention was made in Chapter 4 of the gross milkiness of the serum of the group of chickens on cholesterol diet and receiving 5 mg. epi-oestradiol benzoate per day. This serum appeared to be more viscous than that of the normal chickens and on testing with a Hess viscometer this was confirmed. But in view of the grossly abnormal state of the serum lipids in these birds it seemed that the physico-chemical conditions were too artificially distorted to have real clinical importance.

The viscosity of a liquidmay be increased by the addition of particulate matter. After a fatty meal particulate fat, in the form of chylomicrons, is added to the blood. It is important to know whether this post-prandial-lipaemia increases blood viscosity or not; for an increase in the viscosity of a liquid, other conditions being unchanged, diminishes its rate of flow (Poiseuille's Law). The implications of this is terms of in vivo tendency to coagulation and tissue ischaemia are obvious.

I. LIPAEMIA AND VISCOSITY OF SERUM AND PLASMA

For the purpose of this study the measurement of relative viscosity is adequate. A Hess's viscometer was used. After practice with the instrument and standardisation of technique, which included scrupluous care in cleaning, individual results rarely varied more than ± 0.05 units. The reading taken was the wet glass viscosity, that is the reading obtained after a preliminary

wetting of the viscometer capillaries by the water standard and the material under test (serum, plasma or blood). It was appreciated that the cleaning process between readings, since it involved the cooling effect of evaporating ether, might alter the viscosity values. However this effect must have been negligible, because the variation of ± 0.05 for identical samples included the cleaning manoeuvre. At first the mean of 10 readings was taken but this was extremely tedious and when it was seen to be unnecessarily repetitive only two or three readings were recorded. Lipaemia was measured in an "Eel" photometer using a red filter and water as blank. The optical density was taken as a measure of "units of turbidity".

The subjects were convalescent patients of both sexes. Their ages ranged from 15 years to 78. Blood was withdrawn from an arm vein, by a short wide bore needle, into a dry syringe. Samples were taken while the subjects were fasting and three hours after the ingestion of 50 g. cream fat (250 ml. of dairy cream of tested fat content 19-21 per cent supplied by the hospital milk contractor). To obtain plasma samples, 8 ml. of blood was added to a small quantity of potassium oxalate (about 3 mg.), inverted gently 5 or 6 times, and spun at 3000 r.p.m. for 10 minutes. Oxalate in this quantity has no effect on blood plasma viscosity (Langstroth, 1919). Serum samples were obtained by allowing the blood to clot and then centrifuging as for plasma. Haemolysed samples were discarded.

Although the laboratory temperature varied between 14°C and 22°C during the period of the study, there was no significant variation in temperature while any group of comparative readings was being taken.

TABLE XX. Serum turbidity and viscosity measurements in fasting subjects (F) and 3 hours after ingesting 50 G of cream fat. (25 subjects; 29 tests)

	Serum Turbidity		Serum Viscosity	
	F.	3 hr.	F.	3 hr.
1	8	22	1.60	1.60
2	6	56	1.70	1.70
3	6	37	1.80	1.80
4	7	57	1.80	1.80
5	9	57	1.90	1.90
7a	6	22	2.30	2.40
7b	5	26	2.00	2.00
8a	6	15	2.10	2.10
8b	5	32	1.90	2.00
9	3	15	1.90	1.90
10	11	23	2.10	2.00
11	7	15	1.90	1.90
12	7	20	1.80	1.80
13	7	28	1.90	1.90
14	6	44	1.70	1.70
16	6	39	1.90	1.90
17	4	4	1.80	1.80
19a	5	23	1.70	1.70
19b	5	25	1.80	1.80
20	8	42	1.90	1.90
21	5	14	1.80	1.80
24	4	14	1.80	1.80
25a	5	21	2.00	2.10
25b	5	22	2.00	1.80
26	3	27	1.80	1.80
27	3	14	1.70	1.70
28	5	19	2.40	2.30
29	5	16	1.70	1.60
30	4	12	1.90	1.90
Mean	**5.7**	**26.2**	**1.88**	**1.88**

TABLE XXI. Plasma turbidity and viscosity measurements in fasting subjects (F) and 3 hours after ingesting 50 G of cream fat. (21 subjects; 23 tests).

	Plasma Turbidity		Plasma Viscosity	
	F.	3 hr.	F.	3 hr.
23	6	26	2.10	2.10
28a	6	28	2.20	2.10
28b	6	42	2.20	2.20
31	6	30	2.15	2.20
32	6	53	1.95	2.00
33	7	26	2.25	2.25
38	7	44	2.00	2.10
41	7	21	2.30	2.35
42	6	35	2.00	2.00
43	6	48	1.85	1.85
44a	7	40	2.20	2.25
44b	7	34	2.05	2.05
45	6	37	1.95	2.00
46	7	39	2.00	2.05
48	4	54	2.00	1.95
49	6	31	2.00	2.00
50	4	35	1.75	1.85
51	6	78	1.90	1.95
52	4	33	1.80	1.85
55	4	46	2.05	2.05
56	3	19	1.75	1.75
57	4	15	2.00	2.00
66	7	62	2.00	2.00
Mean	5.7	38.5	2.01	2.04

The results are shown in Tables XX and XXI.

The turbidity readings cover a clinically representative range of lipaemia. It is clear that serum and plasma viscosities were not affected by the presence of particulate fat in the concentrations encountered clinically. The higher value of the mean plasma viscosity as compared with serum viscosity is in conformity with established knowledge (Wintrobe, 1956) and is due to the presence of fibrinogen.

Conclusion

Lipaemia of clinical degree has no effect on serum or plasma viscosity.

II LIPAEMIA AND VISCOSITY OF WHOLE BLOOD

Although lipaemia has no effect on serum or plasma viscosity, it cannot be assumed that it will have no effect on whole blood. Nygaard et al. (1935) have shown that there is a linear relationship between blood viscosity and the haematocrit, for the haematocrit range 15-50 per cent. That is, blood viscosity varies depending on the number of red blood corpuscles present. The effect of chylomicra intermingled with red corpuscles could have quite a different effect from that of small fat particles suspended in plasma by themselves. The aim of this part of the study was to examine this possibility and also to investigate the effect of heparin on blood viscosity in fasting and lipaemic subjects.

Method:

The method and subject sample were as before except that: -

(1) Viscosity readings were made at the bedside immediately after the blood was withdrawn. The four samples from each patient (see later) were allowed

TABLE XXII. Plasma turbidity and blood viscosity readings in 21 subjects to show the effect of lipaemia, clearing action of heparin and standing at room temperature for 1 - 2 hours.

Case No.	Plasma Turbidity				Blood Viscosity							
					Bedside Readings				Delayed readings			
	A	B	C	D	A	B	C	D	A_L	B_L	C_L	D_L
43	6	48	19	4	5.70	5.35	5.25	5.20	5.45	5.50	6.20	5.25
44	7	34	33	7	4.45	4.25	3.95	4.05	4.40	4.40	4.20	4.15
45	6	37	15	7	4.65	4.40	4.25	4.20	4.60	4.60	4.90	4.40
46	7	39	15	4	4.55	4.30	4.20	4.15	4.35	4.20	4.85	4.15
48	4	54	23	-	5.00	4.80	5.00	4.60	4.85	4.80	5.70	-
49	6	31	19	7	5.10	4.70	4.75	4.70	5.10	4.75	6.00	4.75
50	4	35	12	-	4.90	5.00	5.00	-	4.90	5.10	5.10	-
51	6	78	60	11	5.65	5.35	5.50	5.50	5.70	5.65	8.00	5.70
52	4	32	10	5	4.85	4.35	4.35	4.30	4.45	4.45	4.80	4.45
55	4	46	36	5	5.20	5.00	4.90	5.05	5.00	4.90	6.00	5.05
56	3	19	7	3	3.80	4.00	3.90	3.95	3.80	4.00	4.00	3.80
57	4	15	8	4	4.60	4.40	4.40	4.30	4.70	4.45	4.45	4.40
58	5	26	12	10	5.50	5.15	5.00	5.10	5.30	5.20	5.20	5.10
59	4	50	10	7	5.70	5.80	5.60	5.50	5.70	5.80	5.80	5.70
61	4	45	15	7	4.50	4.40	4.20	4.35	4.30	4.20	5.10	4.35
62	4	25	9	4	4.15	4.35	4.25	4.30	4.30	4.35	4.25	4.25
63	4	34	19	5	4.00	4.30	4.20	4.30	3.95	4.25	4.60	4.35
65	5	8	6	5	4.10	3.80	3.75	3.60	-	3.55	3.80	3.65
66	7	62	59	7	4.65	4.65	4.65	4.50	4.50	4.45	5.50	4.50
67	6	26	15	6	4.65	4.60	4.60	4.60	4.50	4.50	5.00	4.60
69	4	34	19	5	4.00	4.30	4.20	4.30	3.95	4.25	4.60	4.30
Mean	5	38	20	6	4.75	4.63	4.54	4.53	4.69	4.63	5.15	4.57

TABLE XXIII. Blood viscosity in 10 fasting subjects before and 15 minutes after 5,000 units of intravenous heparin.

Case No.	Plasma Turbidity		Blood Viscosity			
			Immediate Readings		2 Hours Later	
	Fasting	15 mins.	Fasting	15 mins.	Fasting	15 mins.
47	4	4	3.80	3.70	3.70	3.60
48	5	5	5.40	5.40	5.20	5.15
53	4	4	3.65	3.75	3.60	3.70
54	5	5	5.20	5.10	5.00	4.90
59	4	4	5.30	5.20	5.20	5.25
61	3	3	4.40	4.40	4.25	4.35
63	4	4	4.15	4.05	4.05	4.00
64	4	4	4.45	4.30	4.25	4.10
65	6	6	4.70	4.70	4.50	4.50
68	5	5	5.20	5.20	5.20	5.20
Mean	4.4	4.4	4.62	4.58	4.50	4.48

to stand at room temperature until about 1-2 hours after the last one had been withdrawn, and their viscosities again measured in a group. It was not at first appreciated that this time interval was critical. The specimens were next centrifuged and plasma viscosities estimated.

(2) After withdrawing the 3-hour sample the needle was left in situ and 5000 units of heparin injected. Further samples were taken from the opposite arm 15 and 75 minutes later. For simplicity of reference, the fasting, 3-hour and first and second post-heparin samples are called A, B C and D respectively. Readings taken after the samples had stood in vitro are indicated by the suffix L. V = viscosity and T = turbidity.

e.g. VAL = viscosity of fasting sample after standing at room temperature. The results of 21 experiments are shown in Table XXII.

(3) Two samples of C were taken. One was centrifuged immediately for the estimation of plasma turbidity before further clearing occurred due to the continuing in vitro action of the heparin-lipoprotein lipase complex. The other was used for viscosity measurements. In this way possible alterations in viscosity produced by packing of the red cells in the centrifuge was avoided.

(4) In 10 fasting subjects blood viscosity was measured before and 15 minutes after the intravenous injection of 5000 units of heparin, and the readings repeated about 2 hours later (Table XXIII).

Results

The statistical method used for analysis of the figures was Student's "t" test for paired results.

The bedside readings will reflect more closely the circulating viscosity and are considered first. There is a slight fall in the mean blood viscosity of the lipaemic samples ($t = 2.3$; $0.05 > P > 0.02$). A further slight fall is found 15 minutes after the intravenous injection of heparin ($t = 3.5$; $P < 0.01$). However the fall in viscosity from the mean level of 4.75 in the fasting sample to the mean of 4.54 fifteen minutes after the intravenous administration of heparin, though not great, is highly significant ($t = 3.97$; $P < 0.001$). There is no simple or obvious relationship however between the nature of the viscosity change and the degree of the lipaemia.

Heparin had no effect on the blood viscosity of the fasting subjects and, as one would expect, there was no change in the turbidity of fasting plasma after the intravenous injection of heparin.

When the immediate and delayed readings are compared there is an obvious increase in the viscosity of CL (4.54 to 5.15). This increase must be due to some change associated with the red cells, for no increase was found in the plasma viscosity of these samples. It also seems to require the presence of lipid and heparin together, for it is not caused by lipaemia in the unheparinised samples (compare VB and VBL) nor by heparin in a lipid free sample (compare the immediate and delayed post-heparin viscosities of the fasting subjects) (Table XXIII). It seems therefore that a heparin-lipid complex is formed or that heparin mediates some change in the lipids which is followed by adhesion of the complex or the altered lipids to the red cell. The strongly electro-negative nature of the heparin molecule may be the binding force.

If this hypothesis is correct, and the administered dose of heparin is

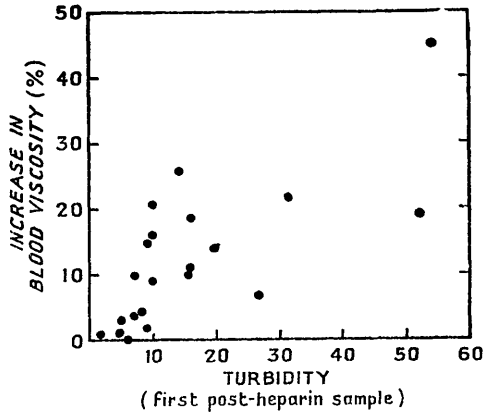


Fig. 25. Relation between the turbidity and in vitro increase in viscosity of sample C.

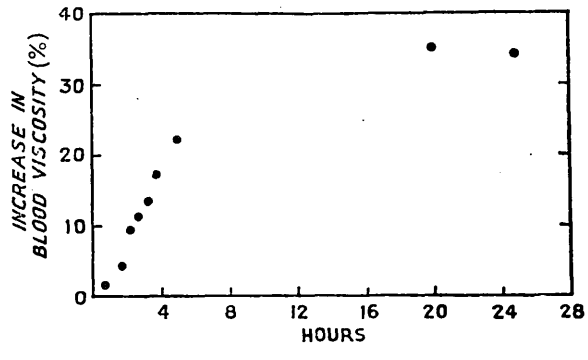


Fig. 26. In vitro increase in viscosity of sample C plotted against time. (Subject 67)

constant (as in this study) there should be some relation between the percentage increase in VC on standing, and its turbidity (i.e. between $\frac{VCL - VC}{VC} \times 100$ and TC); for the increase in viscosity should depend to some extent, although not altogether, on the amount of heparin-lipid complex formed. This relationship is plotted in Fig. 25. It was realised at this point that there might have been closer correlation if the time interval between VC and VCL had been constant. Therefore in subject 67, one of the last to be studied, the viscosity of the first post-heparin sample was measured at intervals, and in Fig. 26 the percentage increase in viscosity is plotted against time. The graph indicates that the viscosity of this sample increased at a constant rate until some point just short of its maximum. Thus the degree of increase in VC seems to depend not only on the amount of heparin-lipid complex formed but on the time allowed for it to adhere to the red cells.

There is no increase in VDL, presumably because the heparin-lipid complex has been cleared from the circulating blood during the intervening hour.

DISCUSSION

It seems clear that post-prandial lipaemia, of the degree encountered in this study, tends on the whole to diminish rather than increase blood viscosity. The difference is not gross however and is unlikely to have a significant effect on blood flow. But it is a reduction and this is at least reassuring in so far as any increase in post-prandial viscosity could theoretically increase the tendency to coagulation.

It must be emphasised however that these findings apply, in a strict sense, only to the fixed and rigid physical conditions of the instrument used, and

cannot be applied without reservations to the more complex problems of blood flow in a system of tubes of varying calibre and elasticity (Fahraeus and Lindqvist, 1931). Therefore one cannot deduce that lipaemia has no effect on blood flow in very narrow tubes such as capillaries and arterioles. Landis (1933) has shown in fact that blood viscosity in frog capillaries is much higher than in small bore arterioles.

It must also be mentioned that the findings of this study are to some extent at variance with those of R.L. Swank who has made a number of contributions to the problem. But the differences are not irreconcilable nor are they in fact strictly comparable. For most of his work has been done on hamsters fed unnaturally high fat diets for two or three weeks with a final loading dose of milk cream 10-15 times as large as the comparable adult dose given in this study (Swank, 1954). It is possible that such regimes produce a degree of lipaemia equivalent to those of the group D chickens of Chapter 4 and it is therefore not surprising that increased viscosities are measured. This is altogether a different experimental dietary scheme from mine, in which an amount of fat not unrepresentative of the normal dietary intake was used. Similarly the dose of heparin he used, and which produced a pronounced fall in the raised viscosity levels, was, at 400 units for a 100 g. hamster approximately 50 times the comparable dose used in this experiment, which again was based on the customary anticoagulant dose requirement.

It is also the case that whereas Swank's measurements continued for 72 hours and showed the peak increase in viscosity to occur between 6 and 9 hours after the final loading diet, and at a time when the lipaemia was beginning to

clear, my readings in effect come to an end with the administration of heparin 3 hours after giving the cream, and at the approximate time of peak lipaemia. In other words the increase in viscosity noted by Swank in his hamsters was occurring at a time when the dietary lipid intake was being assimilated into the general cholesterol-phospholipid-fatty acid pool. But Brundage et al. (1936) detected no relationship between blood viscosity and plasma lipids in a study which took no account of the post-prandial chylomicron lipaemia but dealt specifically with the plasma cholesterol, phospholipid and fatty acid levels.

It might also be argued that the Hess viscometer is hardly a sensitive enough instrument for this work. But it proved sensitive enough to demonstrate the slight but consistent difference between the viscosity of plasma and serum and differences in viscosity of a lesser order than this can hardly be considered clinically important. Holbrook and Watson (1939) have made a good defence of the Hess Viscometer and the ideal conditions for viscosity determinations on whole blood as described by Trevan (1918) were followed by me as closely as possible. And Eckstein et al. (1942) argue that the type of apparatus is not critical where only relative viscosities are being determined. Swank (1954) measured relative viscosity by timing the flow of blood from a needle introduced into the inferior vena cava of the hamster but as Pirofsky (1953) points out a possible major inaccuracy in this method is the effect of changing venous pressure.

The increase in viscosity, on standing at room temperature, of the blood sample taken 15 minutes after the giving of heparin to the lipaemic patient

seems to be an in vitro effect only. It is probably caused by the adhesion of chylomicron aggregates to red blood corpuscles or increased adhesiveness of the red cells to one another (Swank, 1951; Swank and Cullen, 1953). Swank and Levy (1952) suggest that heparin facilitates the transport of neutral fat through the capillary wall. Whatever the mechanism, this heparin-lipid complex is "cleared" in vivo without changes in blood viscosity.

It also seems clear that heparin has no clinically important viscosity effect in either the fasting or lipaemic patient. For although its administration to the latter group does produce a significantly detectable fall in blood viscosity it is of a very small degree. If heparin increases coronary flow volume as reported by Gilbert and Nalefski (1949) and relieves effort angina as reported by Graham et al. (1951) these effects probably depend on mechanisms other than lowering of blood viscosity. Certainly Copley et al. (1942) reported a fall in the "apparent viscosity" of blood after the in vitro addition of heparin, but they used about 200 times the amount of heparin given here.

Finally let me consider briefly two theoretical points. Firstly, if a moderate degree of alimentary lipaemia reduces the blood viscosity, as in fact it seems to do, is there any simple explanation for such an action? Apparently, there may be. It is possible that the small chylomicron particles have a lubricant or ball-bearing effect on the much larger red blood corpuscles. And secondly while this study has assumed the main problem to be one of variations in laminar flow in smooth tubes what might be the effect of atheromatous plaques or vessel constrictions? These physical barriers within the flow system introduce the possibility of turbulence and eddying with perhaps minute backwaters of stagnant

blood in which blood clotting could begin. The last word has almost certainly not been written on the importance of blood viscosity changes as a factor in vascular disease.

Footnote

Following the publication of this work (Watson, 1957) Ahrens (1957) in a review article discussed the divergent results obtained by Swank and myself. He was inclined to accept Swank's work on the grounds that his method was probably more sensitive, one reason why this chapter contains a defence of the Hess technique. However Gousios and Shearn (December 1959) have published an article in which they find no significant reduction in blood viscosity in human subjects following the intravenous injection of heparin. They used a method similar to that employed by Swank in his work on dogs. Further, Shearn and his colleagues (1960, Personal communication) have studied the effect of intravenous fat emulsions on human whole blood viscosity and have been unable to demonstrate an increase.

CHAPTER 11

THE ROLE OF THE TISSUE MAST CELL IN EXPERIMENTAL ATHEROSCLEROSIS

There is evidence which suggests that the presence of mast cells may protect from the occurrence of atherosclerosis, while on the contrary their absence predisposes to it. Atherosclerosis is easily induced in the rabbit and chicken. The rabbit is almost devoid of mast cells (Westphal., 1891; Holmgren and Wilander, 1937) and they are absent from the myocardium of the chicken (Watson and Campbell 1957). On the other hand the rat, which has an abundance of mast cells, particularly in connective tissue and peritoneum, (though not too numerous in the parenchymatous tissues such as lungs, spleen, liver and kidney), (Laguesse, 1919; Arvy, 1956), is notoriously resistant to the production of atherosclerosis. Atheromatous lesions can be induced in the rabbit and chicken by supplementing their normal diet with cholesterol but rigorous experimental regimes have been necessary in the rat and sometimes even these have failed (Page and Brown, 1952). Such regimes have included cholesterol feeding augmented by sodium cholate or cholic acid, and thiouracil or ^{131}I induced hypothyroidism (Page and Brown, 1952; Filios et al., 1956) hypertension (Wissler et al., 1954), steroid administration (Selye, 1958) and choline deficiency (Wilgram et al., 1954). Sundberg (1955) and Cairns and Constantinides (1954) have demonstrated a lower concentration of tissue mast cells in human adults with atherosclerosis compared with those having a healthy vascular tree.

The aim of this study was to observe whether the depletion of mast cells in the rat impaired its relative immunity to experimental atherosclerosis.

METHODS

Twenty-two double-hooded Norwegian rats were divided into 3 groups (A, B and C) of 6, and a group of 4, which were used for intermediate observations. Each rat was housed separately. Group A was fed a standard rat bran throughout group B was fed bran supplemented with 5% cottonseed oil and 2% cholesterol; group C was given the same diet as group B and in addition submitted to the injection regime to be described. Water intake was unlimited but the bran ration was maintained at 20 gms. per day. All animals were weighed weekly.

After 2 weeks of this diet group C began to receive intraperitoneal injections of compound 48/80 in increasing doses, up to 800 μ g. to 1000 μg. per day. Compound 48/80 (generously supplied by Burroughs Wellcome) is a relatively specific histamine liberator of low toxicity. It is a condensation product of p-methoxyphenethylmethylamine and formaldehyde. Full accounts of its pharmacology and its effect on the tissue mast cell have been given by Paton (1951), Feldberg and Miles (1953), Feldberg and Talesnik (1953) and Riley and West (1955). The 4 reserve rats were included in this schedule and killed singly on the 4th, 5th, 6th and 7th days of the injection course to verify that mast cell destruction was taking place.

Seven weeks from the start of the experiment, that is 5 weeks after the injections had begun, 3 rats from each group were killed by ether anaesthesia and the remaining rats 3 weeks later. Injection of compound 48/80 was withheld for the 24 hour period preceding the death of the animal. Blood for cholesterol and lipoprotein analysis was obtained by cardiac puncture. Mesenteric spreads

were prepared as described by Riley (1953) and stained without fixation. The aorta and its main branches were dissected and examined in situ, in some cases before and after infiltration with Sudan Black B as described by Filios et al. (1956). Heart and aorta were then fixed in a 10% formalin, 1% calcium chloride solution, and mounted in gelatin. Frozen sections were cut through the middle and base of the heart and the first part of thoracic aorta. All specimens were stained for mast cells with 0.1% aqueous toluidine blue for 20 secs. Heart and aortic sections were stained for fat with Sudan IV and counterstained with haemalum. Aortic sections were also treated with Weigert's elastic stain. Serum cholesterol was estimated by the micromethod of King (1951) and the lipoprotein pattern demonstrated by paper electrophoresis and staining with Sudan Black B (Chapter 2).

The heart and aortic sections were examined as follows:(a) mast cells were examined and counted in each toluidine stained section; (b) vessels in the corresponding Sudan stained sections were examined for atheromatous change or fatty infiltration; (c) aortic sections were examined with special reference to elastic tissue.

RESULTS

The systemic effect of the intraperitoneal injections of compound 48/80 were in general as described by Feldberg and Talesnik (1953). The animals were severely collapsed following the initial injections, but later in the experiment showed only slight and transient weakness with mild erythema and puffiness of snout, ears and paws.

TABLE XXIV. Summary of experimental data for Chapter 11.

B = Bran; C.O. = 5% Cottonseed Oil; C = 2% Cholesterol.

Rat No.	Diet	48/80	Duration of expt. (wks.)	Weight before (gm)	Weight after (gm)	Average weekly wt.gain (gm)	Serum cholest. (mg.%)	Heart Mast Cell Count Mid.	Base	Total	Lesions
A4	B	0	7	265	305	5.7	83	135	83	218	0
A5	B	0	7	285	310	3.6	90	87	115	202	0
A6	B	0	7	295	300	0.7	92	108	225	333	0
A1	B	0	10	235	255	2.0	91	63	91	154	0
A2	B	0	10	235	305	7.0	101	38	76	114	0
A3	B	0	10	265	320	5.5	70	75	102	177	0
Mean				263	299	4.1	88	84	115	199	0
B1	B + C.O. + C	0	7	305	325	2.9	54	27	114	141	0
B4	B + C.O. + C	0	7	270	315	6.4	104	121	56	177	0
B5	B + C.O. + C	0	7	315	330	2.1	122	82	78	160	0
B2	B + C.O. + C	0	10	240	275	3.5	71	128	137	265	0
B3	B + C.O. + C	0	10	250	250	0	70	44	51	95	0
B6	B + C.O. + C	0	10	295	350	5.5	68	77	104	181	0
Mean				279	308	3.4	82	80	90	170	0
C3	B + C.O. + C	-> 0.8 mg./day	7	315	290	-3.6	133	7	7	14	0
C4	B + C.O. + C	-> 0.8 mg./day	7	270	315	6.4	78	3	7	10	0
C6	B + C.O. + C	-> 0.8 mg./day	7	300	300	0	79	0	0	0	0
C1	B + C.O. + C	-> 1 mg./day	10	230	235	0.5	64	6	5	11	0
C2	B + C.O. + C	-> 1 mg./day	10	245	250	0.5	56	0	1	1	0
C5	B + C.O. + C	-> 1 mg./day	10	285	275	-1.0	70	4	1	5	0
Mean				274	278	0.5	83	3	4	7	0

The results are presented in Table XXIV. No mast cells were seen in the aortic sections of any group. Figs. 27 and 28 give examples of intact and disintegrated mast cells respectively, as seen in the mesenteric spreads. The heart mast cell counts of those animals killed on the 3rd, 4th, 5th and 6th days were 176, 50, 54 and 44 respectively. While most of the heart mast cells in the first animal were still intact the majority of mast cells in the other 3 were disintegrating. It seems a reasonable assumption therefore that the 3 rats killed in the 7th week of the experiment had been in a state of mast cell depletion for 4 weeks, and those killed 3 weeks later for 7 weeks.

There was no significant difference in the cholesterol levels of the three groups and the lipoprotein patterns were unaltered. Details of normal and abnormal lipoprotein patterns in rat serum are given in Chapter 8. There was considerable matting of bowel in each of the 3 rats killed after 8 weeks of compound 48/80 injections. Although the loops were teased apart without difficulty a longer course of injections might produce firm adhesions with intestinal obstruction.

No macroscopic aortic lesions were seen in any animal. There was no naked eye evidence of infiltrations of either liver, kidney or myocardium and histologically no myocardial tissue or vascular lipidosis was seen.

DISCUSSION

Within the limits of this experiment there is no evidence that the eradication of tissue mast cells in the rat diminishes its resistance to the production of atherosclerosis. However these limits are considerable and while the

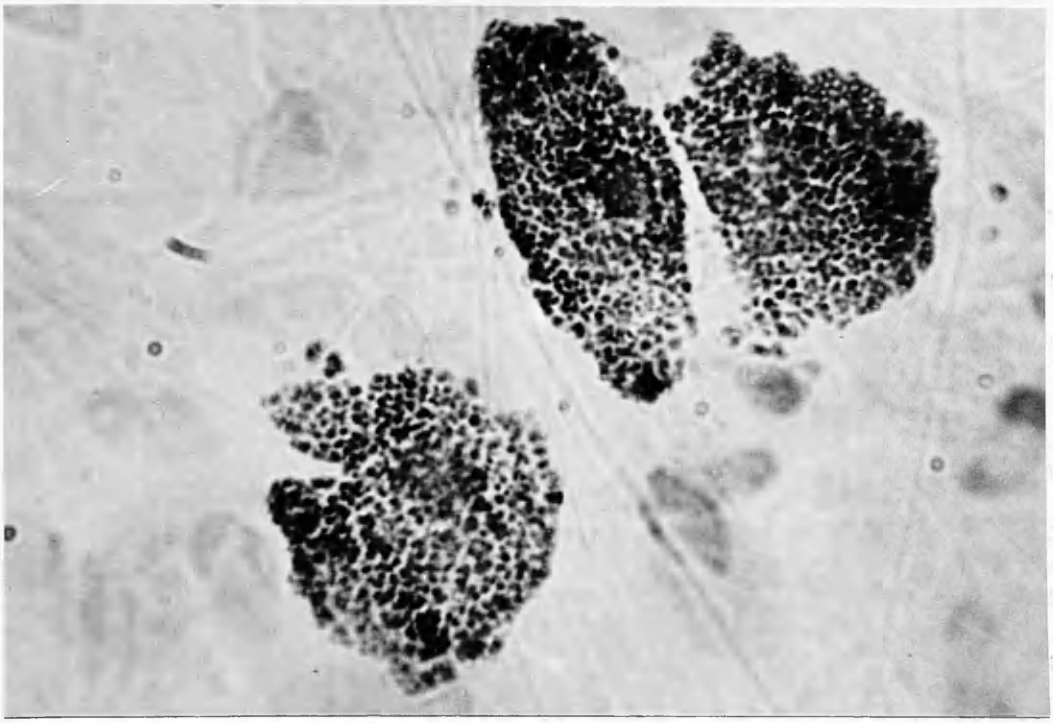


Fig. 27. Normal intact mast cells in rat mesenteric spread. Toluidine blue. x600.

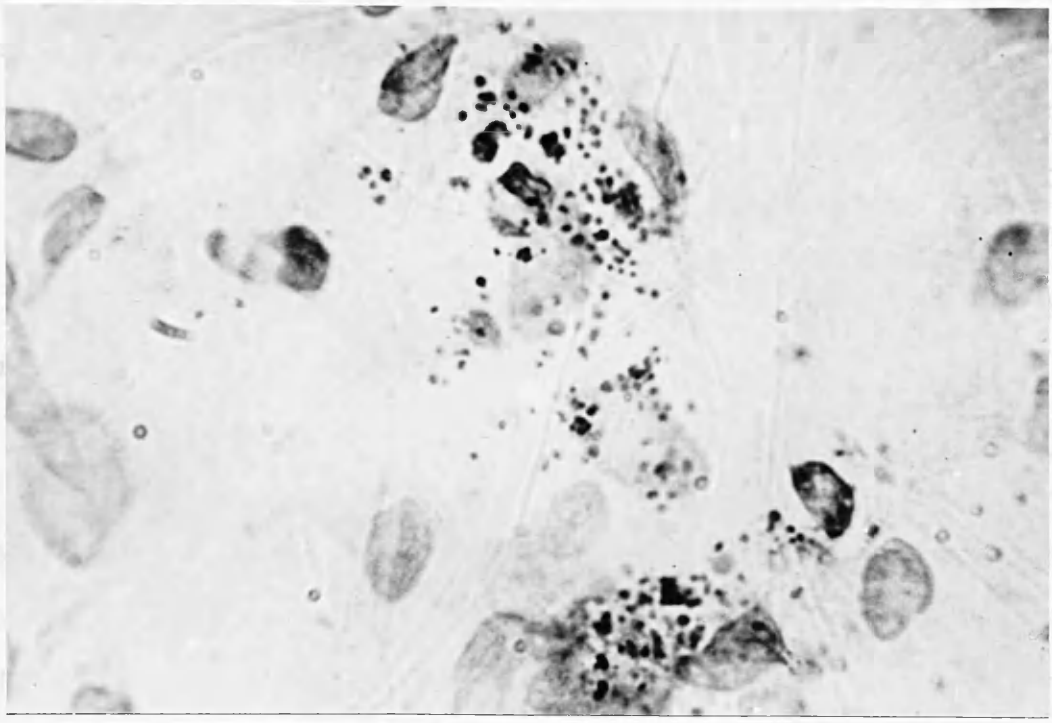


Fig. 28. Mesenteric spread from rat after 5 days of 48/80 injections. Note scanty mast granules and cellular disintegration. Toluidine blue. x600.

ensuing discussion does not explain them away or create significance out of negative results it enlarges the background of this particular experimental approach which at first sight seemed to have something to commend it.

To begin with it may be argued that the experiment was too brief. But arterial lesions can be produced in the rat quite rapidly, for Filios and his co-workers (1956) have produced definite atheromatous changes after only 31 days, others in chickens after a similar period (Rodbard et al., 1950) and Thomas and Hartroft (1959) have produced actual myocardial infarcts in rats in 4 weeks.

A more trenchant objection lies in the absence of proof that the short or long term administration of compound 48/80 eradicates tissue mast cells. It has been too readily assumed that the discharge of granules from the mast cell and the concomitant depletion of tissue histamine following the administration of compound 48/80 means the elimination of the mast cell as a functioning unit. In one of his less well known communications Riley (Riley et al., 1955) shows that whereas almost the entire histamine content of rat skin is lost following mast cell disruption by 48/80 only 53 per cent of the heparin disappears. Heparin and histamine coexist in the mast cell and until recently it was felt that the discharge of histamine from the cell must entail the loss of heparin also. But Riley's observation and the work of Uvnas (Hogberg and Uvnas, 1957; Uvnas, 1958 a and b) seems to indicate that histamine may be released from the mast cell without total loss of heparin.

And it is certainly not the case that prolonged administration of 48/80 to the rat destroys or inactivates the mast cell, for in recent work (Watson and Kennedy, 1960) it was found that after 14 days of 48/80 injections

considerable sulphate uptake could be demonstrated within 24 hours not only in the remaining intact or partially degranulated cells but in cells which were almost impossible to detect and identify as mast cells so complete was the degranulation and loss of normal morphological characteristics.

It is also possible that the experimental regime employed here could protect the animals, for although the heparin discharge of the degranulated mast cells is not comparable in degree to the histamine release as reported by Riley (Riley et al., 1955) it does occur to some extent, and heparin through its effect on blood lipids may have a role in the prevention of atherosclerosis (Boyle, Bragdon and Brown, 1952), the main inference in fact supporting the experimental principle of this study. But the appearance of heparin in the blood as revealed by increased clotting time, following the injection of 48/80, is transitory (Paton, 1951) and unlikely to influence the result.

In this experiment the failure to induce atherosclerosis can almost certainly be attributed to the failure to produce hypercholesterolaemia. A dietary regime such as that employed by Filios et al., (1956) could have been used to produce an increase in the circulating lipids, but was deliberately avoided. A diet similar to that used here produced marked lipaemia in chickens. It was considered that its failure to do so in the rat might be due to mast cell activity, and one expected that if mast cell depletion did induce atherosclerosis the corollary to this would be a rise in serum lipids.

The uninjected rats on the cholesterol enriched diet (group B) showed no qualitative or quantitative change in their mast cells. Since hypercholesterolaemia was not achieved one cannot discount the possibility that such a state

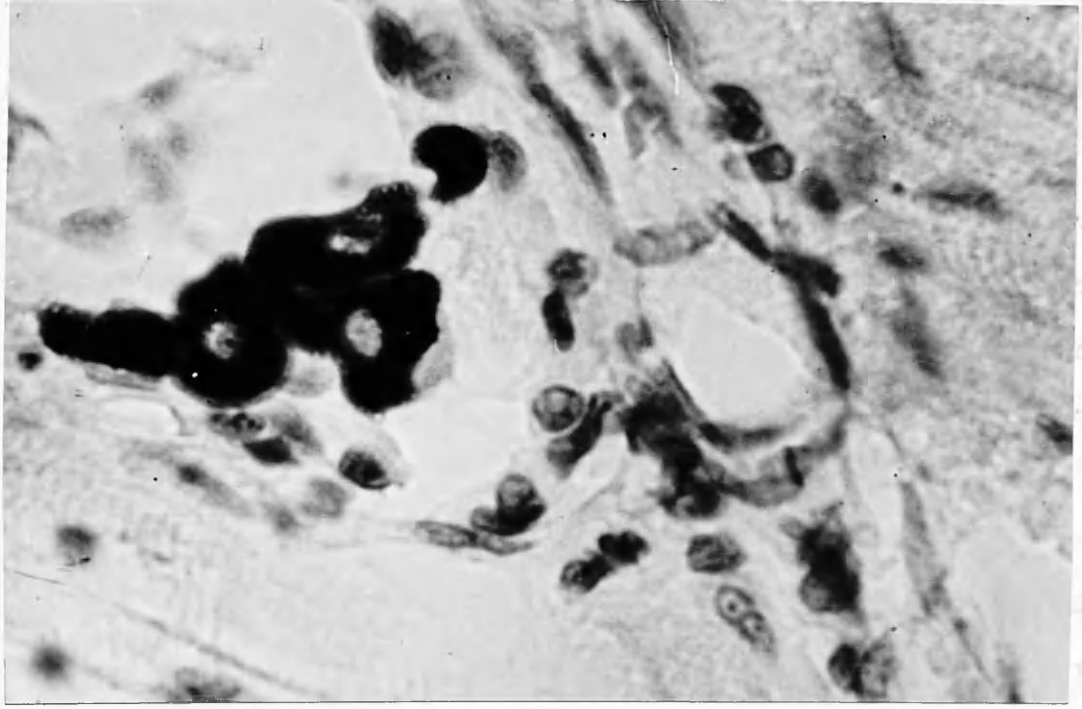


Fig. 29. Mast cells near a coronary arteriole. Toluidine blue. x600

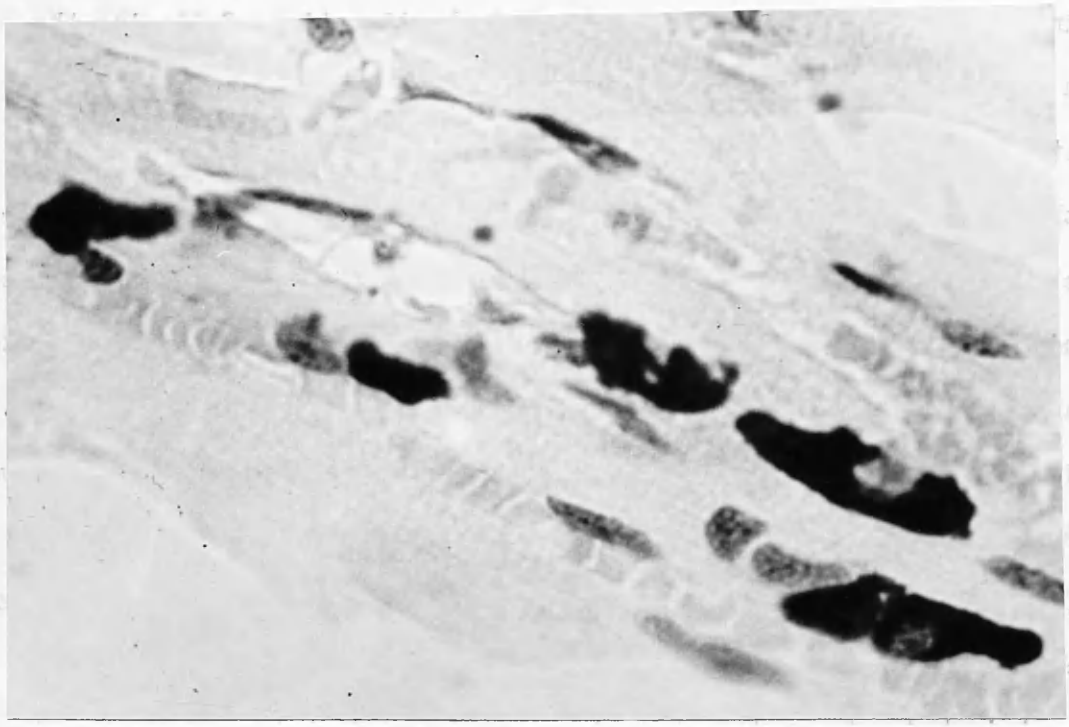


Fig. 30. Mast cells in rat myocardium. These are smaller, more elongate and more densely granular than the mesenteric mast cells. Toluidine blue. x600

may affect the mast cells.

Although mast cells are seen near or around vessels in heart sections (Fig. 29) they also lie in the myocardium (Fig. 30) while a number are seen immediately under the pericardium. The majority of the vessels seen in section had no adjacent mast cells. It seems unlikely therefore, on a purely teleological basis, that the cardiac mast cell has a specific function to protect arteries. The absence of mast cells may contribute in some secondary way to atherogenesis in certain species, but is unlikely to be a primary factor. Alternatively atherogenetic regimes may induce depletion of mast cells. It was the need to test this possibility which led to the work reported in the next chapter.

CHAPTER 12

HYPERCHOLESTEROLAEMIA, CORONARY LIPIDOSIS AND THE TISSUE MAST CELL

The work described in the previous chapter (Watson, 1958) did not support the idea that tissue mast cell depletion promotes hyperlipaemia or atherosclerosis. But it did not examine the possibility that if mast cell deficiency really occurs in association with these states it might be secondary to them and not their cause. Grunbaum et al. (1957) found no difference in the mast cell count of the rat ear when hyperlipaemia had been produced by a diet of butter fat, cholesterol and thiouracil. But they did not achieve particularly high serum cholesterol levels nor, it seems, did they produce vascular lipid infiltration. Shoulders and Meng (1957) on the other hand claimed that the intra-peritoneal injection of fat emulsions caused disruption of mesenteric mast cells. However this was probably due to the non-ionic detergents present in the emulsifying medium and not to the fat.

This study had two main aims, firstly to determine to what extent hypercholesterolaemia and coronary lipidosis affected the myocardial mast cell count, and secondly to investigate the effect of total body depletion of mast cells on the ability of the rat to correct an experimentally produced hyperlipaemia.

Three separate experiments were carried out and these are now described.

EXPERIMENT I

METHODS

Forty, 3 month, male albino rats were divided into 5 groups (K, L, M, N and O) of 8. Weight was distributed comparably in each group. The rats were housed separately and fed as follows.

Group K Normal bran. Rats 1, 3 5 and 7 were killed after 8 weeks and the others after 16.

Group L Bran, 10% corn oil, 2% cholesterol, 2% sodium cholate, and 0.3% methylthiouracil for 8 weeks.

Group M Bran, 2% cholesterol, 2% sodium cholate and 0.3% methylthiouracil for 8 weeks.

Group N Same diet as group M, for 16 weeks.

Group O Same diet as group M for 8 weeks and then normal bran for a further 8 weeks.

Water intake was unlimited, but food was restricted to 20 gms. per day. The animals were weighed at the start and finish of the experiment. At the end of each dietary programme blood for cholesterol estimation was obtained by cardiac puncture and the animals killed with ether. The liver and kidneys were inspected and segments taken for section. The aorta and its main branches were dissected in situ and examined for lesions. The external surface of the heart was inspected for macroscopic lipid infiltration. It was then divided transversely and fixed with the liver and kidney slices in 10 per cent formalin, 1 per cent calcium chloride. Frozen sections of all hearts and 2 liver and kidney specimens from each group were stained for fat with Sudan IV. Paraffin sections of the other half hearts were cut at 5 μ and stained with toluidine blue, as described previously. Preliminary examination of normal rat liver and kidney revealed that these organs were devoid of mast cells, so a systematic study for rat liver and kidney mast cell changes was not included.

This study required a quantitative estimate of mast cell density in myocardial sections. The total mast cell count for a complete cardiac section is

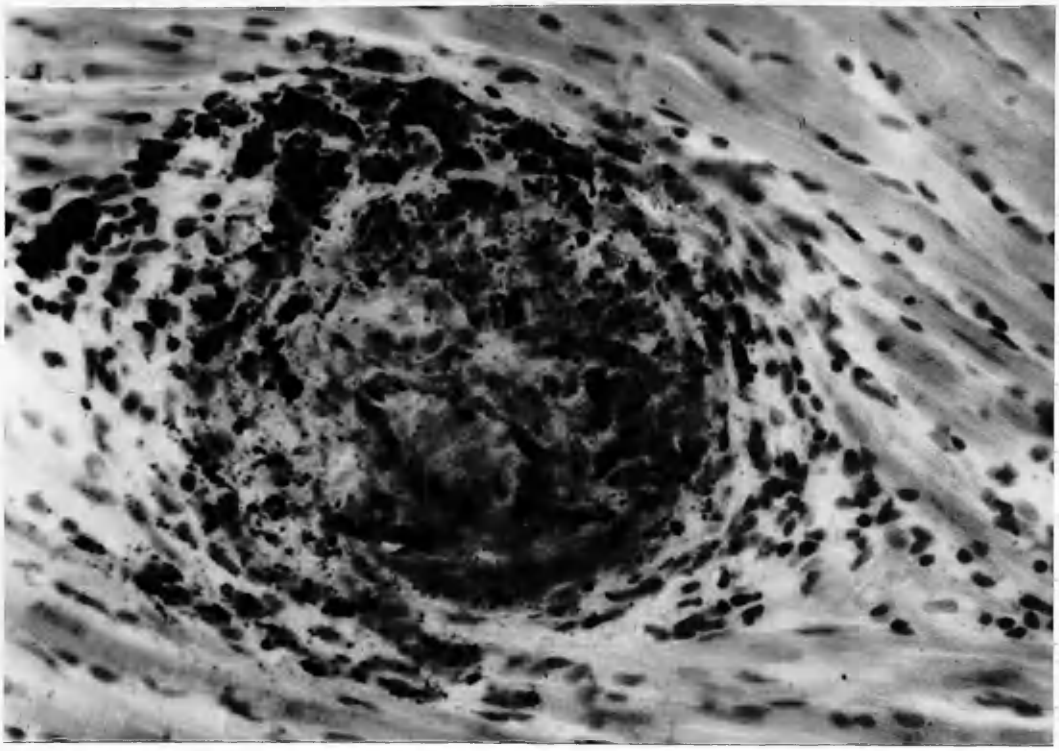


Fig. 31. Intimal and medial lipid infiltration of a coronary artery in a rat from group L. Frozen section: sudan IV and haemalum. x310

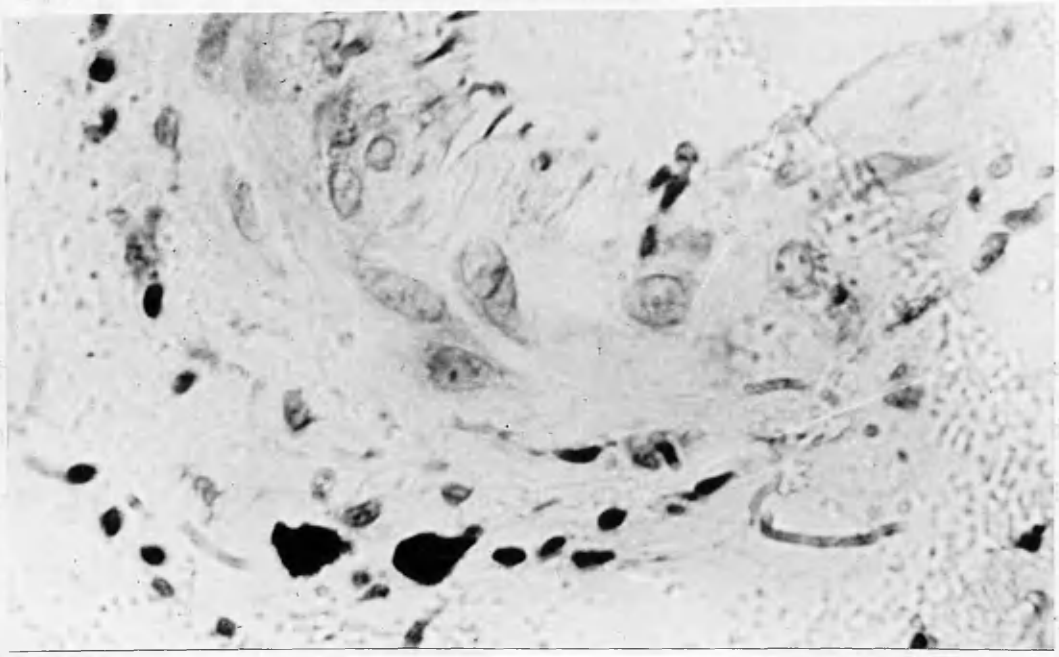


Fig. 32. Mast cells near a coronary artery in rat heart. Toluidine blue. x600

unsuitable for numerical comparison since sections from different hearts vary in area. The method adopted therefore was to count the number of mast cells in an entire transverse section of myocardium, measure the area of the section using a square-lined microscope graticule (Watsons Ltd.), and then calculate the mast cell density of the section as the number of cells per 100 squares. It is of course essential that the sections be of even thickness otherwise a serious fallacy creeps into the method.

RESULTS

No naked eye aortic lesions were seen in any group.

All animals in group L had severe macroscopic fatty infiltration of the liver, mottled infiltrations of the kidneys and a small white plaque of varying size, though averaging about 2 x 2 mm., at the apex of the heart. Frozen sections of the liver and kidney segments showed gross fatty infiltration and in 4 of the 8 hearts intimal and medial lipidosis of coronary vessels was present (Fig. 31). No systematic serial section cutting of hearts was undertaken. There is a reasonable probability that coronary lipidosis was present in all 8 hearts. Coronary lipidosis is the usual arterial lesion produced in rats by the short term experimental diet (Wilgram et al., 1954). This is no more than an infiltration of fat-staining material into the intima, often into the media, and sometimes through to the adventitia and periadventitious tissues. The fibrosis, ulceration and calcification of adult human atherosclerosis are not seen in this particular kind of experimental lesion. Figure 32 shows a cluster of mast cells close to a coronary artery in the heart from L 8.

Similar, but less severe degrees of fatty change were present in the rats of group M, and of a degree intermediate between L and M in group N. These

TABLE XXV. Data for 5 groups of rats on different dietary schedules showing (a) initial weight (Gms), (b) final weight, (c) serum cholesterol when killed (mgms %) and (d) mast cells per unit of area. The mast cell count for each group is compared with group K and the significance values are shown alongside. C.O. = corn oil; C. = cholesterol; S.C. = sodium cholate; M.T. = methyl thiouracil.

Group	Diet	Reading	1	2	3	4	5	6	7	8	Mean	
K	Bran 1,3,5,7 for 8 weeks 2,4,6,8 for 16 weeks	a	275	285	280	295	295	310	275	320	292	
		b	280	310	310	350	315	335	290	360	319	
		c	95	70	61	89	91	88	74	73	80	
		d	23.7	25.3	22.0	20.2	25.2	27.0	25.9	17.9	23.4	
L	Bran + 10% C.O. + 2% C + 2% S.C. + 0.3% M.T. for 8 weeks	a	290	285	290	300	295	310	290	320	298	
		b	235	240	210	215	240	275	245	265	241	
		c	2340	1330	1140	1440	1200	1406	2030	1680	1571	t = 1.33
		d	23.4	27.8	15.3	10.5	22.7	11.1	26.4	21.9	19.9	p > 0.10
M	Bran + 2% C + 2% S.C. + 0.3% M.T. for 8 weeks	a	285	285	290	295	290	325	315	315	300	
		b	240	240	240	230	250	265	220	250	242	
		c	676	1080	1720	1210	773	528	176	680	855	t = 0.90
		d	8.7	23.9	23.8	26.9	14.9	27.8	18.7	23.8	21.1	p > 0.25
N	As M for 16 weeks	a	280	290	295	295	305	305	310	330	301	
		b	230	245	230	240	250	250	260	270	247	
		c	811	-	604	1109	1006	974	978	900	912	t = 1.36
		d	16.9	-	11.3	13.1	30.6	21.2	27.3	15.9	19.5	p > 0.10
O	As M for 8 weeks then bran for 8 weeks	a	280	285	295	295	295	305	305	320	298	
		b	300	305	300	300	300	330	310	345	311	
		c	89	59	73	95	75	61	85	46	73	t = 0.24
		d	22.4	21.6	28.2	22.4	21.4	23.0	22.4	23.7	23.1	p > 0.25

gradations can be explained by the different degrees and durations of hypercholesterolaemia in the 3 groups of animals.

In group O considerable regression of tissue changes had occurred. The livers were only slightly paler than normal and no lipid infiltrations were seen in hearts or kidneys. The liver sections showed only traces of lipid staining and the hearts none.

The animals' weights, cholesterol levels and myocardial mast cell counts are given in Table XXV. Rat N2 died 3 days before its due time. No blood was obtained for cholesterol estimation and although the heart was sectioned and stained for mast cells, post-mortem changes had occurred and only faintly staining granular remnants were visible.

The myocardial mast cell counts for the groups L, M, N and O are each compared with group K and the significance values shown in Table XXV. The slight variations in the average mast cell counts for each group are obviously not significantly different from group K or from one another. But it can be seen from a study of Table XXV that there is a bigger range between the mast cell counts of individual animals in groups L, M and N. The differences are not related to cholesterol levels or degree of weight loss (all of the animals on the lipogenic diet lost weight). Individual myocardial mast cell counts might have varied according to the extent of the coronary lipid infiltrations. Therefore the 23 animals in these 3 groups were divided into 2 categories, 16 in which the coronary artery changes were slight or absent, and 7 in which they were severe. The mean mast cell counts for these groups are 19.0 and 22.9 respectively ($t = 1.50$; $p > 0.10$). The difference is not significant.

The conclusions which can be drawn from this experiment are:-

From Groups L, M and N

'Acute' hypercholesterolaemia and coronary lipidosis of short duration and of a moderate or severe degree neither increase nor decrease the concentration of mast cells in the myocardium. The slight difference in the mean count of groups M and N is not significant ($p > 0.25$) and provides no evidence that prolongation of a lipogenic regime might lower the count. No morphological changes were observed in the mast cells.

From Group O

Recovery from moderately severe hyperlipaemia and minor degrees of coronary lipidosis, (the results of group M represent this) allowed to continue for 8 weeks after the withdrawal of a lipogenic diet, is accompanied neither by an increase nor a decrease in the myocardial mast cell count. An increase might have occurred as part of some "normalising" mechanism, in which a mast cell lipid scavenger effect came into play; a decrease might have followed, for example, exhaustion of the cells through utilisation of their heparin.

EXPERIMENT II

The last experiment left a doubt as to whether a possible normalising or scavenger role of the mast cells had been adequately tested, or the effect looked for at the optimum time. The coronary lipidosis in Group M (the group whose serum and tissue changes probably corresponded with those of group O at the point of withdrawal of the lipogenic diet) was not gross. Again the recovery period of 2 months which elapsed might have been long enough to permit a transient

TABLE XXVI. Serum cholesterol and myocardial mast cell count of normal rats (Group P) and of rats at the 24th. day of spontaneous recovery from severe hypercholesterolaemia and coronary lipidosis. a = serum cholesterol after 2 months of lipogenic diet; b = serum cholesterol when killed; c = myocardial mast cell count per unit of area.

	Group P		Group V		
No.	b	c	a	b	c
1	78	20.0	1280	82	12.0
2	76	23.6	1280	95	16.4
3	76	28.3	1570	80	16.2
4	82	29.5	1310	80	28.8
5	63	21.2	2340	92	14.3
6	97	19.6	1320	80	25.4
7	71	27.4	1390	90	12.4
8	69	27.5	1710	61	16.5
9	92	17.8	1600	73	10.6
11			2270	84	25.1
Mean	78.2	23.9	1617.0	81.7	17.8

change in the mast cells and their subsequent return to normal. A second experiment was therefore devised and carried out as follows.

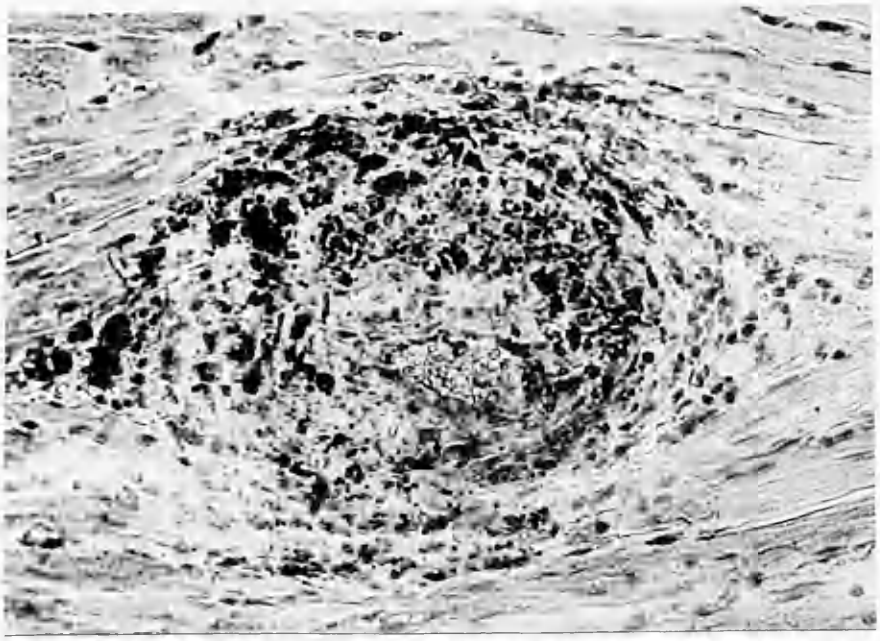
METHODS

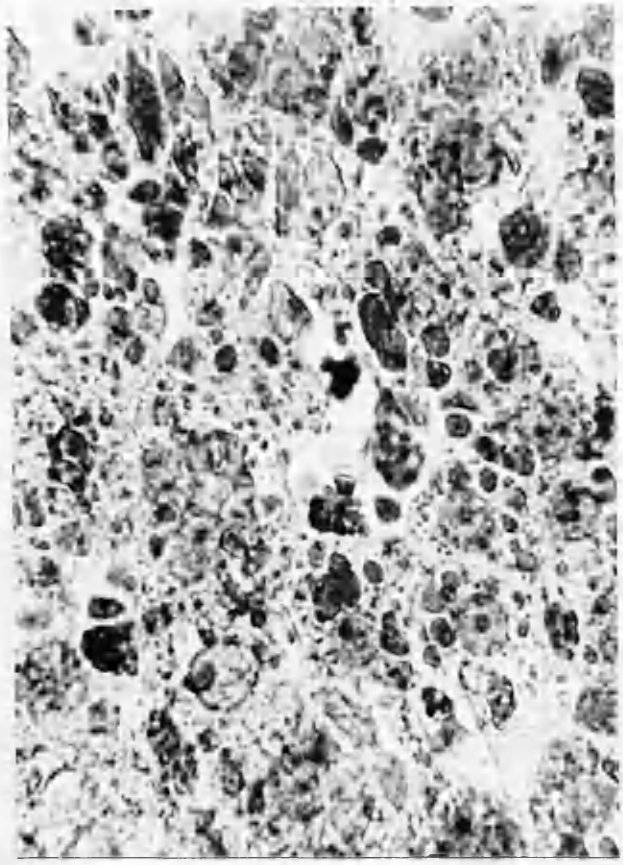
Twenty-two, 1 year old, male albino rats were divided into 2 groups (P and V) of 10 and 12. P is the control group referred to in Chapter 8 and in the experiment described after this. One of the rats in group P died during the experiment. Group P was given normal bran throughout. Group V was given bran supplemented with 10% corn oil, 2% cholesterol, 2% sodium cholate and 0.3% methyl thiouracil, for 8 weeks. At the end of this time blood was withdrawn by cardiac puncture, under ether anaesthesia, for serum cholesterol estimations, and all animals allowed to recover. The 2 rats with the lowest cholesterol levels (910 and 1030 per cent) were killed and their hearts, livers and kidneys examined macroscopically and microscopically as described in Experiment I. Both had gross lipid infiltrations of these organs. Fig. 33 shows sections stained for fat from heart, liver and kidney of one of them.

The remaining 10 rats, whose average serum cholesterol level at this point was 1617.0 mg. per cent, were returned to normal feeding for a further 24 days at which time blood was again withdrawn and the animals killed while still anaesthetised. The organs were inspected and sectioned as already described.

RESULTS

The serum cholesterol and myocardial mast cell counts for the two groups are given in Table XXVI. It can be seen that a remarkable drop in the cholesterol levels of group V has occurred in this short time (from 1617.0 to 81.7 mg%: normal average 78.2 mg.%). However although the serum lipids had returned to





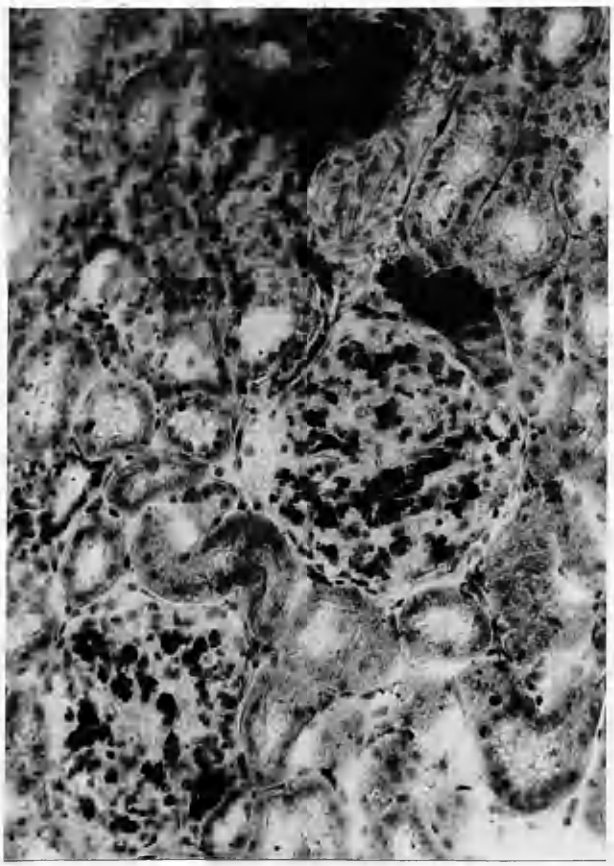
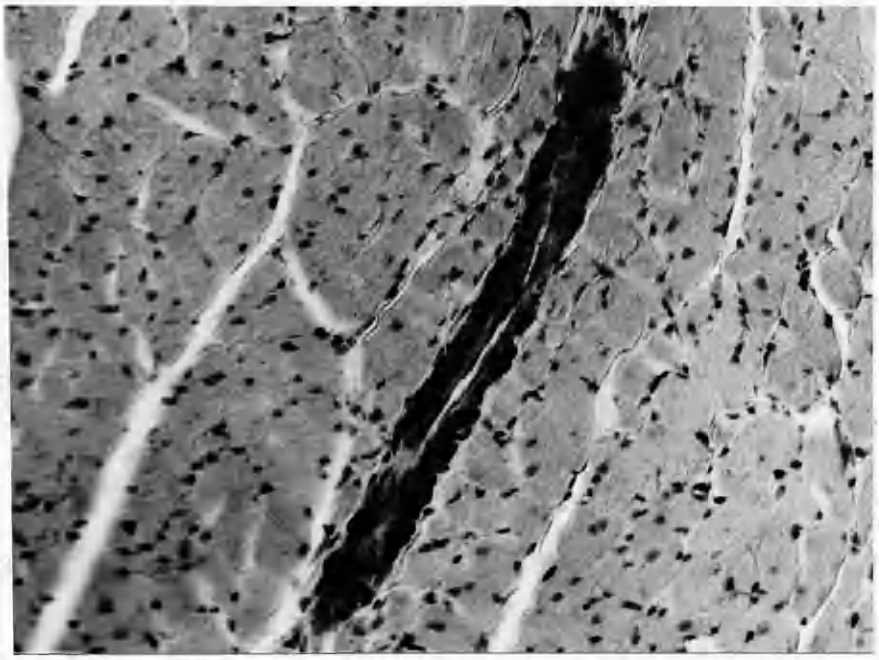
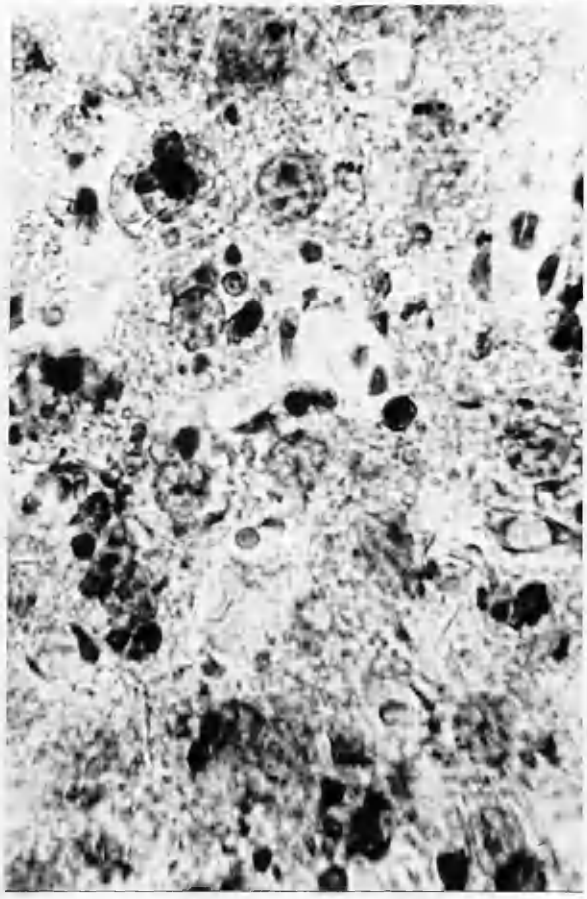


Fig. 33. Frozen sections of (a) heart, (b) liver and (c) kidney from rat V12 to show the degree of fatty infiltration after 8 weeks on a lipogenic diet. Serum cholesterol 1030 mg. per cent. Fat is present in coronary intima and media, in liver cells and renal glomerular tufts and arterioles. Sudan IV and haemalum. (Heart and kidney x220; liver x600)

normal levels the tissue lipids had not. (Fig. 34). The liver was still pale, but showing the red mottling of lipid losing areas, and in hearts and kidneys microscopic lipid deposits were present to a considerable degree. The animal at the time of death was thus still in the phase of recovery from the dietetically induced organ lipidosis.

The average myocardial mast cell concentration at this point of recovery was 17.8 cells/unit area. This may be significantly less than the average mast cell concentration of the control group which was 23.9/unit area, ($t = 2.32$; $0.05 > P > 0.02$) but is not a convincing reduction. Nevertheless when one examines the mast cell figures for all groups of this and the previous experiment the mean figures for the two normal groups (K and P) come very close to each other, (23.4 and 23.9, respectively) while the mean figures for the 5 groups which have been exposed to some degree of tissue lipid infiltration are to a greater or lesser degree below these values. If the figures for the two experiments are considered together the mean mast cell tissue concentration for the 17 animals of the 2 normal groups K and P is 23.7 per unit area and that for the 41 animals of groups L, M, N, and O and V is 20.1 per unit area. Their mean difference of 3.6 is again in the marginal area of significance ($t = 2.36$ $0.05 > p > 0.02$). The balance of probability, however, considering the individual group statistics of Experiment I and the apparent lack of correlation between mast cell counts and degree of vascular change in individual animals seems to be against any significant involvement of the tissue mast cell in the deposition or clearing of tissue lipid. Any claim that the greater lipid infiltration of liver and kidney reflects a vulnerability to lipid, due to the absence





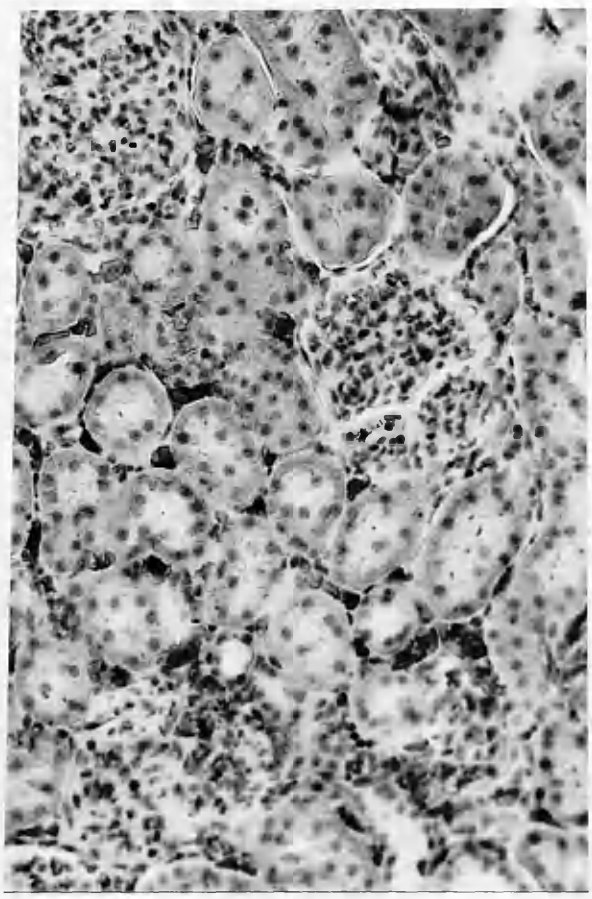


Fig. 34. Frozen sections of (a) heart, (b) liver and (c) kidney from rat V11 showing persistence of lipid in these tissues 24 days after the lipogenic diet had been withdrawn. The lipidosis in liver and kidney is much less than in V12. Sudan IV and haemalum. (Heart and kidney x220; liver x600)

of mast cells, would be a superficial interpretation of the facts. For these organs by virtue of their stucture, vascularity and functions are at greater risk than the myocardium.

The third and final experiment now described seems to show clearly that, so far as relatively acute changes in the rat are concerned, the tissue mast cell is not involved in tissue and blood lipid derangements.

EXPERIMENT III

Recovery from a hyperlipaemic state might occur without a demonstrable change in the number of mast cells, the cells participating in this recovery as a normal function and without such a degree of functional stress as would affect their concentration in the tissues. On the other hand if the mast cell does assist in the recovery their absence might retard it, because of impaired mobilisation of tissue lipid and perhaps also because of impaired transport for excretion.

METHODS

The rats of Group P, as mentioned previously, acted as the control group for this experiment. Another 20 animals from the same batch, of the same age and of comparable weight, were divided into 2 groups (Q and R) of 10 each. One animal in group R died before the experiment was completed. Both groups were given a diet consisting of normal bran containing 2% cholesterol, 2% sodium cholate and 0.3% methylthiouracil for 8 weeks. The ration was 20 gms. per day and water intake was unlimited. A similar diet for a similar period had produced a mean cholesterol level of 855 mg.% in a younger group of rats (group M, Experiment I) and it seemed reasonable to expect a comparable increase.

TABLE XXVII. Serum cholesterol levels of normal rats (Group P), lipaemic rats after spontaneous recovery (Group Q) and lipaemic rats after 24 days of compound 48/80.

	Cholesterol (mg %)		
No.	P	Q	R
1	78	74	65
2	76	71	87
3	76	93	76
4	82	83	77
5	63	66	94
6	97	64	73
7	71	55	79
8	69	78	79
9	92	57	80
10	-	80	-
Mean	**78.2**	**72.1**	**78.9**

This expectation is largely confirmed by the rise to a mean level of 1501 mg.% for the 12 animals in group V whose diets contained 10% corn oil in addition.

After 8 weeks of special diet both groups (Q and R) were returned to normal feeding. At this point of transition intraperitoneal injections of compound 48/80 were started in group R, the dose increasing from an initial dose of 0.1 mg. to 1 mg. over a period of 8 days. The animals showed the usual effects, (see previous chapter) but none succumbed. No intermediate histological studies were carried out to observe the progress of mast cell disruption. By this time sufficient experience of the method had been obtained to permit confidence in its effects. The injections were given on 6 days of each week.

Twenty eight days after the return to normal diet both groups of animals were bled and killed, the main organs inspected and sectioned as described earlier, and mesenteric spreads prepared from some of the animals in group R to confirm that the expected mast cell change had occurred.

RESULTS

It can be seen from Table XXVII that there is no significant difference between the cholesterol levels of the 3 groups of rats. Total tissue mast cell depletion has not prevented, although of course it may have delayed by a few days, the return to normal from high cholesterol levels. There was no difference in the degree of coronary lipidosis in the hearts of the group Q and R animals. In both groups it was either negligible or absent. The naked eye appearance of liver in both groups was comparable and microscopically there were equivalent degrees of fatty cellular infiltration.

It does not seem that the tissue mast cell is essential either for the mobilisation of tissue lipids or their excretion from the blood.

GENERAL DISCUSSION

This work does not advance the idea that the tissue mast cell may be involved in atherosclerosis. It does not completely rebut it, for the lipid changes produced in the foregoing experiments come critically short of atherosclerosis as opposed to the mere infiltration of fat into vascular tissue, the earliest stage in the development of the atheromatous plaque. It seems clear nevertheless that this initial stage can occur in the presence of normal concentrations of mast cells and without causing their disappearance, depletion or morphological derangement. Any mast cell disturbance which occurs later than this may be no more than the normal response to tissue reaction.

Pollak, (1957) in a careful and detailed study of mast cell incidence and distribution in the normal and diseased human vascular tree, was unable to correlate clinical and autopsy data with aortic mast cell counts. Without quoting figures, however, he gives it as his impression that the incidence of periadventitial mast cells around atheromatous coronary arteries is higher than around normal vessels. The closing sentence of his account comes as a surprising non-sequitur in an otherwise conservative and balanced discussion. He writes "The presence of mast cells in oedematous tissues and their absence in atheromatous plaques possibly implicate these cells as participants in atherogenesis".

The ultimate experimental answer to this problem may be found in a long term study where there is time for coronary atherosclerosis, with fibrosis or calcification, to develop. But there are some drawbacks to this, for on diets

like that used here, the animals go out of condition, lose weight and have chronic diarrhoea. In addition the thiouracil in the diet must cause profound changes in the animal's normal metabolism. After only 2 months of the diet the rat thyroid is greatly hypertrophied.

Because mast cells contain heparin and because in unphysiological doses heparin modifies lipoprotein structure there is the temptation to incriminate them in a tissue lipid defect. The mast cell presumably has some role in tissue metabolism and this, among other things, may include lipid regulation or utilisation, but on the basis of the experimental evidence produced here, the verdict for or against such a function seems to be not proven.

CHAPTER 13

SERUM LIPOPROTEINS AND ELECTROCARDIOGRAPHIC CHANGES AFTER PARTIAL GASTRECTOMY

Following partial gastrectomy of the Polya type many patients lose weight and most are below their expected weight (Anderson et al. 1955). This is probably due to a low caloric intake and post-gastrectomy symptoms, but impaired digestion and absorption of fat may be a contributory factor. Recent work in gastrectomy patients using ^{131}I labelled fats has shown increased fat excretion and diminished absorption after fatty meals, and that fat loss is greater after the Polya operation than after the Billroth I (Everson, 1954; Shingleton et al., 1957). Bearing in mind the possible relationship between dietary fat, serum lipids and myocardial ischaemia which was discussed in the introductory chapter, this impaired fat absorption might be reflected in a lower incidence of coronary artery disease and a corresponding alteration in the serum lipoproteins of gastrectomy patients.

This study was carried out in association with Mr. J. K. Watt and Dr. R. S. Walker who together were responsible for the organisation of the experimental routine and selection of patients.

MATERIAL

Of 53 patients who had undergone the Polya type of partial gastrectomy 40 were traced and examined. All were males between 45 and 65 years who had been operated on because of severe duodenal ulcer symptoms. The mean lapse of time from operation to interview was 9 years 10 months (range: 7 years 1 month to 13 years 4 months). At the same time 40 men in the same age group

with long-standing symptoms of duodenal ulcer (mean duration 17 years) were studied; 15 of these patients had suffered major complications such as perforation and haemorrhage. As a "normal" control group 40 males, admitted for operative treatment of minor surgical conditions and without ulcer or cardiac symptoms were examined. In the latter two groups the last 5 or 6 patients were selected on an age basis to ensure that the mean ages of each group were closely related (Table XXVIII).

METHODS

A clinical history and examination of each patient were undertaken; height, weight, pulse rate and blood pressure were recorded and a sample of venous blood withdrawn for haemoglobin and lipoprotein estimations. Electrocardiography was performed using a direct writer (Mingograph) before and after Master's double two step test (Master, 1950). The amount of exercise in this test is graduated according to the patient's weight and age. One man with a previous myocardial infarction was not exercised, in two others the exercise was discontinued because of angina and in a third because of dyspnoea; all others completed the test. To facilitate rapid recording after exercise only nine leads were used; the 3 standard leads, aVR, aVL, aVF, V2, V4 and V6. The records were studied independently by two of us without reference to the patient's group, and the findings compared. A further independent opinion was obtained where a difference in interpretation occurred.

It is difficult to apply rigid standards to the interpretation of electrocardiograms but the following criteria of ischaemia were adopted as a guide: ST

TABLE XXVIII. Summary of clinical, E.C.G. and biochemical data contained in Appendix III. There are 40 subjects in each group.

Group	Mean age (Yrs.)	Mean Hb %	Mean weight (lbs.)	No. with E.C.G. or clinical ischaemia	Mean beta:alpha ratio	No. with prebeta Lipoprotein
Polya Gastrectomy	53.2	88.2	132	11	1.59	14
Range	45 - 65	59-103	103-183		0.56-3.95	
Control	53.6	96.7	154	15	1.70	19
Range	46- 64	77-118	114-208		0.82-2.83	
Ulcer	53.5	97.7	147	21	1.73	24
Range	47- 64	80-112	110-202		0.59-5.02	

depression of 1 mm. or more where the increase in pulse rate did not exceed 20/min. and of 1.5 mm. or more where it did; flat or inverted T waves in appropriate leads; and multiple extrasystoles. All other abnormalities were noted and care was taken to distinguish changes due to tachycardia alone, namely a low RS-T junction with upward sloping ST segment. The distinction between the lateral ischaemia of coronary artery disease and that of left ventricular strain is taken into account in the analysis of the results.

The beta:alpha and pre-beta:beta lipoprotein ratios were estimated as described in Chapter 2.

RESULTS

The individual results are listed in Appendix III and a summary of the principal findings is presented in Table XXVIII. Only blood pressures greater than 139/89 are recorded.

The present account of this study differs from the published version (Walker et al., 1958) in 2 respects. Firstly a different convention was used for measuring the beta-lipoprotein area, so that it included the neutral fat band. This gave higher values for the beta:alpha ratio than the standard method adopted for this thesis. Secondly in assessing the incidence of myocardial ischaemia in each of the experimental groups, I have included symptoms as well as E.C.G. findings. Thus one man in the gastrectomy group (No. 42) who had a normal exercise electrocardiogram complained of chest discomfort during the test, while another (No. 53) with a normal E.C. G. and no test symptoms gave a history of exertional dyspnoea and occasional effort angina. On this basis

of assessment the former interpretation of the experimental findings requires modification. This revised view of the significance of the study, which I believe is more likely to be the correct one, is presented here.

The incidence of myocardial ischaemia in the gastrectomy group (11 cases) is less than in the control group (15 cases), but the difference is not significant ($\chi^2 = 0.91$; $P > 0.10$). Likewise there is no significance in the difference between the ulcer patients with ischaemia (21 cases) and the controls. ($\chi^2 = 2.59$; $P > 0.10$). But the difference between the ulcer and gastrectomy patients is greater than either of these and may have a degree of significance ($\chi^2 = 5.21$; $0.05 > P > 0.02$). However it is clear that no claim can be made that the cardiac status of any one of these 3 groups is fundamentally better than another. Nevertheless there is an interesting trend in the figures which a larger and more detailed study might confirm.

Lipoproteins

The lipoproteins are more directly involved in alterations in fat metabolism and one might have expected greater differences between the groups in respect of their lipoproteins. This aspect of the problem is examined briefly in the following paragraphs.

Lipoproteins of the Clinical Groups

The beta:alpha lipoprotein ratio of the gastrectomy group was 1.59, and pre-beta lipoprotein was present in 14 cases. The corresponding figures for the control group were 1.70 and 19, and for the ulcer group 1.73 and 24. Again

TABLE XXIX. Lipoprotein data of 120 subjects grouped according to weight.

	Standard weight -15 lbs.or more	Standard weight ± 14 lbs.	Standard weight +15 lbs.or more
No. in group	52	58	10
Average beta:alpha ratio	1.56	1.69	2.08
No.with prebeta lipoprotein	19 (36.6%)	30 (51.8%)	8 (80.0%)

there is no definite significance between any pair of figures (e.g. comparing ulcer and gastrectomy beta:alpha ratios $t = 0.95$ and $P > 0.10$) but the trend of the values follows the incidence of group ischaemia. However the subsequent alignments of the figures show that in certain specific respects this similarity of trends breaks down.

Lipoproteins and Ischaemia:

The lipoproteins were next examined with reference to the individual occurrence of ischaemia. The figures can be broken down as follows:

a) Average beta:alpha ratio of 47 ischaemic patients = 1.63

Average beta:alpha ratio of 73 non-ischaemic patients = 1.69

b) Of the 47 ischaemic cases 27 had a pre-beta lipoprotein band and 20 had none.

c) Of 57 patients with a pre-beta lipoprotein band 27 had evidence of myocardial ischaemia and 30 had none.

It is obvious from these figures that no correlation can be established between either the beta:alpha or pre-beta:beta lipoprotein ratios, and the presence or absence of myocardial ischaemia.

Lipoproteins and Weight:

The 120 patients were divided into 3 groups; those whose weights were 15 lbs. or more below their expected weight (group I), those whose weights were 15 lbs. or more above their expected weight (group III) and those between (group II). The results are shown in Table XXIX. The average beta:alpha lipoprotein ratios of the groups in ascending order of weight is 1.56, 1.69 and 2.08 while the incidence of the occurrence of pre-beta bands is 36.6, 51.8 and 80.0 per cent

respectively. Unfortunately group III is rather small for statistical comparison with the other groups, but the figures do suggest that these two lipoprotein parameters are related to an individual's over or under weightness. As a matter of interest, statistical comparison between the incidence of pre-beta lipoprotein in the under and overweight groups gives these values: $\chi^2=6.08$ $p < 0.02$. Examination of weight with reference to the distribution of ischaemia among the groups showed no ostensible correlation.

None of the other aspects investigated showed any significant difference between the groups, apart from the lower average haemoglobin level of the gastrectomy patients (88.2 per cent). This is actually an adverse factor which would tend to increase the number of positive electrocardiograms in this group.

DISCUSSION

The incidence of abnormal electrocardiograms in this study seems to be unduly high, and one wonders if the criteria of abnormality are stringent enough. Certainly Master et al. (1942) found no significant changes in the electrocardiogram following the double two-step test in 34 normal subjects of unspecified age. Against this, Riseman et al. (1940) employing criteria of abnormality more severe than Master's and comparable to those used here, found significant changes in 10 of 15 normal patients whose ages ranged from 25 to 73 years. In a review of the literature they draw attention to the divergent findings of a number of authors. Such differences cannot be reconciled. The results reported here were obtained from a standard test consistently applied to each of the clinical groups. But it is true that the reporting of electrocardiograms is

more of an art than a science and rigid mathematical standards are probably misleading in the realm of marginal alterations. Certainly on having a second look at the electrocardiograms of this study there are some which in spite of significant metrical alterations I would be less inclined to regard as abnormal.

Although there are no lipoprotein changes which seem to be characteristic of the clinical groups, an analysis of the lipoproteins in other respects suggests that both the beta:alpha lipoprotein ratio and the incidence of the pre-beta band bear some relation to weight, not absolute weight, but to the degree of over or underweightness, of the individual patient. It will be remembered that higher lipoprotein ratios and a greater incidence of pre-beta lipoprotein occurred in pregnant women whose weight gain was regarded as abnormally high.

No relationship has been demonstrated between the lipoprotein parameters and myocardial ischaemia. But for the reasons given above this should not be regarded as contradicting the work of Besterman (1957, 1958) who in his analysis of lipoproteins is dealing with patients with gross coronary artery disease.

Finally although there is no statistical proof of the fact it seems possible that lower lipoprotein values occur in patients who are underweight and higher values in those who are overweight. Whether this is due to a specific dietary factor such as fat absorption, or a non specific factor such as total calorie intake, cannot be determined from this study. But it is an observation which tends to confirm that weight reduction is a valuable contribution to the control of blood lipids.

CHAPTER 14

GENERAL CONCLUSIONS

1. There is good evidence that the blood lipids are implicated in atherosclerosis, probably implicated in thrombosis and are perhaps concerned with impaired fibrinolysis. It is generally agreed that some kind of lipid modification might advance the therapy of atherosclerosis and its clinical sequelae. The main lipid derangements usually, but not always, found in affected subjects are a raised total serum cholesterol; an increase in the low density lipoproteins, especially pre-beta lipoprotein; an elevated cholesterol : phospholipid ratio; and delayed clearing of post-prandial lipaemia.

2. The lipid parameter most frequently used as an index of therapeutic effectiveness is the total serum cholesterol level. An understanding of the metabolism and synthesis of cholesterol, and some knowledge of physiological and ethnic variations, suggest possible methods of control.

3. Experimental hypercholesterolaemia in the chicken and rat is associated with the formation of an unusual alpha 2-lipoprotein. In rat serum this lipoprotein, which carries almost all of the extra cholesterol, is deficient in protein and is highly unstable in vitro. Comparable in vivo instability would partly account for the speed with which tissue lipidosis develops. The substances most necessary for the surface stability of lipoproteins are protein and phospholipid.

4. When a rise or fall in the cholesterol level is the primary change in human blood lipids it is usually accompanied by a rise or fall in the beta:

alpha lipoprotein ratio. This is because beta lipoprotein seems to be the main carrier-receptor for the "tidal" cholesterol of human serum. But when the serum lipid changes are complex, as when hypocholesterolaemia is achieved by the ingestion of fatty acids or accompanied by changes in serum phospholipid, the lipoprotein response is not simply or directly related to cholesterol change.

5. Pre-beta lipoprotein is found almost invariably in patients who have had a myocardial infarction, but it is also found in clinically normal individuals and in primi g ravidae. It increases relative to beta lipoprotein in subjects receiving corn oil for long periods. This may indicate some change in the esterification of the cholesterol contained in this lipoprotein complex. Other explanations are of course possible.

6. Oestrogens affect the serum cholesterol in different ways in different species. One consistent effect seems to be an increase in circulating phospholipid relative to cholesterol. This may stabilise the hypercholesterolaemic lipoprotein complex and so diminish its pathogenicity. Although oestrogens are effective as long term hypocholesterolaemic agents in adult males there is no evidence yet of clinical benefit, and frequently side effects are troublesome.

7. Thyroid extract and a number of thyroid analogues are powerful hypocholesterolaemic agents, but none has yet been found which is devoid of hypermetabolic tissue effects. This vitiates their use in patients with myocardial ischaemia. It may be that the hypocholesterolaemic and hypermetabolic effects of thyroid compounds reside in a common molecular group and are indivisible.

But it is nevertheless clear that a dissociation of the degree of activity of these separate functions can be achieved by molecular alterations.

8. Certain marine and vegetable oils are effective hypocholesterolaemic substances, both in man and in the experimental animal. Their degree of unsaturation is related to their effectiveness in this respect. There may be a non-lipid factor (1: 2-dienolglucose or something else) which augments the effect. Extracted maize germ is a potent hypercholesterolaemic agent in chickens. This suggests an important interplay of cholesterol promoting and inhibiting factors in this particular food raw material.

9. The hypocholesterolaemic effect of these oils enhances, or is enhanced by, the cholesterol reducing effect of a low animal fat diet. Such a diet, supplemented by 2 ozs. of corn oil per day is acceptable as a long term dietary mode and approaches the dietary pattern of certain racial groups or societies in whom atherosclerotic vascular disease is unusual and serum cholesterol levels are low or normal, compared with British and American figures. Such a regime is available to all, is free of the risks which limit the usefulness of anticoagulants and requires no laboratory control. However no assessment has yet been made of its clinical success.

Corn oil consumed without restriction of dietary animal fat will usually produce hypercholesterolaemia. If large enough quantities are taken patients will gain weight and myocardial ischaemia or strain will be aggravated.

0. Heparin is a powerful anti-lipaemic substance, but does not lower the serum cholesterol. Any merit which heparin may have in the treatment of angina is unlikely to be due to the effect of its anti-lipaemic action on blood viscosity.

For post-prandial lipaemia does not increase blood viscosity, and may actually reduce it, and although blood viscosity is further reduced by the intravenous injection of heparin during the lipaemic state, the effect is so slight as to be clinically insignificant. Heparin, 50 mg. I.V. has no effect on the blood viscosity of fasting subjects.

11. Tissue mast cells are an important source of heparin. There is some evidence in the literature that their concentration in human perivascular tissue is low in the presence of atherosclerosis. But on the basis of experimental work reported here there is no evidence that they are affected by, or that their absence predisposes vascular tissue to, the early stages of lipid infiltration. Also the clearing of arterial lipidosis is not delayed by total body depletion of mast cells.

12. The serum cholesterol level and $beta_a$:alpha lipoprotein ratio bear some relationship to weight, tending to be lower in underweight subjects and higher in those who are overweight. The maintenance of ideal weight is probably an important contribution towards the control of blood lipids.

13. The ultimate value of attempts to correct the lipid derangements associated with atherosclerosis depends on the capacity of affected tissues to recover from the lesions. This will of course depend on the severity of the individual lesion. Calcified atheromatous plaques are probably too resistant to normalising processes. But with less severe degrees of arterial damage there is convincing evidence that regression of lesions does occur in the experimental animal. There is only indirect evidence that similar changes occur in humans.

14. Because of the difficulty which is likely to be experienced in persuading a nation to alter its eating habits it is a matter of urgency that an effective cheap, palatable and non-toxic agent for the control of blood lipids be discovered. A dietary principle or food factor is the substance most likely to satisfy these requirements.

ACKNOWLEDGEMENTS

The work reported here was carried out partly at the Department of Materia Medica and partly at the Muirhead Department of Medicine, Glasgow Royal Infirmary. I am particularly indebted to Professor Alstead and Professor Davis for unfailing encouragement, for the facilities and equipment which they so readily made available, and for permission to study patients under their care. Dr. McKay Hart kindly allowed me to study patients attending his ante-natal clinic and admitted to his wards at Stobhill Hospital, and Sister Dawes skilfully coped with the organisation which this involved. I am grateful to Dr. J. W. Chambers for the cholesterol estimations of Chapter 3, and to Dr. G. Church and Mr. M. Dick for the cholesterol and phospholipid figures quoted in Chapters 4 and 5.

It was a pleasure to work with Mr. R. S. F. Campbell of the Department of Veterinary Pathology during the experimental work on chickens. This training was an invaluable prelude to the personal experimental work on rats which followed. Some of the frozen sections (Chapter 12) were prepared in the Department of Pathology, Glasgow Royal Infirmary, and I am grateful to Dr. W. P. Duguid for such assistance. Drs. Robb and Silvey of the Mathematics Department gave unstinted help and advice in statistical problems. Messrs. Boots, Glaxo and Burrough's Wellcome were most generous in the supply of certain drugs and dietary components.

Dr. Winternitz of the Department of Scientific and Industrial Research (Hydraulics Division) at East Kilbride helped to clarify some of the difficult theoretical problems in the unfamiliar field of viscosity, and Dr. J. H. Wright

who showed a sustained and stimulating interest in much of this work, most graciously allowed me to make extensive use of the Electrocardiography Department in Glasgow Royal Infirmary. I am indebted to Dr. E. M. McGirr for the radioiodine studies.

I must especially thank Mr. R. McGregor of the Department of Medicine for the photography in the preparation of this thesis and last of all I cannot forget the large number of patients whose cheerful and willing co-operation in this work made it possible.

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APPENDIX I. MONTHLY CHOLESTEROL LEVELS (mg.per cent) AND AVERAGE WEEKLY WEIGHT GAIN (in lbs.) OF 42 NORMAL PRIMIGRAVIDAE.

Patient No.	Age	Weeks 13-16	Weeks 17-20	Weeks 21-24	Weeks 25-28	Weeks 29-32	Weeks 33-36	Weeks 37-38	Week 39	Labour	Post-Natal 5-7 days	Post-Natal 5-7 weeks	Weekly weight gain
46	23						231	186	166	-	250	242	1.82
11	19						236	250	-	-	173	220	1.10
17	20						279	267	-	-	278	200	1.04
2	20					202	228	214	-	-	150	159	1.02
9	17					270	218	215	-	228	186	156	0.64
28	17					267	281	207	212	-	240	162	1.0
29	21					236	-	250	186	-	207	190	1.31
33	22					312	232	228	-	270	208	299	0.33
1	20				250	284	260	243	313	-	-	266	1.85
5	21				210	212	200	186	-	-	171	-	1.46
16	23				242	243	159	218	-	-	258	232	1.26
25	19				164	243	193	303	-	208	207	175	1.00
34	27				256	240	243	-	-	228	204	162	1.25
35	23				284	240	256	266	-	-	258	248	1.40
40	23				212	200	206	238	-	-	260	219	0.66
8	27			284	288	232	225	257	-	-	193	262	1.63
26	20			250	312	307	343	-	-	320	228	219	1.42
42	20			262	268	346	268	-	348	-	358	242	1.00
47	23			175	-	260	179	-	184	236	156	235	1.25
6	29		173	172	161	173	186	174	-	179	181	224	0.81
12	21		186	194	188	217	-	219	-	255	250	225	1.70
14	17		204	231	199	313	207	276	-	275	250	258	1.38
21	25		224	212	179	272	308	-	297	-	237	235	1.33
30	27		343	288	268	250	273	-	300	333	250	250	0.95
32	22		255	216	250	276	328	-	274	-	218	273	0.90
39	20		184	186	210	224	-	-	255	-	181	208	1.15
50	27		240	221	234	265	300	-	250	314	312	358	0.75
52	20		253	306	330	367	394	-	328	-	320	328	1.14
53	18		157	188	219	208	236	279	228	-	221	-	2.00
54	25		201	250	267	264	250	250	-	-	184	201	0.76
7	24	186	198	192	229	224	301	291	225	250	272	228	1.05
15	21	219	207	288	243	-	319	296	345	340	310	265	1.00
18	21	143	142	125	212	183	208	-	162	212	228	169	1.24
19	22	185	189	172	257	226	243	-	268	288	220	221	1.05
31	21	226	216	214	250	335	285	275	-	-	301	236	1.20
36	19	170	139	168	158	-	158	165	154	194	235	190	1.20
37	23	218	198	235	258	275	255	-	250	-	338	279	0.75
38	20	205	198	210	232	268	269	294	250	-	250	258	0.75
43	26	156	147	192	194	234	183	235	204	209	235	170	1.40
44	26	174	237	250	-	237	280	298	280	310	280	264	0.90
48	20	219	200	296	207	278	256	336	268	258	312	260	0.76
51	27	197	-	235	308	306	271	271	250	-	287	-	1.26
Mean	22	191	206	218	229	252	243	250	246	256	239	228	1.10

APPENDIX II. MONTHLY BETA:ALPHA LIPOPROTEIN RATIOS OF 42 NORMAL PRIMIGRAVIDAE AND THE BABIES' BIRTH WEIGHTS (in pounds and ounces)

Patient No.	Weeks 13-16	Weeks 17-20	Weeks 21-24	Weeks 25-28	Weeks 29-32	Weeks 33-36	Weeks 37-38	Week 39	Labour	Post-natal 5-7 days	Post-natal 5-7 Weeks	Babies' weights Lbs.	Babies' weights Ozs.
46						2.00	2.31	1.40		1.56	1.47	8	5
11												6	6
17						1.50	1.80			2.20	1.77	6	5
2					1.25	1.33	2.40			1.49	1.55	6	14
9					2.36	2.70	2.87		1.94	1.77	1.20	7	11
28						1.87	2.08	1.93		2.10	1.15	7	1
29					1.73		1.85	1.32		2.39	1.50	6	11
33					1.64	1.52	2.42		2.48	2.35	2.28	6	7
1					2.35	2.62	2.27	1.64			2.21	8	4
5						1.29	1.45			1.32		6	4
16					1.45	1.45	1.85			1.56	1.37	7	9
25				1.38	1.82	1.40	1.14		1.93	1.50	1.41	6	14
34				1.35	1.41	1.56			1.53	1.10	0.94	7	6
35				1.16	1.54	2.16	1.93			1.94	1.98	6	1
40				1.08	1.73	2.18	1.88			1.77	1.32	7	9
8			1.64	1.76		1.87	1.85			2.02	1.76	6	11
26				2.29		2.75			2.82	1.92	1.59	7	10
42			2.03	1.80	2.38	1.87		1.80		1.95	1.32	6	13
47			1.39		0.90	1.30		1.41	1.34	1.36	1.27	5	12
6			1.14	0.85	1.25	1.04	1.15		0.97	1.10	0.80	7	11
12		1.40	1.16		1.31		1.31		1.68	1.34	1.32	7	6
14			1.20	1.38	1.63	2.14	2.50		1.84	1.80	2.16	8	0
21		0.93	1.37	1.28	1.22	0.98		1.03		1.29	1.08	7	8
30		1.84	1.27	1.96	1.65	2.16		1.52	2.36	1.26	1.93	8	3
32		1.56		1.37	2.30	2.14		1.31		1.44	1.44	8	1
39			1.11		1.43			1.09		1.28	1.03	7	12
50		1.48	1.34	1.63	1.50	1.57		1.62	1.39	1.76	1.65	5	15
52		0.89	1.35	2.06	2.12	2.00		2.56		2.24	1.96	7	3
53		1.08	0.97	0.79	0.90	1.10	1.31	1.36		1.54	1.50	7	9
54		0.79	0.77	1.05	1.45	1.25	1.22			1.14	1.05	6	15
7	1.02	1.03	1.44	1.31	1.30	1.28	1.60	1.63	1.75	2.24	0.95	8	2
15	1.30	1.23		1.72		1.92	1.60	1.52	1.50	1.66	1.28	7	0
18		0.62	0.66	1.27	1.16	1.62		1.00	1.17	0.98	1.30	7	0
19	1.20	1.45	0.78	1.26	2.00	2.06		1.30	1.96	1.72	1.00	7	4
31	1.14	1.26	1.18	1.90	1.98	2.11	2.30			1.40	1.16	7	12
36	1.06	1.19	1.05	1.00		1.75	1.85	1.12	1.36	1.15	1.23	8	7
37	0.95	1.24	1.12		1.13	1.06		1.16		1.24	1.00	8	15
38	1.11	1.19	2.01	1.81	1.54	1.75	1.84	1.45		0.97	1.31	6	14
43	0.95	0.93	1.26	1.66	1.72	1.15	1.26	1.44	2.00	1.32	0.78	8	7
44	1.60	1.47	1.13		1.38	1.55	1.52	1.75	1.39	1.50	1.46	7	14
48	1.10	1.28	1.25	1.18	1.65	1.21	1.60	1.58	1.28	1.88	1.54	6	4
51	0.75		0.91	1.03	0.94	1.01	1.00	1.39		1.08		6	10
Mean	1.14	1.20	1.26	1.44	1.62	1.72	1.85	1.43	1.70	1.59	1.37		

APPENDIX III. ULCER GROUP

Case	Age	Hb.	B.P.	Exp. wt. (lbs)	Wt. (lbs)	Beta/ alpha	Prebeta/ beta	E.C.G.	Remarks
1	51	103		139	128	1.73	0.14	-	
2	49	85		164	171	1.60		-	
3	54	88		140	119	0.94		-	
4	55	100	200/100	157	130	1.84	0.15	Lat.ischaemia or left vent.strain	Pulmonary T.B. 1948-55
5	47	94		146	136	0.79		-	
6	48	101		155	157	0.71		Posterior ischaemia after exercise	
7	51	101		174	166	1.38	0.10	-	Tinge of cyanosis Exertional dyspnoea
8	61	98		162	157	1.20	0.12	Lat.ischaemia after exercise	
9	55	97		140	142	0.91	0.10	Post.ischaemia after exercise	
10	62	98	140/95	172	175	1.06	0.12	-	
11	58	80		149	112	1.85	0.14	-	Praecordial pain 1 mth.before test
17	47	112		155	117	1.32	0.14	Post.ischaemia after exercise	Mild angina
21	50	94		165	176	2.43	0.10	-	
23	53	100		144	110	0.96		Post.ischaemia after exercise	Exertional dyspnoea
24	58	91	160/120	157	146	1.52	0.16	Left vent.strain and/or ant.lat. ischaemia	
25	56	100		140	138	2.88	0.24	Ant.ischaemia after exercise	
26	56	90	200/100	181	142	1.42	0.13	Post.ischaemia after exercise	Dyspnoea and oedema Apex beat displaced to left
27	58	96		153	118	0.59		-	
28	63	91	180/110	167	169	2.50	0.19	Left B.B.B. Antero-lateral ischaemia after exercise	
29	49	100		147	132	1.24		-	
37	64	106		140	150	1.55	0.09	Lateral ischaemia after exercise	Test abandoned because of dyspnoea
38	52	100		170	178	1.17		Post.ischaemia after exercise	
39	58	97		153	144	1.61		-	
40	55	100	210/120	140	135	1.27		Post.ischaemia after exercise	Cardiac enlargement Exertional dyspnoea
54	53	115	170/95	161	169	5.02	0.17	Lat.ischaemia after exercise	
55	56	97		181	158	2.12	0.10	Post.lat.ischaemia after exercise	
56	47	103		168	202	2.95	0.14	-	
57	54	97		140	155	1.87	0.12	Healed posterior myocard.infarction	Myocardial infarction in 1949 & 1957. Not exercised
59	54	97		140	141	2.59		-	
65	63	112		162	189	2.63	0.10	-	
66	54	97		166	156	1.60	0.07	-	
67	51	97		156	166	1.04		-	
69	52	100	160/95	152	152	1.67	0.12	-	
75	52	100		152	126	2.01	0.16	Post.lat.ischaemia after exercise	
104	48	100		159	154	1.10		-	
105	51	91	155/90	144	130	4.25	0.09	-	
106	47	88		163	168	1.38		Post.ischaemia after exercise	
107	52	97	160/80	149	126	1.78		Post.ischaemia after exercise	
108	49	97		143	130	1.13		-	
151	47	97	150/100	138	122	1.59	0.09	Post.ischaemia after exercise	
Av.	53.5	97.7			147.5	1.73			

APPENDIX III. GASTRECTOMY GROUP

Case	Age	Hb.	B.P.	Exp. wt. (lbs)	wt. (lbs)	Beta/ alpha	Prebeta/ beta	E.C.G.	Remarks
12	56	91		157	114	1.65		-	
13	53	85		144	128	1.43		-	
14	51	91		170	164	1.13		-	
15	45	82		158	133	1.12		-	
16	47	100	145/100	173	140	2.46	0.11	-	
18	58	97		149	123	1.35	0.10	Occasional vent.extrasystoles	Exertional dyspnoea. Slight ankle swelling.
19	56	94	140/90	140	138	1.35	0.11	-	
20	49	97		139	128	1.72	0.11	-	
22	53	88		166	126	1.16		Post.Ischaemia after exercise	
30	51	77	150/100	139	134	1.89	0.11	-	
31	58	91		153	128	1.26		-	
32	52	91		165	130	1.14		-	Slight exertional dyspnoea.
33	51	88		170	151	3.16	0.08	-	
34	49	63		155	142	1.28	0.16	-	
35	64	79	160/85	145	118	1.36		Vent.extrasystoles.	
36	49	91		155	121	1.07		-	
41	55	103		153	110	1.09		Vent.extrasystoles after exercise.	
42	54	85	160/90	140	118	1.09		Normal.	Angina during test.
43	50	77		160	142	1.32	0.19	-	
44	52	68		149	136	0.56		-	
45	56	94		176	161	1.78		Post.ischaemia after exercise.	
46	48	94		138	102	2.10		-	
47	51	80		188	165	1.04		Post.ischaemia after exercise.	
48	52	59		156	130	0.92		-	
49	50	85		148	126	2.02		? Low volt. of emphysema.	Exertional dyspnoea. Slight ankle swelling.
50	63	97		162	138	2.64	0.23	-	
51	62	94		140	111	1.11		-	
52	49	91		183	152	1.37		-	
53	50	94		139	122	1.62	0.23	-	Exertional dyspnoea. Occasional angina.
60	46	91		154	136	3.95	0.17	-	
61	47	103		159	154	2.52		-	
62	45	88	150/90	162	138	1.60		-	
63	65	82		149	103	1.44		-	
64	48	91	180/120	155	142	1.17		Frequent V.E.S.after exercise.	Angina during test.
68	50	97		139	116	2.36	0.13	-	Exertional dyspnoea.
70	49	85		139	130	0.94		-	Effort angina.
96	63	94		157	133	1.50		-	
97	65	91		162	168	1.86	0.17	Post.lat.ischaemia after exercise.	
98	57	100		157	108	1.80		-	
99	57	91		149	122	1.34	0.13	-	Exertional dyspnoea.
Av.	53.2	88.2			132	1.59			

APPENDIX III. CONTROL GROUP

Case	Age	Hb.	B.P.	Exp. wt. (lbs)	Wt. (lbs)	Beta/ alpha	Prebeta/ beta	E.C.G.	Remarks
71	54	100	140/90	171	165	1.12	0.10	Postero-lat.ischaemia after exercise	Patients in this group were selected because they had no symptoms attributable to cardiovascular disease.
72	53	77	160/100	171	168	2.23	0.11	-	
73	61	115	140/100	162	166	2.38	0.15	-	
74	52	97	140/100	149	172	1.37	0.11	Postero-lat.ischaemia after exercise	
76	52	100		156	160	1.00		-	
77	64	91	170/125	176	160	1.68	0.10		
78	56	85		153	156	1.92	0.17		
80	52	97		156	140	2.82	0.13		
81	53	94		171	172	1.82	0.18		
82	60	77		145	127	1.46		Posterior ischaemia after exercise	
83	60	82	145/90	145	114	1.26		Postero-lat.ischaemia after exercise	
84	48	106		143	170	1.35	0.08	Anterior ischaemia and/or left vent. strain	
85	52	94		175	160	1.27		Post.ischaemia after exercise	
86	47	94		150	168	1.95	0.15	Postero-lat.ischaemia after exercise	
87	59	97		153	165	1.52		-	
88	56	99		153	135	1.33		-	
89	56	94		176	140	0.97		-	
90	47	103		150	161	1.93	0.12	Posterior ischaemia after exercise	
94	57	109	150/90	162	176	2.70	0.13	-	
95	56	97		166	151	2.46		-	
100	53	91		161	144	2.42	0.15	Posterior ischaemia after exercise	
101	60	91		176	171	1.46	0.14	Posterior ischaemia after exercise	
102	47	88		146	138	0.93		-	
109	60	91	160/90	149	162	1.26		-	
110	50	91	180/130	156	161	2.08		-	
111	48	103	140/90	143	138	1.49		Postero-lateral ischaemia after exercise	
112	57	88		167	178	1.99	0.17		
113	55	106	150/90	176	180	0.82		Right ventricular strain pattern	
114	47	91		138	138	1.96	0.13	Posterior ischaemia after exercise	
115	46	97		146	140	1.58		Posterior ischaemia after exercise	
116	52	97		152	115	0.90		-	
117	59	97	190/90	145	116	1.02		Left ventricular strain pattern	
118	49	80	150/80	155	146	1.58			
119	51	94		139	136	2.28		-	
120	49	112	260/145	160	208	2.17	0.11	-	
121	57	100		153	149	1.21	0.09		
122	53	106		144	140	2.83	0.10	Posterior ischaemia after exercise	
123	48	103	140/100	173	189	2.54		-	
124	54	118	160/100	144	138	1.78		Posterior ischaemia after exercise	
130	54	103	200/110	161	154	1.20		-	
Av.	53.6	96.7			154	1.70			