SOME FACTORS INFLUENCING SERUM TRIGLYCERIDE IN MAN

A T H E S I S

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CONTENTS

PART II

EPIDEMIOLOGICAL FACTORS ASSOCIATED WITH SERUM TRIGLYCERIDE

PART III

THE RELATIONSHIP BETWEEN DIETARY CARBOHYDRATE AND SERUM LIPIDS IN MAN

PREFACE

For the past two years I have been privileged to have been a member of the University of Cape Town Clinical Nutrition Research Unit, headed by Professor J.F. Brock. During these years, in which all the work described in this thesis was carried out, I worked under the direction of Dr. A.S. Truswell who was appointed by the University as the Supervisor of my thesis. He has been not only a constant supervisor but also a friend during this period and for both his supervision and friendship, I am grateful.

Part I of this thesis deals with general methodology and the experimental work can be clearly divided into two sections. Part II deals with studies carried out chiefly to determine further the epidemiological factors influencing serum lipid (and in particular, serum triglyceride) levels in the population groups of Southern Africa. The original objectives are described on page 70 and the main conclusions summarised on page 114.

Part III describes three studies which were conducted in an attempt to fill some of the gaps in the considerable literature on the relationship between dietary carbohydrate and serum lipids in man. Both in the review of the literature at the beginning of this section and in the interpretation of the results of each of the studies, discussion has been chiefly limited to experiments conducted in man. There is a great deal of information available on studies carried out in experimental animals which show marked species differences from man with regards kinetic behaviour of serum and liver triglycerides[1]. Where relevant, of course, reference has been made to these studies. The significance of each of the three studies has been discussed separately, but the principal objectives are mentioned on page 138 and the general conclusions are summarised on page 205.

(1) Nikkilä, E.A. 1969
Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin.
Adv. Lipid Res. 7: 65.

ABBREVIATIONS

Certain abbreviations will be used throughout this study:

cm	-	centimetre(s)
g	-	gram(s)
kCal	-	kilocalorie(s)
kg	-	kilogram(s)
mg	-	milligram(s)
ml	-	millilitre(s)
rpm	-	revolutions per minute
µg	-	microgram(s)
GLC	-	gas-liquid chromatography

Nomenclature of Fatty Acids

The classification proposed by Dole et al[1] has been adopted. The fatty acids will, in general, be designated by a number referable to the number of carbon atoms in the molecule. This will be followed by a colon and the number of double bonds in the molecule, also indicated by a number. Thus myristic acid is C14:0, palmitic acid is C16:0, palmitoleic acid is C16:1, stearic acid is C18:0, oleic acid is C18:1, linoleic acid is C18:2, linolenic acid is C18:3 and arachidonic acid is C20:4.

Certain statistical abbreviations used are defined in the chapter on statistical methods.

(1) Dole, V.P., A.T. James, J.P.W. Webb, M.A. Rizack and M.F. Struman, 1959 The fatty acid patterns of plasma lipids during alimentary lipaemia. J.Clin.Invest. 38: 1544.

PART I

GENERAL METHODOLOGY

CHAPTER I - LABORATORY TECHNIQUES

The concentration of the lipid components of the serum (cholesterol, triglyceride and phospholipid) were determined chemically. Duplicate determinations were performed on all samples and the result for each lipid component expressed as mg/100ml of serum. A semi-quantitative estimation of serum lipoprotein distribution was obtained by electrophoresis on filter paper. The fatty acid pattern of the serum triglyceride was examined by the technique of gas-liquid chromatography, after separation of the lipid fractions by thin-layer chromatography. Blood sugar and serum insulin concentrations were determined in two of the investigations conducted. The calorific value and total nitrogen content of certain dietary constituents were determined. Each of these laboratory techniques will be considered in some detail.

(A) SERUM TRIGLYCERIDE DETERMINATION

The serum triglyceride level was determined by the method of Young and Eastman[32]. This is a local modification of the Van Handel and Zilversmit method[29] and was devised as doucil, the substance used for the removal of phospholipid was not readily available in South Africa.

1 ml of serum was extracted in 2 : 1 chloroform : methanol, according to the method of Folch et al[8] and brought to a final volume of 25 ml. 2 ml aliquots of this extract were pipetted in duplicate and all traces of solvent removed by evaporation in a water bath at a low temperature. The phospholipid was then removed by adsorption on silicic acid as follows. About 320 mg of silicic acid (Mallinckrodt silicic acid, 100 mesh, suitable for chromatographic analysis, sieved to yield 72-350 mesh, dried overnight, and stored in an air-tight bottle) was added to each tube, followed by 5 ml of chloroform from a pipette. The tubes were vortexed for about 1 minute and then centrifuged at 3000 rpm for 1 minute. A 3 ml aliquot of the supernatant was pipetted into a test-tube fitted with a

ground glass joint, care being taken to avoid disturbing the silicic acid, with its adosrbed phospholipid. A chloroform blank and triolein standards were included from this point. Glycerol trioleate obtained from British Drug Houses as recommended by Young and Eastman was used as standard. 100 mg of this triolein was diluted to 100 ml in a volumetric flask with chloroform. 5 ml of this stock solution was diluted to 200 ml with chloroform, giving a working solution with a concentration of 25 µg/ml. 1, 2, 3 and 4 ml aliquots pipetted in duplicate, were run as standards in each triglyceride run together with a chloroform blank. The chloroform was evaporated in a water bath at a low temperature and 1 ml absolute ethanol and one drop 5% potassium hydroxide added to each tube. The tubes were stoppered and the mixture saponified for 30 minutes at 60°C. Four drops of 6% acetic acid in methanol were then added and the mixture evaporated to dryness in a water bath. 1 ml of 0.67 molar sulphuric acid was pipetted into each tube. The glycerol formed was then oxidised to formaldehyde and formic acid by the addition of 0.3 ml of 0.02 molar sodium metaperiodate. The iodate was reduced by the addition of 0.3 ml of 0.2 molar sodium arsenite after the reaction was completed. Five minutes later 8.4 ml of chromatropic acid reagent was added. The chromatropic acid reagent was prepared by mixing 66% sulphuric acid with chromatropic acid (1 g chromatropic acid was dissolved in 100 ml distilled water and the solution filtered) in the proportion 9 : 2. The tubes were stoppered, shaken and kept in a boiling water bath for 30 minutes. After cooling the optical density was read at 570 mµ in a Zeiss spectrophotometer. A standard curve (optical density versus concentration) was then plotted and the triolein concentration in micrograms in an unknown sample, was read off the graph. The results were then expressed as mg triolein/100ml serum and calculated by the following formula:-

$$\text{mg triolein/100ml serum} = \mu\text{g triolein} \times \frac{5}{3} \times \frac{25}{2} \times \frac{100}{1} \times \frac{1}{1000}.$$

As the colour reaction estimates glyceride glycerol, this method in fact measures tri-, di- and monoglycerides but since the latter two components are present

in serum in only small amounts[5], the term triglyceride is used to describe the results of this procedure.

In principle this method differs from that of Van Handel and Zilversmit in only one respect. These authors describe extraction in chloroform and simultaneous removal of phospholipid by adsorption on doucil; whereas in the Young and Eastman method extraction is carried out in 2 : 1 chloroform : methanol and phospholipid removed subsequently by adsorption on silicic acid. The unsaponified blank which van Handel and Zilversmit originally described was found to give the same values as the reagent blank and we therefore dispensed with it. Zilversmit too, no longer determines this unsaponified blank[34]. It is, in principle, also similar to the method described by Carlson[4].

I considered it necessary to establish that in my hands complete extraction of triglyceride could be achieved without dissolving the serum phospholipid. To investigate this problem, I^{131} labelled triolein was emulsified with a small amount of phosphotide and added to serum[29]. An aliquot of the serum was extracted and treated in the usual way. In three experiments between 97 and 98% of the added I^{131} triglyceride was recovered in the chloroform supernatant, after the silicic acid stage of the experiment. Chemical analysis for phospholipid phosphorus showed that no measurable amount of phospholipid was present in the chloroform supernatant at this stage.

In order to check the precision of the method, a large pooled serum extract was prepared and duplicate 2 ml aliquots included in each run as an internal standard. Two such pooled serum extracts were used during the eighteen month period in which all the triglyceride estimations described in this study were carried out. There was an overlap period of one week while the one extract was standardised against the next. The first extract was run from 26th February to 14th July 1969 and the second from 9th July 1969 to 15th June 1970. The standard error of a single determination and the coefficient of variation for each of the two pooled serum extracts were determined and the values shown in Table I.

TABLE I

	Number of determinations	Mean value mg/100ml	Range	S.E. of a single determination	C.V. %
1st extract	33	110	101-119	4.34	3.95
2nd extract	51	72	65-79	6.60	9.17

The concentration of serum triglyceride (mg/100ml serum) as determined on aliquots of the two pooled serum extracts used as the internal standards. The range of values, standard error (S.E.) of a single determination and coefficient of variation (C.V.) expressed as a percentage, are indicated

I. I.

In view of the fact that the determinations on the two pooled serum extracts were carried out during the course of five and eleven months respectively, the degree of reproducibility was considered to be satisfactory.

The coefficient of variation as calculated above does not, however, provide any indication of the degree of reproducibility of the extraction procedure. This was therefore determined as follows. Two extracts of the same serum sample were prepared and analysis performed on each extract. Twenty serum samples were treated in this way covering a range of serum triglyceride levels from 59-304 mg/100ml. The mean difference between duplicate analyses expressed as a percentage of mean triglyceride vale was 3.78% (range 0-10.13; S.E.M. 0.68). The mean difference between duplicate analyses of the same serum extract expressed in a similar way was 2.14% (range 0-5.02; S.E.M. 0.32). In such cases, when the difference was greater than 5%, analysis was repeated.

Throughout this study the stock solution of triolein was prepared from the same bottle of glycerol trioleate. It was, however, considered necessary to check the purity of the standard in order to compare the results of the surveys described in this study with those obtained by other workers who had used pure glycerol trioleate and tripalmitate as standards. When analysed by gas-liquid chromatography,

the triolein (supplied by British Drug Houses) was found to consist of 74.2% oleic acid, whereas triolein (obtained from Applied Science Laboratory in the United States of America) contained 100% oleic acid, Figure 1 shows fatty acid patterns of the two standards and Table II, the percentage of fatty acids present in each of them.

TABLE II

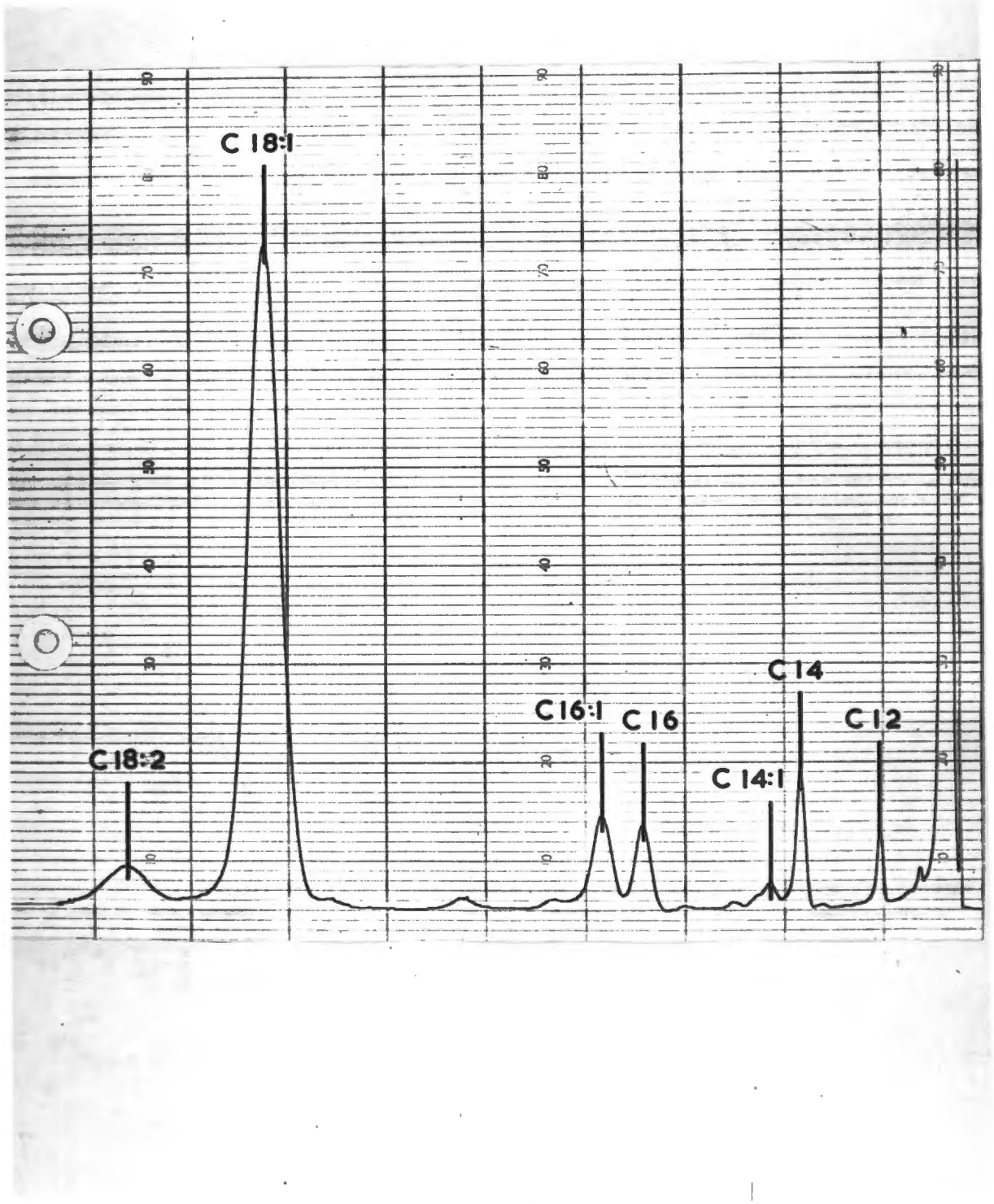
	Percentage of Fatty Acid Present						
	C12: 0	C14 : 0	C14: 1	C16: 0	C16: 1	C18: 1	C18: 2
BDH Triolein	1.7	4.2	1.6	4.7	6.3	74.2	7.3
Pure Triolein	-	-	-	-	-	100	-

Percentage of fatty acids present in triolein standard obtained from British Drug Houses (B.D.H.) and Applied Science Laboratory (pure triolein).

I. II.

The two standards were also compared by separating the different lipid fractions on thin-layer chromatography plates (See Figure 8). The pure triolein is seen to contain only a triglyceride fraction whereas the impure triolein in addition to triglyceride contains free fatty acid, 1,3-diglyceride, 1,2-diglyceride, monoglyceride and a small amount of an unidentified component.

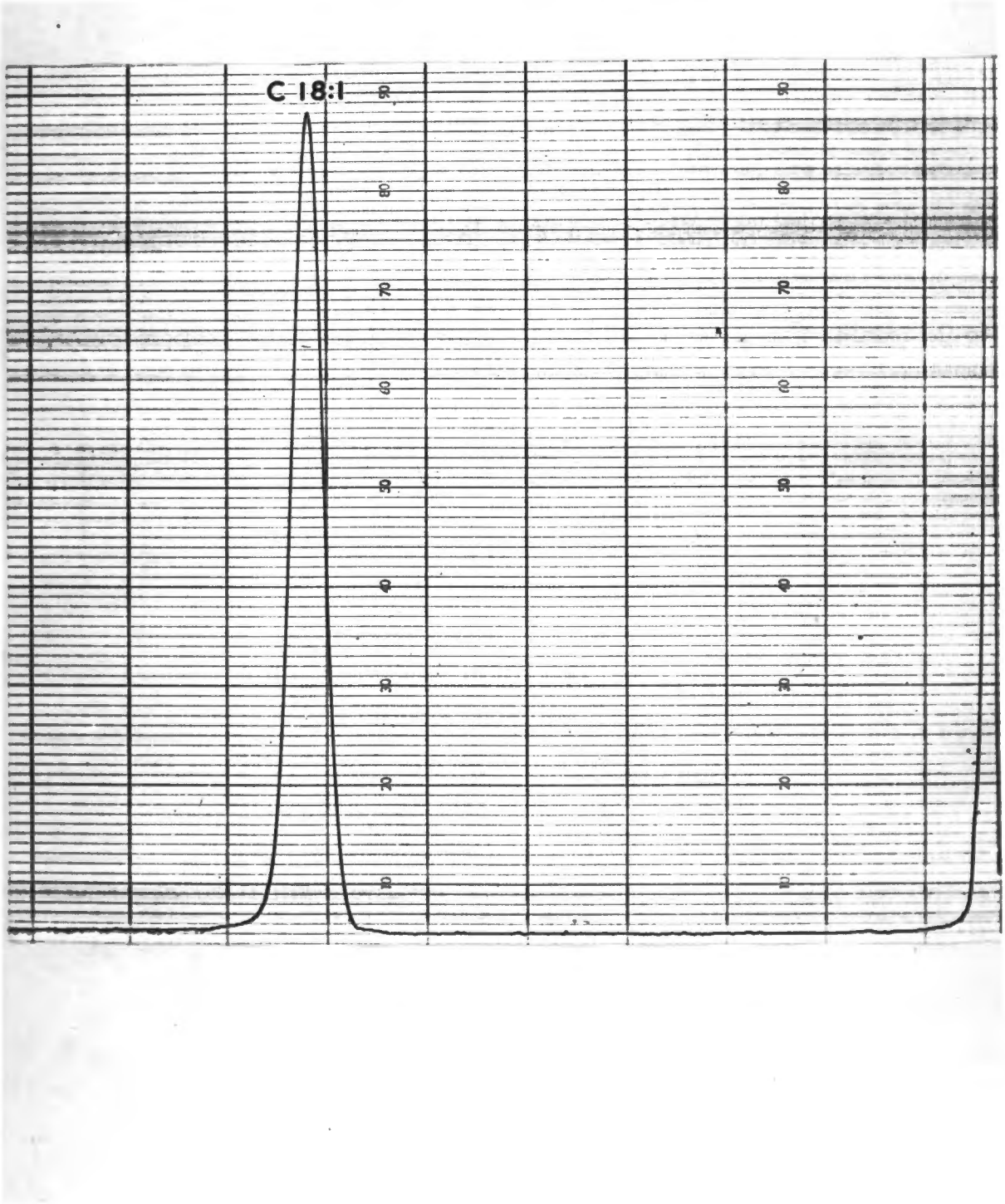
In order to obtain a correction factor whereby the triglyceride results obtained in this study could be expressed in terms of pure triolein, a standard solution of the pure triolein was prepared and the standard curves of the pure and impure standards compared. This was done on three occasions and the correction factor was found to be 1.20, 1.19 and 1.21 on each of these occasions. The factor 1.20 was therefore employed to express our results in terms of pure triolein. As the molecular weights of triolein and tripalmitate are 885 and 807 respectively,



(a)

Figure 1: Gas-liquid chromatographic analysis of the triolein standard supplied by (a) British Drug House and
(b) Applied Science Laboratory.

I. 1.



(b)

it was calculated that the factor to convert to tripalmitin is 0.91. An almost identical value was obtained when plotting a standard curve with readings obtained from pure tripalmitin standard, which was also obtained from the Applied Science Laboratory. An illustration of the three standard curves described is shown in Figure 2.

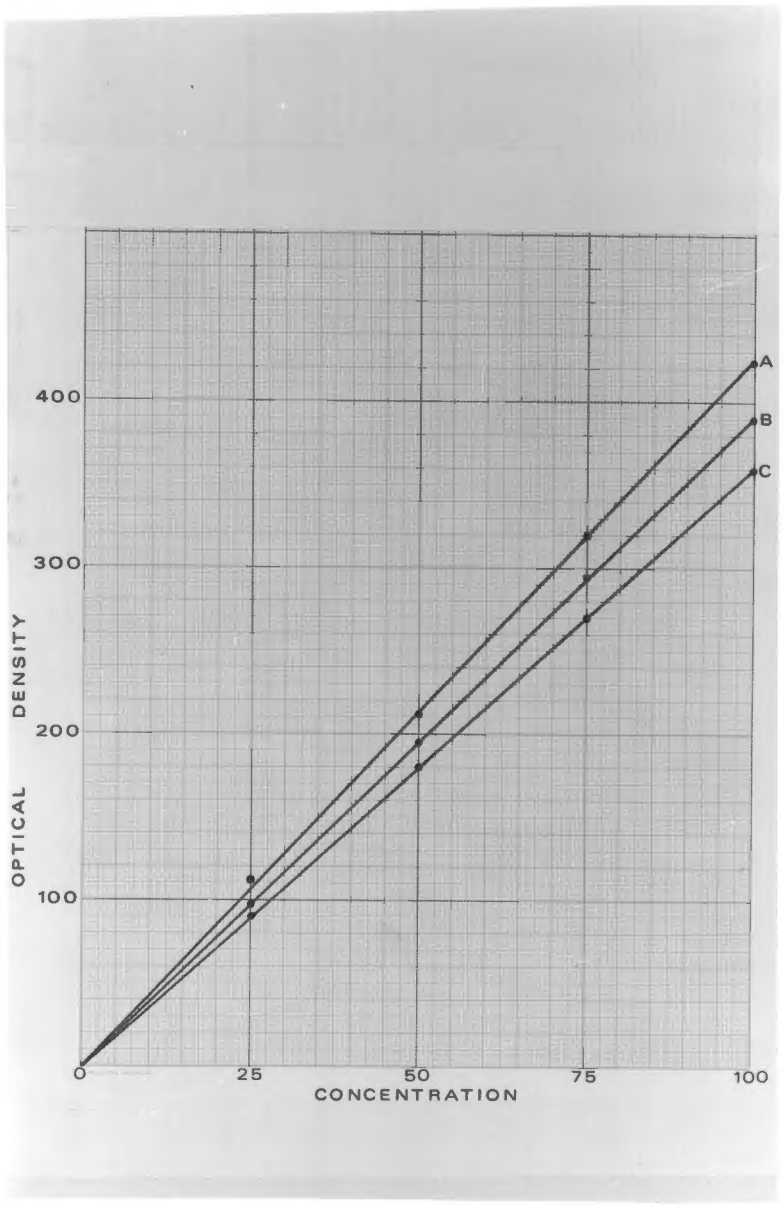


Figure 2: The standard curves (optical density at 570mμ versus concentration in μg triolein and tripalmitin) of
(a) British Drug Houses triolein
(b) Pure tripalmitin and
(c) Pure triolein.

(B) SERUM CHOLESTEROL DETERMINATION

The serum cholesterol was determined by the method of Abell et al[1]. This method measures total serum cholesterol in mg/100ml. 0.1 ml samples of serum were pipetted in duplicate (using a micro-pipette) into test-tubes fitted with ground glass joints. 2 ml of the standard cholesterol solution was also pipetted in duplicate. The standard cholesterol solution was prepared by dissolving 0.0600 grams of pure dry cholesterol (recrystallised four times from absolute alcohol) in absolute alcohol and diluted to 500 ml. 2 ml of alcoholic potassium hydroxide (3 ml 33% aqueous potassium hydroxide and 47 ml absolute alcohol) were added to each of the tubes containing serum. 0.12 ml 33% potassium hydroxide was added to the standard tubes. A reagent blank was included and contained 2 ml of alcoholic potassium hydroxide. An internal standard serum was included in duplicate in each run. The tubes were then stoppered and saponified in a water bath at 37°C for at least two hours. This liberated the cholesterol from lipoprotein complexes and saponified the cholesterol esters.

The cholesterol was extracted into 4 ml of petroleum ether (boiling point 60-80°C), after 2 ml of distilled water had been added to dilute the alcoholic solution, by stoppering the tubes and vortexing them for 30 seconds. After the layers had separated the upperlayer (petroleum ether) was transferred to a 50 ml conical flask. The extraction was repeated a second time. To each flask of extract one drop of 25% acetic acid in petroleum ether was added. The flasks were placed on a hot plate and allowed to evaporate to dryness. The cholesterol was then measured by means of the Liebermann-Burchard colour reaction.

The Liebermann-Burchard reagent (20 volumes acetic anhydride, 1 volume sulphuric acid and 10 volumes glacial acetic acid) was prepared and 6 ml added to each of the flasks. The flasks were placed in a water bath at 25-28°C. Fifteen minutes after the reagent had been added to the first flask, the optical density of each sample was read against the blank, in a photoelectric colorimeter at 620 mμ. The cholesterol value in mg/100ml serum was calculated using the

value of the standard as 240 mg/100ml.

This method is internationally used and direct comparisons may therefore be made with the results obtained by the workers in many parts of the world. The method is an extremely precise one. The precision was checked by the inclusion of duplicate aliquots of a pooled serum sample in each run. Five such pooled samples were used as internal standards in the period during which the cholesterol estimations described in this study were performed. There was always an overlap period while the one internal standard was standardised against the next. The first was used from 3rd April to 10th June 1969, the second from 2nd June to 19th August 1969, the third from 12th August to 28th November 1969 and the fourth from 22nd November 1969 to 4th April 1970 and the fifth from 1st April to 29th July 1970.

The standard error of a single determination and the coefficient of variation, expressed as a percentage, for each of these five pooled sera are shown in Table III.

TABLE III

	Number of determinations	Mean value (mg/100ml)	Range	S.E. of a single determination	C.V. %
1st pooled serum	24	211	206-216	2.93	1.39
2nd pooled serum	22	204	199-210	2.50	1.23
3rd pooled serum	34	111	104-116	2.71	2.44
4th pooled serum	12	209	205-212	2.20	1.05
5th pooled serum	16	230	226-235	2.62	1.14

The concentration of serum cholesterol (mg/100ml serum) as determined on duplicate serum samples of the five pooled serum samples used as internal standards. The range of values, standard error (S.E.) of a single determination and coefficient of variation (C.V.) expressed as a percentage are shown.

The purity of the cholesterol standard on thin-layer chromatography is shown in Figures 7 and 9.

(C) SERUM PHOSPHOLIPID DETERMINATION

Phospholipid was determined by the method of Fiske and Subbarow[7], modified for micro-analysis by Bartlett[2]. The standard solution was prepared according to the method of Marinetti et al[19]. The results were obtained as milligrams of phosphorus and expressed as milligrams of lecithin per 100 ml of serum. (Milligrams lecithin = milligrams phosphorus x 25).

The following data was obtained on the same serum sample which was analysed on 10 occasions -

Mean :-	211 mg/100ml
Range :-	206 - 215 mg/100ml
S.E. of a single determination:-	3.81
C.V. (%) :-	1.82

(D) SEPARATION OF LIPOPROTEIN SPECIES BY PAPER ELECTROPHORESIS IN ALBUMIN CONTAINING BUFFER

Lipoprotein species were separated by paper electrophoresis according to the method of Lees and Hatch[16]. Forty microlitres of serum was applied to the paper strip (Beckman No. 320046) and electrophoresis was carried out at room temperature in a Durrum hanging strip cell for 16 hours at 110 volts with a current of approximately 7/8 of a milli-amp per strip. Barbital buffer of ionic strength 0.1 and pH 8.6, containing 0.001 molar E.D.T.A. and 1% human serum albumin was used. After electrophoresis the strips were dried in an oven at 120°C for one half hour and were then stained with oil-red O at 35°C for 18 hours according to the method of Jencks and Durrum[15].

The addition of albumin to the buffer solution results in a sharper separation of the lipoprotein species. The mobility of the lipoproteins during electrophoresis is not altered from the normal positions relative to other serum proteins in buffer without additive. The high density (alpha) lipoprotein

moves between albumin and alpha-1 globulin while the low density (beta) lipoprotein moves with the beta globulin. This is shown in Figure 3.

The very low density (pre-beta) lipoprotein is usually identifiable and sometimes separated from the beta-lipoprotein in serum samples which contain appreciable quantities of this lipoprotein fraction. Chylomicrons if present in the serum sample, remain at the origin. This is shown in Figure 4.

Lees and Hatch have shown a satisfactory correlation between the density of lipoprotein fractions separated with the ultracentrifuge and their electrophoretic behaviour[16].

Jencks and Durrum[15] found that direct optical scanning of the dry strips was unsatisfactory as the considerable background variation resulted in difficulty in selecting a true baseline, and also because of the non-linearity of the relationship between dye concentration and optical density on paper. They did, however, elute the dye (with a solution of glacial acetic acid, diluted with absolute ethanol) from the sections of the paper strip containing the alpha and beta lipoprotein bands and expressed the quantity of dye taken up by these two lipoprotein fractions in terms of optical density which was read in a Beckman Model D.U. spectrophotometer at 520 mμ. The concentration of oil-red O was found to have a linear relationship to optical density up to a reading of 1.3 in this instrument. No attempt was made to convert the results to actual quantity of dye bound because of the heterogeneity of the dye preparation.

It was felt that a quantitative method as described by these authors was of little value in this study as it is not possible to distinguish quantitatively between beta and pre-beta lipoprotein in this way. In this study, this technique was therefore utilised in the following two ways. Firstly, it was used together with serum cholesterol and triglyceride estimations to classify those individuals with hyperlipoproteinaemia, into five different types as described by Fredrickson et al[9]. Type I in which there is a lipoprotein lipase deficiency and consequently impaired chylomicron clearance, is charact-

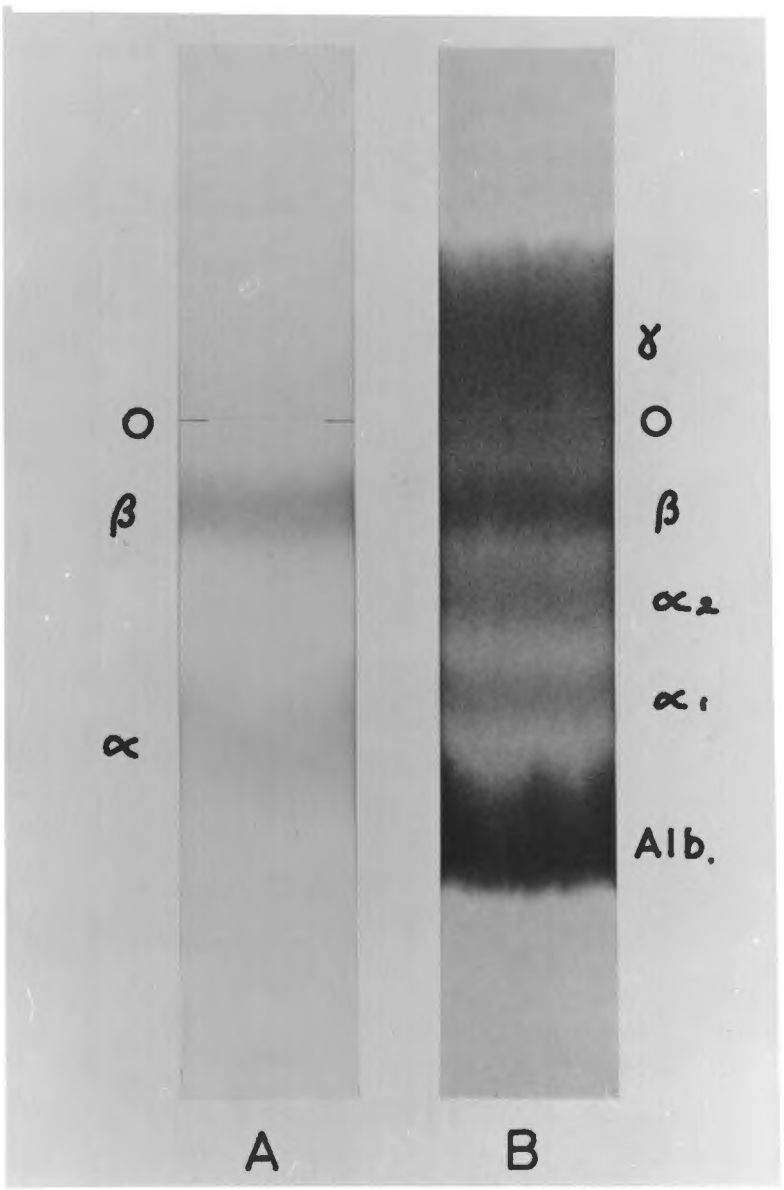


Figure 3: An electrophoretic strip run in buffer containing albumin and stained in oil-red O[(A)] is compared with a strip[(B)] run in buffer without albumin and stained with amido-schwartz. The oil-red O, a lipid stain, stains the lipoproteins and amido-schwartz, the serum protein fractions. The high density (alpha) lipoprotein moves between albumin and alpha-1 globulin while the low density beta lipoprotein moves with the beta globulin.

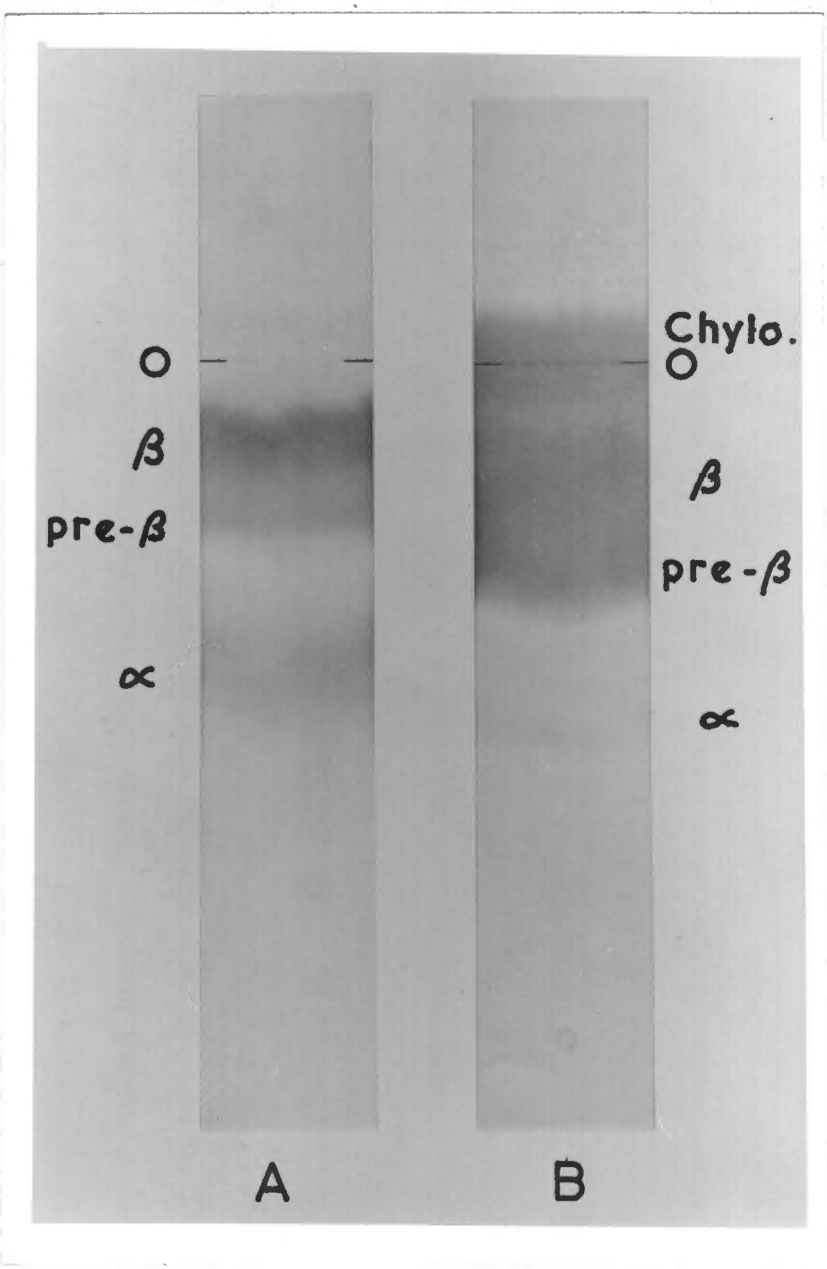


Figure 4: (A) An electrophoretic strip, run in albumin containing buffer stained in oil-red O, illustrating the beta, pre-beta and alpha lipoprotein fractions.

(B) An electrophoretic strip, showing the presence of chylomicrons at the origin (O), and also an appreciable amount of pre-beta lipoprotein.

erised by the presence of chylomicrons, which are present at the origin of the electrophoretic strip in the fasting serum. Fasting serum triglyceride levels are markedly elevated with a normal or only slightly elevated serum cholesterol. In Type II, an elevated serum cholesterol is found and an intensely staining band of beta-lipoprotein is noted on the electrophoretic strip. Some degree of pre-beta lipoproteinaemia may also occur in this type and will be apparent on the electrophoretic strip as a band of pre-beta lipoprotein. This abnormality has been labelled as type IIb for the purposes of this study. Type III is characterised by a "broad beta lipoprotein band" on electrophoresis and is due to the presence in large quantities of an abnormal lipoprotein which has the mobility of a beta-lipoprotein on electrophoresis, but is abnormally laden with triglyceride, the bulk of which is usually found in the pre-beta lipoprotein fraction. Final diagnosis of this type can only be made by identification of the abnormal lipoprotein using the technique of ultracentrifugation. Type IV is characterised by an increase in endogenous triglyceride and the serum pre-beta lipoprotein which is responsible for transporting it. Consequently the pre-beta band lipoprotein on the electrophoretic strip is increased. In type V there is an increase in the endogenous and exogenous triglyceride and consequently electrophoresis on the fasting serum shows a chylomicron band and increased pre-beta lipoprotein band. An example of the electrophoretic strip of each of these different types is shown, together with a normal strip, in Figure 5.

The technique was furthermore used as a semi-quantitative method of expressing serum pre-beta lipoprotein. An arbitrary system was devised by which the pre-beta lipoprotein band on the electrophoretic strip was graded as being absent (0), present in very small amounts (trace) or given a grading of one plus to four plus (+, ++, +++, ++++), according to the width and intensity of the pre-beta band on the paper strip. This grading system is shown in Figure 6. Pre-beta bands which did not conform fairly closely to the above grading were given an intermediate grading (e.g. + ⟶ ++).

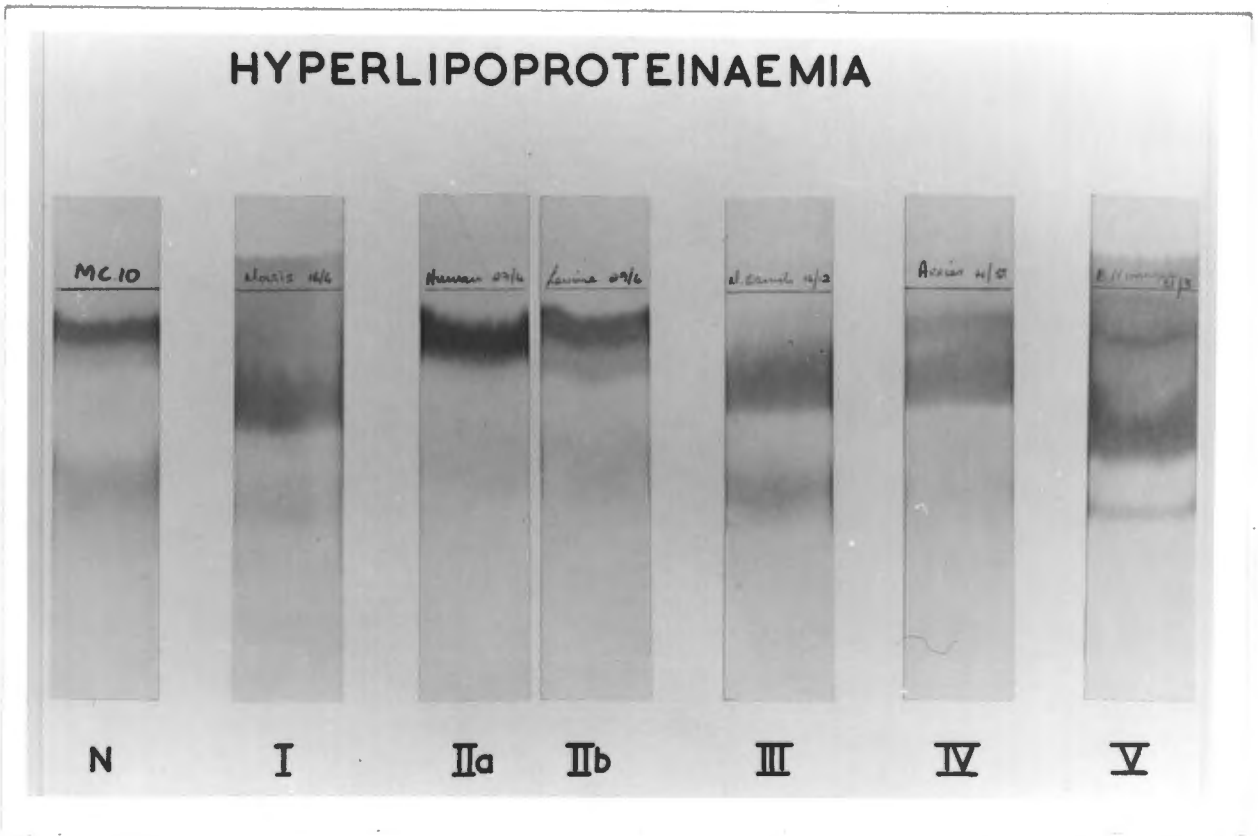


Figure 5: A series of lipoprotein electrophoretic strips, stained in oil-red O, comparing the normal pattern with the five types of hyperlipoproteinaemia as described by Fredrickson. Type II may be associated with some degree of hyperprebetalipoproteinaemia and the two types of strips seen are shown in the figure as a in which no pre-beta lipoprotein is present, and b in which some degree of prebetalipoprotein is present. This latter abnormality has been labelled as Type IIb for the purposes of this study.

As most of the triglyceride present in the serum in the fasting state is transported by the pre-beta lipoprotein[9], a relationship between fasting serum triglyceride and the grade of pre-beta lipoprotein, as expressed above, should exist. This was tested in the following way. Serum triglyceride level and the lipoprotein electrophoretic pattern of 621 fasting serum samples (including many of the samples analysed in this study) were determined. The pre-beta band on the electrophoretic strip was graded before the serum triglyceride level had been estimated. The correlation between the grading of pre-beta lipoprotein and the triglyceride value was found to be highly significant, $p < 0.001$ (correlation coefficient, $r = 0.87$). The mean triglyceride value ($\pm$ S.E.M.) and range of values found in each grading of pre-beta lipoprotein is shown in Table IV.

TABLE IV

	Grading of pre-beta lipoprotein band on electrophoretic strip					
	0	trace	+	++	+++	++++
Mean triglyceride value in mg/100ml	62	80	112	169	276	464
S.D.	15.51	18.82	21.04	44.64	55.24	
S.E.M.	1.68	1.42	1.59	4.58	11.28	
Range	40-89	50-125	63-156	116-292	171-385	
Number	85	240	176	95	24	1

The mean triglyceride value (in mg/100ml), standard error of the mean (S.E.M.) and range of values found in each grading of pre-beta lipoprotein. For the purpose of this table, any intermediate gradings were given the lower grading (e.g. + ⟶ ++, graded as +).

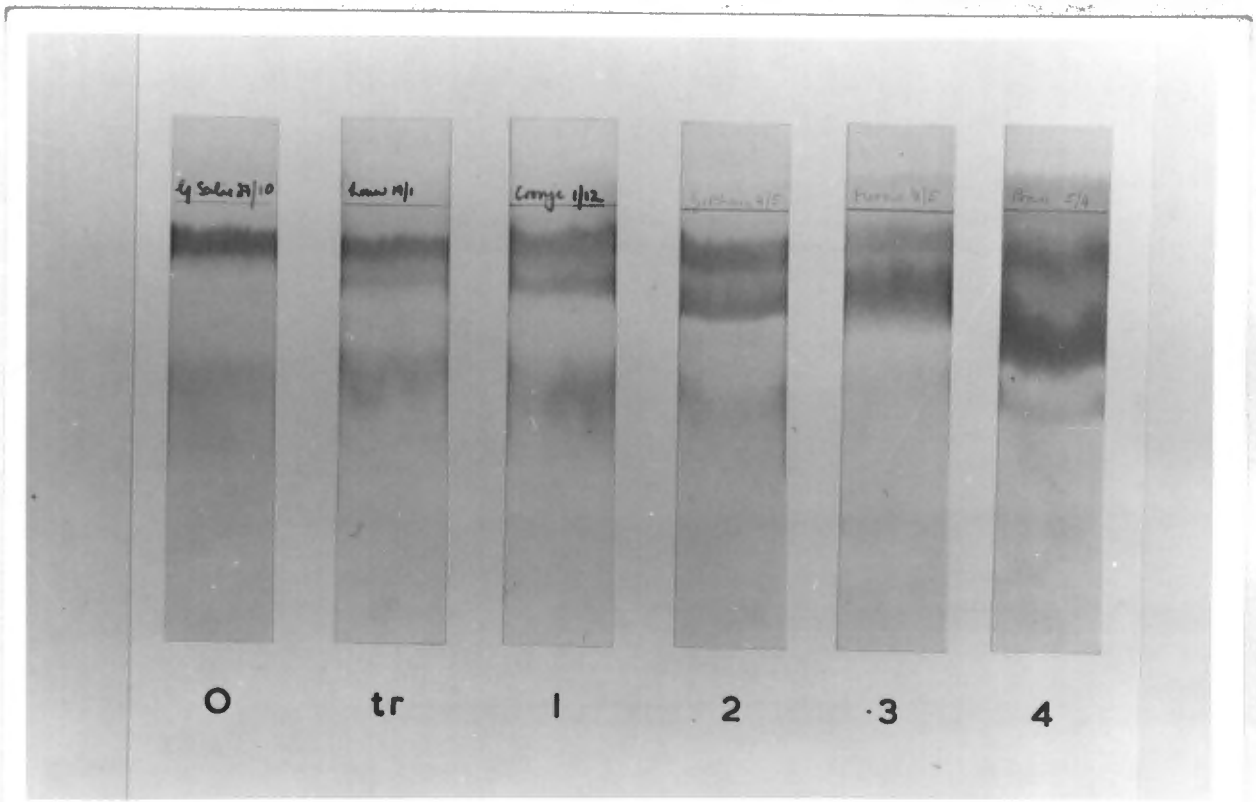


Figure 6: The grading of the pre-beta lipoprotein band, according to its width and intensity.

In view of the good correlation between fasting serum triglyceride level and pre-beta lipoprotein grading, it is suggested that this method may have a role as a screening method before determining the serum triglyceride values on routine clinical samples. The determination of serum triglycerides as a routine laboratory procedure is a tedious process and there seems little value in routinely determining the triglyceride value on a patient whose serum shows + or lower pre-beta lipoprotein grading. In this fairly large sample no triglyceride value of greater than 156 mg/100ml was found in serum which had been given + or lower grading. Serum triglyceride would then only be determined in clinical cases if the serum was overtly lipaemic, if chylomicrons were present on electrophoresis of a fasting serum sample or a pre-beta lipoprotein grading of greater than + was noted.

(E) THIN-LAYER ADSORPTION CHROMATOGRAPHY OF LIPIDS

The principal lipid fractions of the serum were identified by thin-layer adsorption chromatography. This technique was employed to investigate the purity of certain lipid standards in this study and also to separate the triglyceride fraction from the other lipid components of the serum so that triglyceride fatty acid composition could be determined. The technique employed was based on the methods described by Stahl[24] and Mangold[18].

(a) Preparation of the plate

Silica Gel G* was used as adsorbent and slurried with distilled water (1 : 2 weight volume) immediately before use. A DESAGA applicator was used to apply the adsorbent to the glass plate in a layer 0.25 mm thick. The plates were allowed to air dry and then activated at 110°C for one hour before use.

(b) Preparation of the sample

The lipid standards were applied to the plates with a Hamilton micro-

* Silica Gel G (for thin-layer chromatography according to Stahl), E. Merck, AG, Darmstadt, Germany.

syringe as approximately 1% solutions in petroleum hydrocarbon (boiling point 40-60°C).

Lipids were extracted from 2 ml of serum in 2 : 1 chloroform : methanol according to the method of Folch et al[8]. The whole extract was evaporated to dryness in a rotary evaporator, redissolved in a small quantity of petroleum hydrocarbon (boiling point 40-60°C) and applied to the plate with a micro-syringe. A mixed lipid standard was run with the serum on each plate.

(c) Developing the plates

The plates were developed at room temperature in ascending technique. A saturated atmosphere was maintained by lining the developing tank with filter paper.

A wide variety of solvents have been described for chromatography of lipids. A mixture containing petroleum hydrocarbon (boiling point 40-60°C), diethyl ether and glacial acetic acid was found to be the most useful for the separations performed in this study. These solvents may be mixed in several different proportions.

The most extensively used mixture appears to be the one in which petroleum hydrocarbon, diethyl ether and glacial acetic acid are mixed in the proportions 85 : 15 : 1 (by volume)[30]. Clear separation is obtained between cholesterol ester, triglyceride and free fatty acid and as the principal use of this technique in this study was the identification and isolation of the triglyceride fraction, this system was utilised for the separation of the lipid fractions of most of the serum samples studied. The mean Rf values for each lipid fraction using the solvent system are compared with the values obtained by Pie and Giner[22] for the same system in Table V.

TABLE V

	Cholesterol Ester	Triglyceride	Free Fatty Acids	Cholesterol
Present Study	92.6 (0.92)	62.1 (0.75)	25.3 (0.50)	9.6 (0.15)
Pie and Giner[22]	98.3 (0.17)	58.8 (0.42)	18.2 (0.46)	9.8 (0.20)

Rf values ($\pm$ S.E.M.) obtained in this study, using the mixed solvent system, petroleum hydrocarbon (B.P. 40-60°C), diethyl ether and acetic acid (85:15:1), by volume, compared with values obtained by Pie and Giner using the same solvent system.

I. V.

Figure 7 shows an example of a thin-layer chromatography plate on which cholesterol ester, cholesterol, free fatty acid and triglyceride (pure and impure) standards were run.

This system did not, however, give good separation of the free fatty acid, free cholesterol, monoglyceride and di-glyceride fractions and therefore, when the purity of the triglyceride standards was investigated, the polarity of the mixture of solvents was increased by increasing the diethyl ether component. The proportions used were petroleum hydrocarbon (boiling point 40-60°C): diethyl ether : glacial acetic acid, 60 : 40 : 1 (by volume) as described by Meinertz and Dole[20]. As seen in Figure 8, the triolein standard obtained from Applied Science Laboratory contained only a triglyceride fraction whereas the triolein from British Drug Houses contained five additional fractions clearly separated by this solvent system. These fractions were identified from Rf values obtained from previous work in this Department in 1963[26] when 1,3 diglyceride, 1,2 diglyceride and monoglyceride standards were available and run using this solvent system. In order of decreasing polarity therefore, these fractions are free fatty acid, 1,3 diglyceride, 1,2 diglyceride, a small unidentified component and monoglyceride. Table VI shows the Rf values obtained using this solvent system.

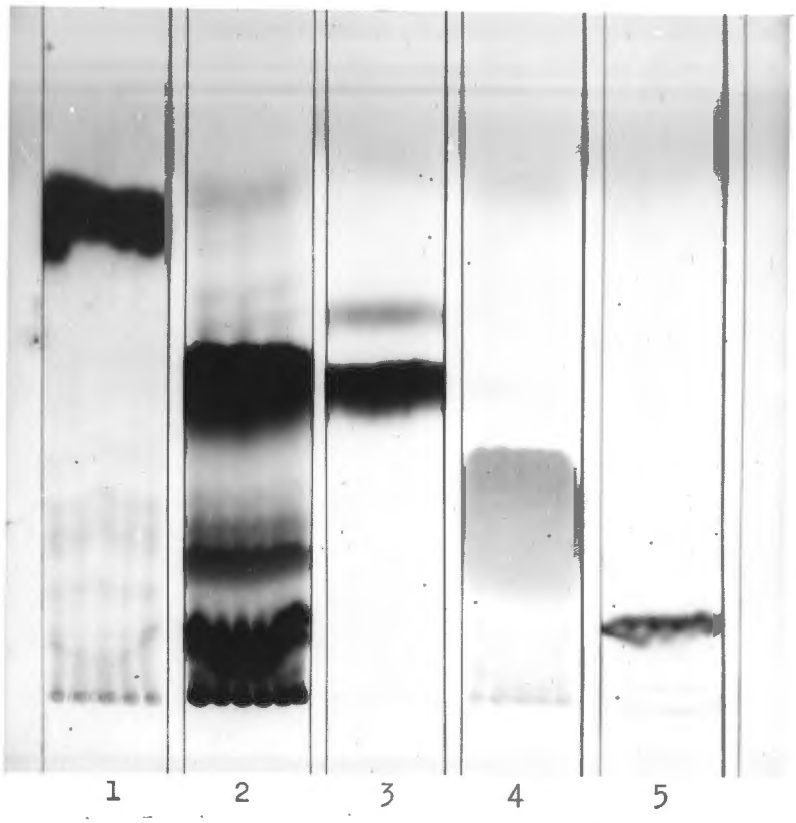


Figure 7: Thin-layer chromatography plate, developed in a mixture of petroleum hydrocarbon, diethyl ether and glacial acetic acid (85:15:1 by volume) and the lipid fractions identified by spraying with 50% Sulphuric Acid.

Column 1. Impure cholesterol ester standard (British Drug Houses).

Column 2. Triolein standard (British Drug Houses) seen to contain fatty acid and at least two other impurities, in addition to triglyceride.

Column 3. Pure triolein standard (Applied Science Laboratory) contains a triglyceride fraction.

Column 4. Palmitic acid standard (British Drug Houses). An ill-defined band of free fatty acid is seen.

Column 5. Cholesterol standard (prepared in our laboratory by recrystallization from absolute ethanol) contains only a cholesterol fraction.

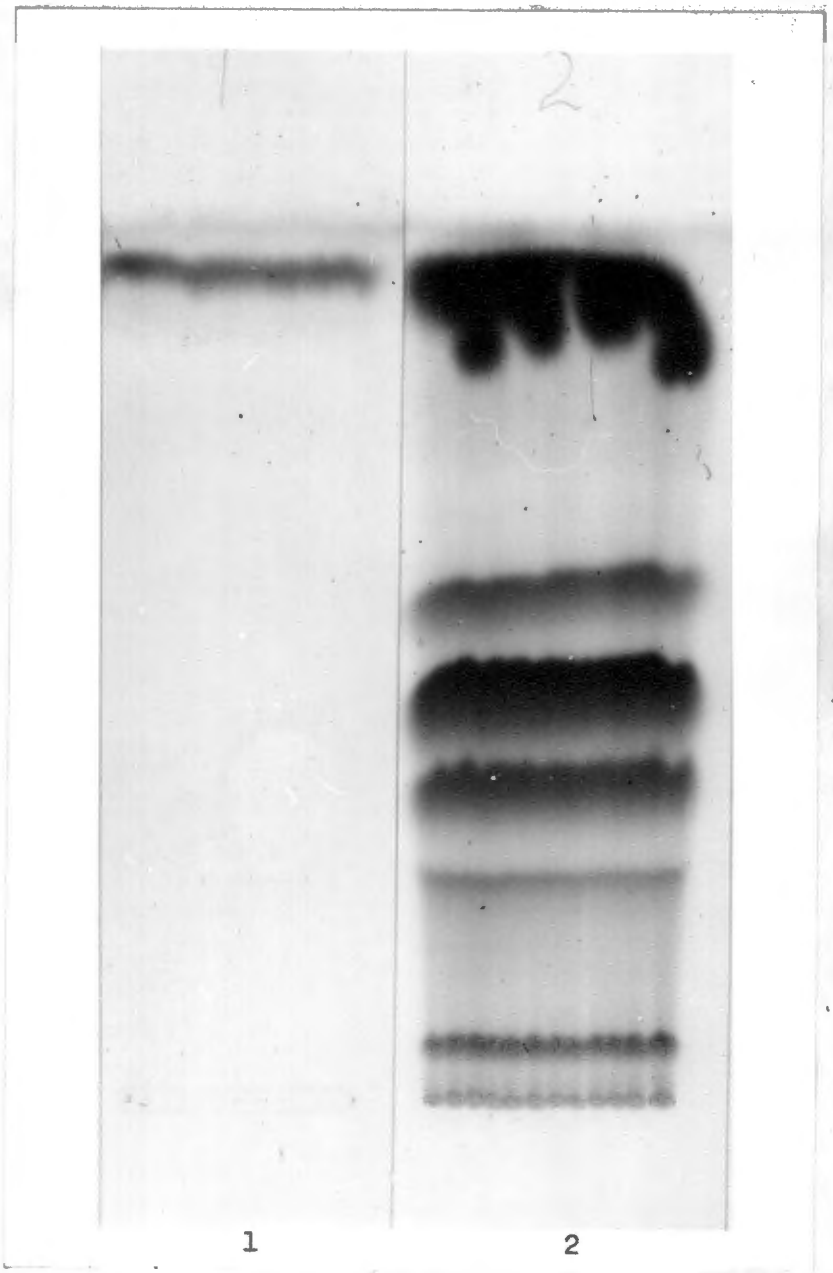


Figure 8: A thin-layer chromatography plate developed in a mixture of petroleum hydrocarbon (B.P. 40-60°C), diethyl ether and acetic acid (60:40:1, by volume) and the lipid fractions identified by spraying with sulphuric acid (50%).

Column 1. Triolein standard (Applied Science Laboratory) only a triglyceride fraction is present.

Column 2, Triolein standard (British Drug Houses). A triglyceride fraction is present together with the following fractions in order of decreasing polarity.

(a) Free fatty acid
(b) 1,3 diglyceride
(c) 1,2 diglyceride
(d) unidentified fraction
(e) monoglyceride

TABLE VI

	Triglyceride	Free fatty acids	1,3 diglyceride	1,2 diglyceride	Unidentified component	Monoglyceride
Present Study	98.6	61.4	49.3	37.1	26.4	7.1
1963	99.8	64.0	51.9	38.4	-	6.3

Rf values obtained using a solvent system of petroleum hydrocarbon (B.P. 40-60°C) diethyl ether and acetic acid (60:40:1 by volume) in the present study and in 1963.

I. VI.

The solvent system of Meinertz and Dole as described above provides excellent separation of free fatty acid and mono- and diglyceride but is of little value for identifying all the serum lipid fractions as no separation is obtained between cholesterol ester and triglycerides. Pie and Giner[22] have described an excellent (and surprisingly little used) solvent system (petroleum hydrocarbon, diethyl ether and acetic acid, 85 : 15 : 7.5 by volume) which gives clear separation of all lipid fractions of serum except between cholesterol and 1,3 diglyceride. However, as mono- and diglycerides are present in only very small amounts in the fasting serum[5], this rarely presents a practical problem. Separation of the lipid fractions of a small number of serum samples in this study was performed using this system. Table VII shows the Rf values obtained using this solvent system, and Figure 9 a thin-layer chromatography plate on which standards were run and developed in this system.

TABLE VII

	Cholesterol ester	Triglyceride	Free fatty acid	1,3 diglyceride	Cholesterol	1,2 diglyceride	Monoglyceride
Present Study	99.9	88.0 (1.13)	65.1 (0.87)	46.8 (0.49)	45.1 (0.45)	41.3 (0.51)	11.1 (0.64)
Pie and Giner	99.0	67.7 (0.79)	38.9 (1.20)	-	28.8 (0.98)	-	-

Rf values ($\pm$ S.E.M.) obtained in this study using the mixed solvent system, petroleum hydrocarbon (B.P. 40-60°C), diethyl ether and acetic acid (85:15:7.5 by volume) compared with the values obtained by the authors who described the system.

I. VII.

The phospholipid fraction remains at the origin when all the solvent systems described above are used.

(d) Visualisation and Identification of Lipids

Many indicators are available for the identification of lipids on thin-layer chromatography plates. In this study, identification was carried out as recommended by Mangold[18] by the use of iodine vapours or by charing the plates at 200°C after spraying with 50% sulphuric acid. (Spraying the plates with sulphuric acid is facilitated by heating them at 100°C for 50 minutes before spraying). Sulphuric acid has been used in the plates shown in the photographs in this study.

When serum lipid fractionation was carried out prior to eluting the serum triglyceride fraction from silicic acid for gas-liquid chromatographic analysis of the fatty acid patterns, the plates were sprayed with Rhodamine 6G and visualised and lipid fractions demarcated under ultraviolet light[23]. Gas-liquid chromatographic analysis is in no way affected by this substance[30].

(F) GAS-LIQUID CHROMATOGRAPHY

The fatty acid pattern of the serum triglyceride fraction was examined

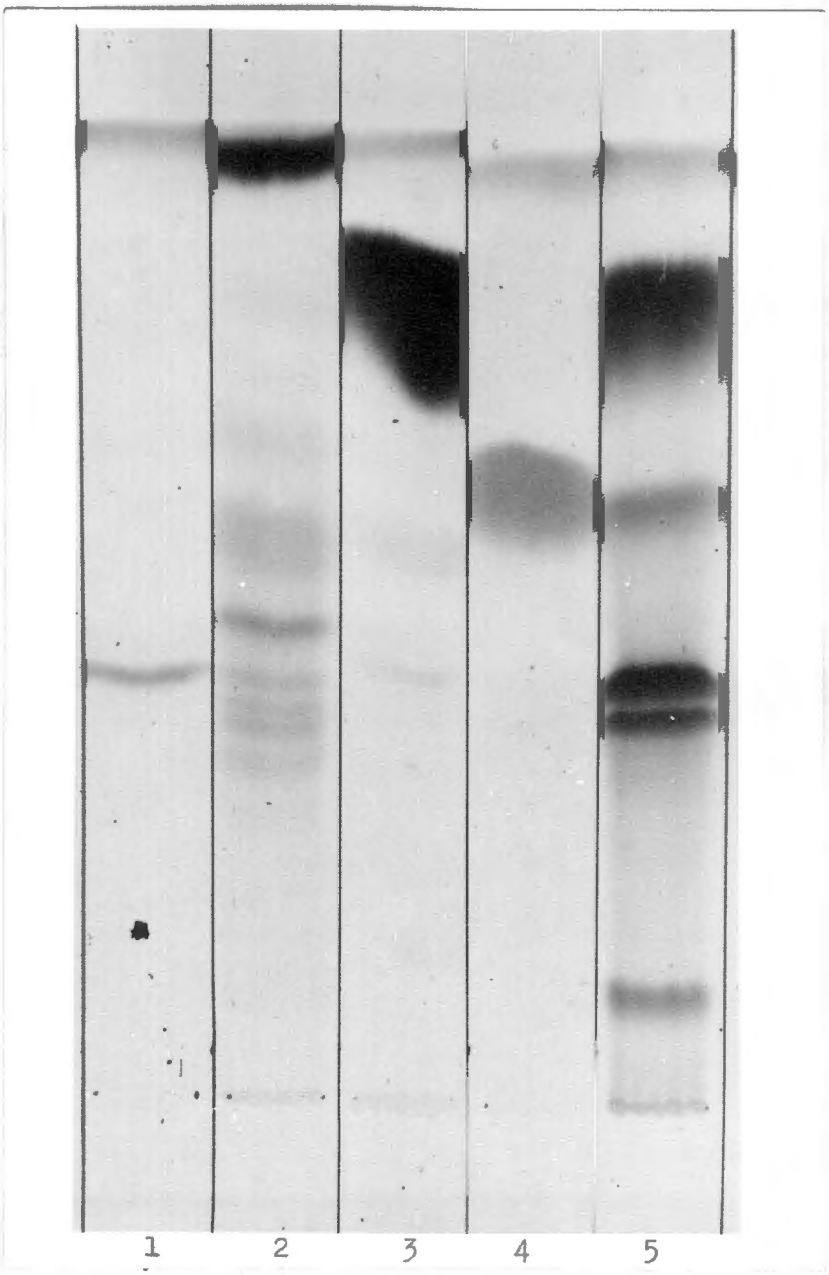


Figure 9: Thin-layer chromatography plate developed in a mixture of petroleum hydrocarbon (B.P. 40-60°C) diethyl ether and acetic acid (85:15:7.5 by volume) and the lipid fractions identified by spraying with 50% sulphuric acid.

Column 1. Cholesterol standard (prepared in our laboratory).

Column 2. Impure cholesterol ester standard (British Drug Houses).

Column 3. Triolein standard (Applied Science Laboratory).

Column 4. Palmitic acid standard (British Drug Houses).

Column 5. Triolein standard (British Drug Houses) contains triglyceride, free fatty acid, 1,3 diglyceride; 1,2 diglyceride and monoglyceride.

by the technique of gas-liquid chromatography (G.L.C.) which was developed by James and Martin[12] in 1952 and modified by them in 1956[13].

(a) Preparation of sample

The principal lipid fractions of 2 ml of serum were separated by thin-layer chromatography as described above. The triglyceride fractions were identified and demarcated under ultraviolet light after spraying with Rhodamine 6G. Gas-liquid chromatographic analysis is performed on methyl esters. The technique used for methylation based on a micro-method described by Stoffel et al[25] was performed as follows.

The silicic acid in the demarcated area was carefully scraped off the plates and transferred to a test-tube fitted with a B19 ground glass joint. 6 ml of 5% (V/V) sulphuric acid in super dry methanol and 1.5 ml of petroleum hydrocarbon (boiling point 40-60°C) were added to the tube. The mixture was refluxed in the silicone bath at 100° for three hours. After cooling to room temperature, 3 ml of distilled water were added and the methyl esters extracted three times with 4 ml of petroleum hydrocarbon (boiling point 40-60°C). The combined extracts were simultaneously dried and neutralised over a sufficient quantity of 4 : 1 (W/W) sodium sulphate: sodium bicarbonate. The extract containing methyl esters was then transferred to a small conical tube and the solvent evaporated at room temperature under a stream of nitrogen, immediately prior to analysis.

During all the procedures involved in preparing samples for G.L.C. great care was exercised to avoid any possibility of oxidation occurring. Samples were not left to stand in a dried condition, but always contained solvent and air was expelled by nitrogen. All samples not being processed were stored at 4°C under nitrogen.

(b) The technique of gas-liquid chromatography

(i) The apparatus

The apparatus used in this study was the Pye Argon chromatograph which

has been modified to give an uninterrupted gas flow during the loading of the sample. This apparatus incorporates the Argon beta-ray ionisation detector developed by Lovelock[17], and contains a Radium D source. The detector signals are amplified by an electronic circuit and recorded by a Sunvic recorder which responds linearly with mass.

(ii) Preparation of the stationary (liquid) phase

The stationary (liquid) phase used throughout was the polyester of ethylene glycol adipate (E.G.A.), prepared according to the method of James[14]. The E.G.A. was coated on to a solid support (Chromosorb W, acid washed and size graded to 80-100 mesh). 20% (W/W) E.G.A. was coated on to the Chromosorb W by dissolving the E.G.A. in an excess of chloroform, adding the proportional amount of Chromosorb W with thorough mixing and evaporating chloroform on a rotary evaporator. The E.G.A.-coated Chromosorb W was packed into a glass column which is a standard fitting for this chromatograph (column length 4 ft; internal diameter 4 millimetres; external diameter 6 millimetres). The constricted end of the column was plugged with a small quantity of teased-out glass fibre and the coated Chromosorb W poured into the column and packed with gentle tapping to a height of about 3 ft. 8 ins. About one inch of uncoated Chromosorb W was packed above this and a plug of glass fibre placed on top of it. The column was conditioned by 'baking' for 24-48 hours at 200°C in an auxiliary heating jacket with argon (gas phase) flowing under a pressure of 20 pounds per square inch. This conditioning process ensures the removal of any remaining traces of chloroform and also allows any unabsorbed liquid phase to 'bleed' off. Since this 'bleeding' of the stationary phase and initial heating causes temporary baseline instability, it is essential that the conditioning process continues until the baseline of the recorder settles to a constant level before starting on any analytical run.

(iii) Operating conditions

The conditional column was used at an operating temperature of 180°C

with an Argon pressure of from 10-15 pounds per square inch giving gas flow rates of 40-60 ml per minute. The detector voltage was maintained at a constant 1000 volts. The degree of amplification (which is controlled by means of a built-in sensitivity switch with a ratio of 1 : 3 : 10, corresponding to the positions X 10, X 3 and X 1 respectively on the instrument panel) was adjusted according to the time of elution of the components. From the time of injection to elution of C18: 2 was recorded on the lowest sensitivity (X 10) and beyond the elution of C18: 2 on higher sensitivity (X 3). The speed of the chart in the recorder was 15 inches per hour. The gas pressure was adjusted so as to give good separation between components.

(e) Standardisation and calibration of apparatus

(i) Linearity of response

While the detector and amplifier-recorder systems in the apparatus are designed to give linearity of response, it is essential to test whether the ionization current recorded on the moving chart is directly proportional to the amount of fatty acid methyl ester applied to and eluted from the column under the conditions of operation. Certain mixtures of pure methyl esters of fatty acids have been devised specially for assessing this parameter of apparatus performance. Before commencing the analysis and at frequent intervals during the course of this study, standard mixtures were run on the apparatus to verify that linearity of response was being maintained. It was also checked by means of suitable standard mixture that the calculated proportions of the fatty acid components in the mixture reflect its composition by weight. All the above fatty acid methyl ester standards were obtained from Applied Science Laboratories, U.S.A.

The results of repeated analysis of a standard mixture are shown in Table VIII.

TABLE VIII

	% by weight	% by G.L.C.	
		Mean	S.D.
C14: 0	11.8	11.7	0.41
C16: 0	23.6	24.8	0.53
C16: 1	6.9	6.4	0.31
C18: 0	13.1	13.1	0.49
C18: 1	44.6	44.0	0.93

The percentage composition of a standard mixture as given by weight (information supplied by Applied Science Laboratory, U.S.A.) and determined by G.L.C. The mean and S.D. for 10 gas chromatographic analyses are shown.

I. VIII.

The response of the apparatus under the operating condition can thus be accepted as being linear to the concentration, by weight, of the fatty acid methyl esters.

(ii) Resolving the power of the column

The efficiency of separation can be determined on the basis of the number of theoretical plates in the columns. This is calculated according to the formula of Farquhar et al[6] as follows:

$$n = 16 \left(\frac{tR}{W}\right)^2$$ where

n = number of theoretical plates

tR = retention time (or distance)

w = base width of components in the same units as tR.

In this study, theoretical plates were measured at C18:0 and the average value was approximately 1600 theoretical plates, which is somewhat lower than the value of 2000 advocated for maximum efficiency by Farquhar et al. However, it has been suggested that it may be more meaningful, in evaluating the efficiency of polyester columns, to measure their resolution of C18:0 from C18:1 and of C16:0

from C16:1, rather than to calculate only the theoretical plates of the column[33]. Resolution is then calculated according to the formula of Farquhar as follows:

$$\text{Component resolution} = \frac{2\,\Delta Y}{Ya+Yb}$$

Where ΔY = distance between any two peak maxima

Ya and Yb = base widths of components a and b.

A value greater than 1 indicates complete resolution[33]. The average value for C18:0/C18:1 was 1.06 and for C16:0/C16:1, 1.04, indicating that satisfactory resolution was obtained. (Figure 10).

When the degree of separation between these peaks fell below 1 the column was replaced.

(d) Loading of sample

The fatty acid methyl esters, dissolved in a small volume ($\pm$ 0.1 ml) of petroleum ether (boiling point 40-60°C) were contained in a tapered test-tube fitted with a ground glass joint. From 0.1 microlitres to 0.5 microlitres, depending upon the concentration of the sample, was taken up in a Hamilton micro-syringe and injected through the silicone rubber seal at the top of the glass column.

(e) Calculation of percentage composition of fatty acids

Since the recording system used draws a differential curve, the areas of the peaks represent the relative proportions of the component fatty acid methyl esters. Areas under peaks can be measured by planimetry, by cutting out the curve and weighing the papers, by triangulation, or by automatic integration. The method of triangulation as described by Farquhar[6] was applied here. The area under each peak was calculated (height x width at half height). The sum of the peak areas was obtained and each component expressed as a percentage of the total peak areas. This method was compared with planimetry and almost identical results were obtained.

(f) Identification of fatty acids

Identification of fatty acids in gas-liquid chromatography is based on

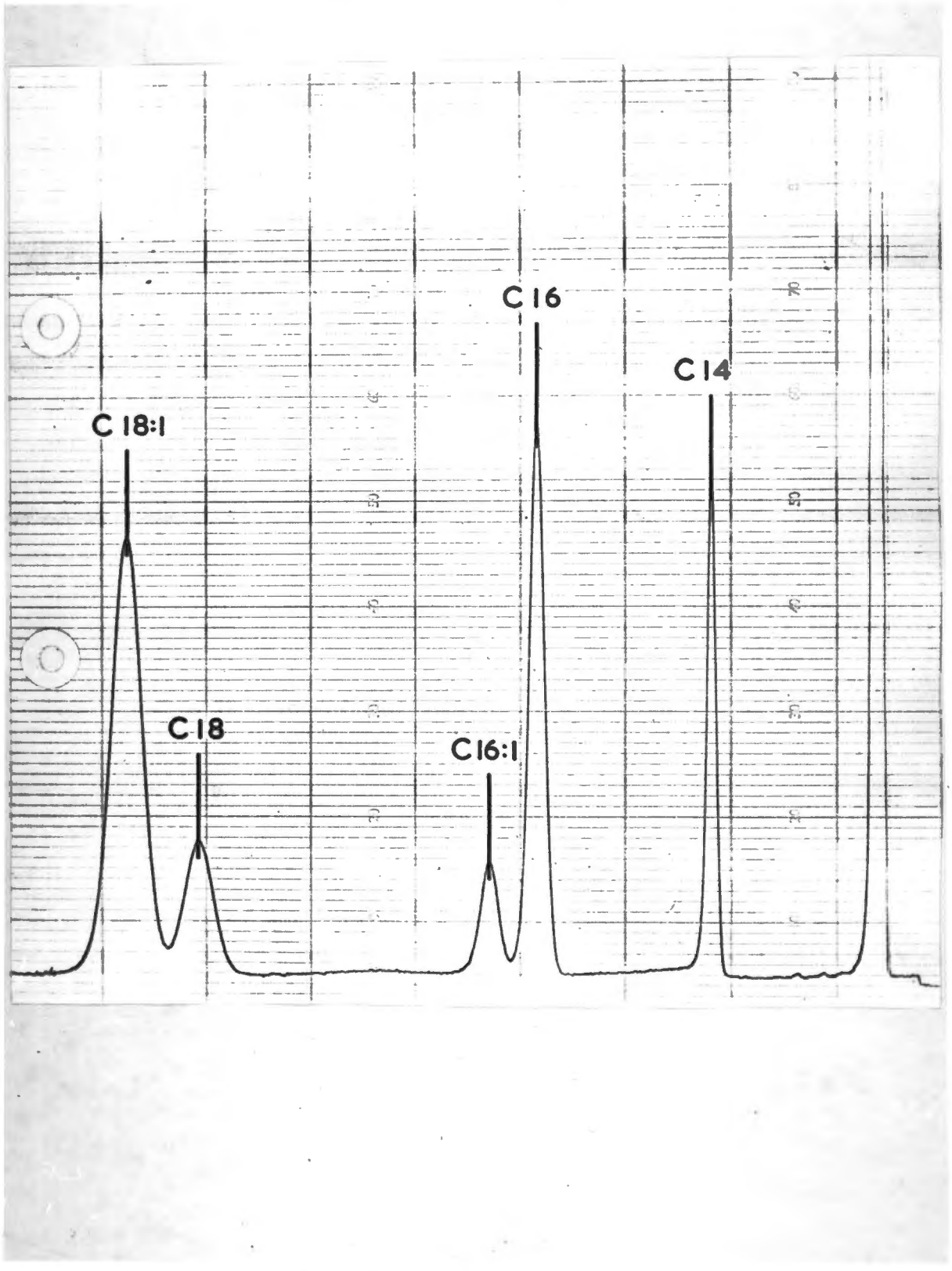


Figure 10: A typical chromatogram of a standard mixture of fatty acid methyl esters showing the degree of separation between components.

I. 10.

the principle that a particular liquid phase will have specific retentive properties for each fatty acid methyl ester under given conditions of temperature and pressure. Factors which determine the degree of retention are the chain length (number of carbon atoms) of the fatty acid and whether the fatty acid is saturated or unsaturated. The degree of retention increases with increasing chain length of the saturated components. When a polar phase, such as the polyester of E.G.A. is used, the methyl esters of unsaturated fatty acids emerge later than those of saturated fatty acids with the same chain length and increasing unsaturation causes a progressive increase in retention. In addition, it has been shown that plotting of either log retention volume or log retention volume relative to a standard substance against the number of carbon atoms in the molecule, gives a straight line for members of an homologous series[12]. This applies to saturated and mono-, di-, and tri-unsaturated fatty acids of an homologous series. Retention volume is obtained by multiplying the flow rates by the retention time. Since in any run flow rate is constant, the retention volume is directly proportional to the retention time. The log retention time can thus be directly related to the number of carbon atoms in the molecules. In practice, the retention time is measured as distance from the point of injection to centre of the relevant peak[6]. In different runs the absolute retention time will vary with differences in gas flow rate. However, at a given temperature, the retention time of fatty acid methyl esters relative to one another are constant. Thus the relative retention time can be calculated for each component. This is commonly expressed as the retention time of the component relative to that of C18:0 (Stearic acid)[6].

Applying these principles it is possible to identify components by (1) comparing their absolute and relative retention time under certain conditions with those of pure methyl esters of known fatty acids under the same conditions. (2) Plotting the log retention time or log relative retention time against the number of carbon atoms in the molecule for members of an homogolous series.

(3) Identification of the unsaturated components can, in addition, be verified by hydrogenation of the sample of natural methyl esters. In this way the unsaturated fatty acid esters are converted to their respective saturated compounds. By comparing the chromatograms before and after hydrogenation, it can be established which components of the original sample were unsaturated. Samples of methyl esters were hydrogenated according to the method of Farquhar[6].

It was the object of this study to compare the fatty acid patterns of the serum triglyceride fraction of the different population groups surveyed. It was found that with the exception of two components, all the fatty acids present in the different groups were present in the serum triglyceride fraction of the group of Bushmen surveyed. Identification of the fatty acids in all groups was therefore based primarily on the identification of these components in the Bushman sample, using the techniques described.

(g) Application of methods for identification of fatty acids

(i) Comparison of relative retention times

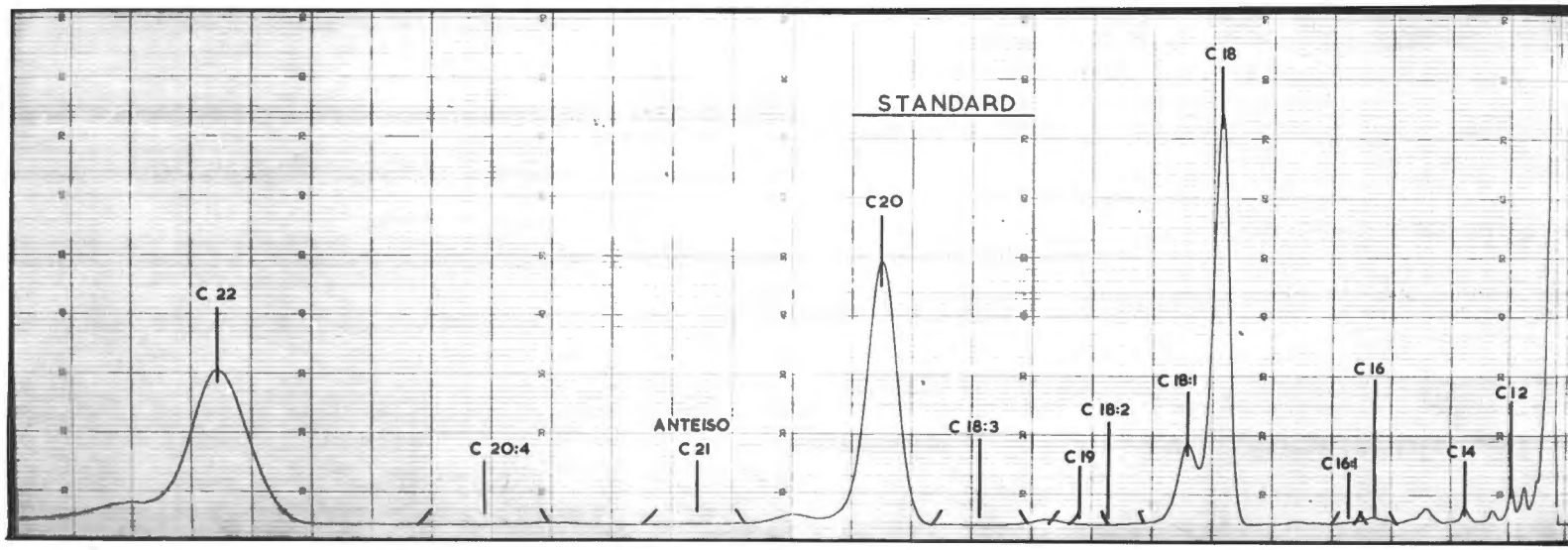
The relative retention times of fatty acid components of the Bushman triglyceride fraction have been compared with those of pure fatty acid methyl esters. The values are shown in Table IX. The chromatograms of a standard fatty acid mixture (on which the position of other standard fatty acids run has also been indicated) and of the fatty acids of the Bushmen serum triglyceride fraction are compared in Figure 11.

TABLE IX

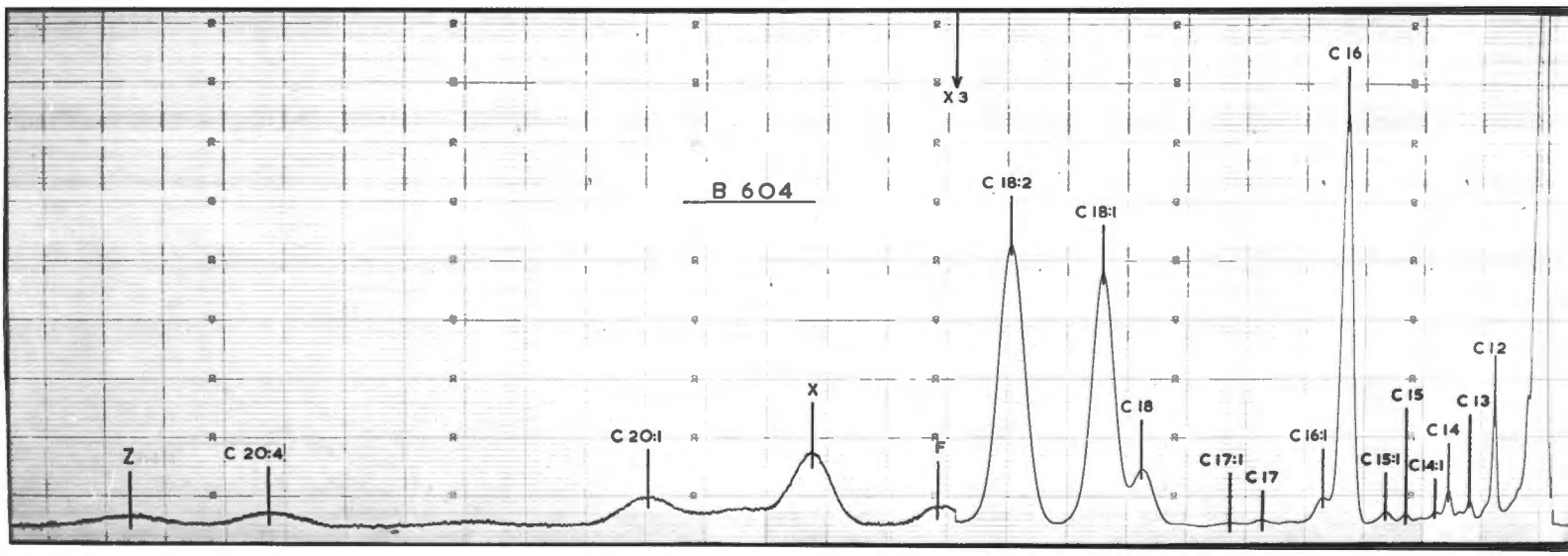
Standard Fatty Acids		Bushman Triglyceride Fatty Acids	
Fatty Acid	d/C18:0	d/C18:0	Identification
C12:0	0.13	0.13	C12:0
C13:0	0.18	0.19	C13:0
-	-	0.23	A
C14:0	0.26	0.26	C14:0
-	-	0.29	C14:1
C15:0	0.36	0.35	C15:0
-	-	0.41	C15:1
C16:0	0.51	0.51	C16:0
C16:1	0.58	0.57	C16:1
C17:0	0.71	0.71	C17:0
-	-	0.79	C17:1
Iso C18	0.86	-	-
C18:0	1.00	1.00	C18:0
C18:1	1.10	1.10	C18:1
Anteiso C19	1.28	-	-
C18:2	1.33	1.33	C18:2
C19:0	1.40	-	-
-	-	1.51	F
C18:3	1.70	-	-
Iso C20	1.71	-	-
-	-	1.79	X
C20:0	1.98	-	-
-	-	2.19	C20:1
Anteiso C21	2.52	-	-
C20:4	3.16	3.16	C20:4
C22:0	3.88	-	-
Identified by plotting of leg retention time			

Comparison of relative retention times for fatty acid components in the Bushman serum triglyceride fraction with those found for standard fatty acids.
d/C18:0 = relative retention time, i.e. retention time (in cm) relative to retention time of C18:0.

I. IX.



(A)



(B)

Figure 11: (A) Chromatogram of a mixture of standard fatty acids methyl esters. The position of other standard fatty acids run has also been indicated.

(B) Chromatogram of the fatty acid pattern of a Bushman serum triglyceride fraction.

The data in Table IX shows that C12:0, C13:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:0, C18:1, C18:2 and C20:4 found in the Bushman serum triglyceride fraction can positively be identified by comparing their relative retention times with those of standard fatty acids run at the same temperature.

In the serum triglyceride of the group of rural Bantu surveyed C18:3, easily identified by its relative retention time, was also found to be present.

Eight peaks (relative retention times 0.23, 0.29, 0.41, 0.79, 1.51, 1.79, 2.19 and 3.45 remained unidentified and the following attempts were made to identify them.

(ii) Plotting of log retention time

The log retention time expressed as log distance (in cm) has been plotted against the number of carbon atoms in the molecule and shown in Figure 12. It is apparent from this figure that the saturated fatty acid components identified by comparison of their relative retention times with the relative retention times of standard fall on a straight line. On plotting the distances of the two monounsaturated fatty acids identified as C16:1 and C18:1 it was found that the peaks with relative retention times 0.29, 0.41, 0.79 and 2.19 fell on this straight line. These peaks, falling on the monounsaturated fatty acid lines have therefore been identified as C14:1, C15:1, C17:1 and C20:1 respectively.

The peaks with relative retention times 0.23, 1.51, 1.79 and 3.45 did not fall on either the saturated or mono unsaturated fatty acid line and as there were not sufficient values to plot the log retention times against carbon chain length for the polyunsaturated fatty acids, these peaks have been labelled as A, F, X and Z. Peaks A and Z were very small and not constant and further attempts at identifying them were therefore not made.

(iii) Hydrogenation

An attempt to identify the peaks F and X was made by using the hydrogenation technique. Peak F was a fairly constant peak in the rural Bantu and X fairly constant in the Bushmen.

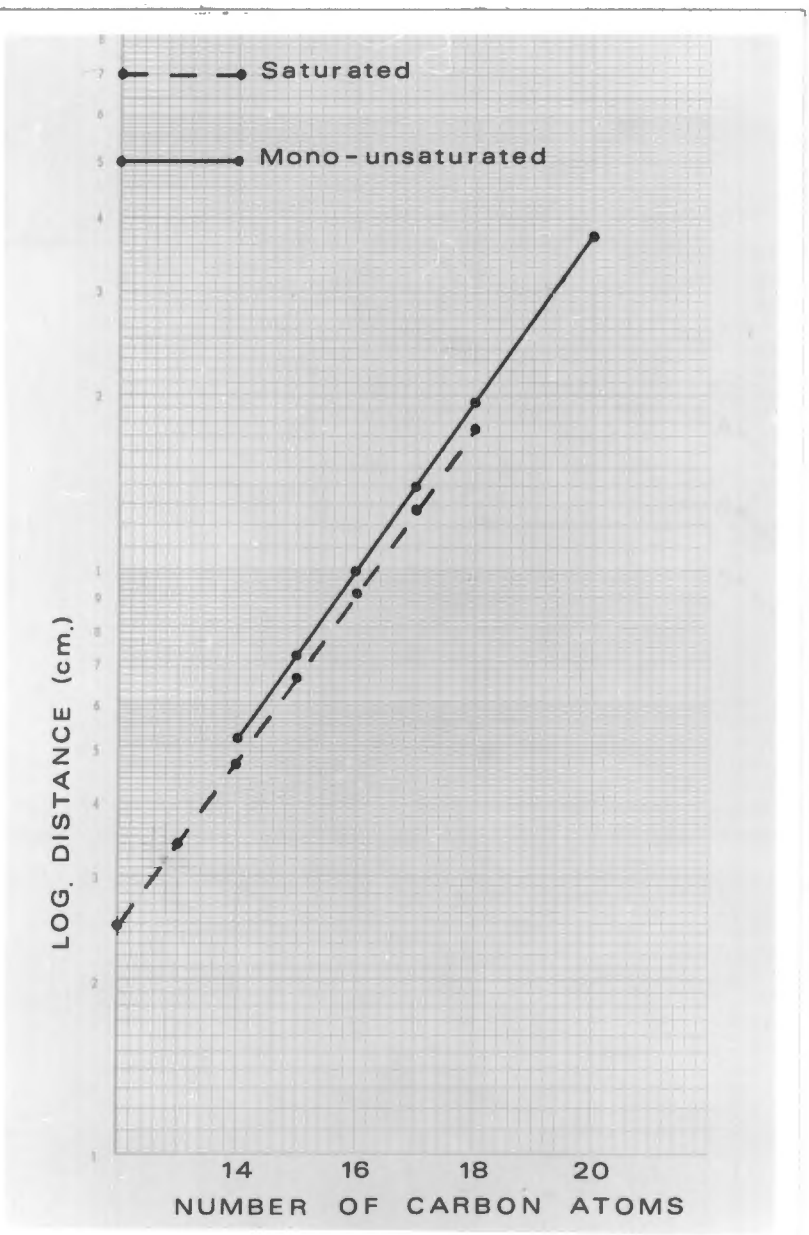


Figure 12: Plot of the log distance of the peaks shown in the chromatogram of the Bushman serum triglyceride fatty acids (Figure 11b) against the chain length of the components.
Note that the saturated and monounsaturated fatty acids fall on to separate straight lines.

Figure 13 compares the natural and hydrogenated fatty acids of the serum triglyceride in a Bushman. The relative retention times for the saturated components are identical in the two states. In the hydrogenated sample, all the unsaturated fatty acid components have disappeared. Here, however, there is an increase in proportions of the saturated fatty acids. Peak X has disappeared. Since the polyester of E.G.A. has been used as the stationary phase, the methyl esters of unsaturated fatty acids emerge later than the saturated fatty acids of the same chain length. X has therefore been identified as an unsaturated fatty acid with an 18 carbon chain length. C20:4 peak has disappeared and C20:0 which was not present in the natural form has appeared.

Using the same technique, Peak F has also been identified as an unsaturated fatty acid with an 18 carbon chain length.

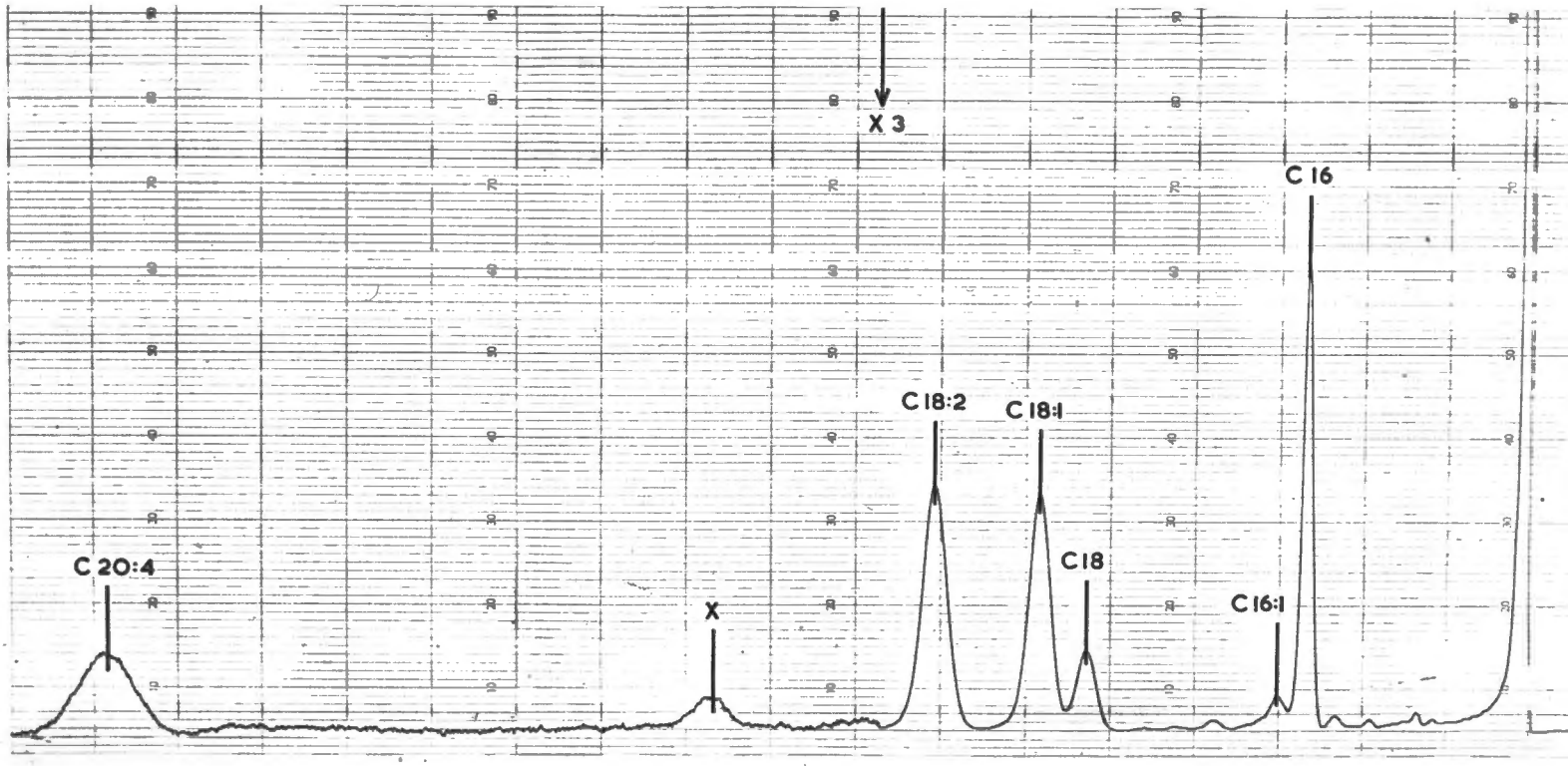
(h) Reproducibility of fatty acid analysis

In order to determine the degree of reproducibility of fatty acid estimations by G.L.C., a standard mixture was analysed ten times at random intervals during the course of this study. The percentage of each fatty acid was determined and the coefficient of variation calculated for each component. The data given in Table X shows that there is a high degree of reproducibility in estimating the fatty acid composition by G.L.C.

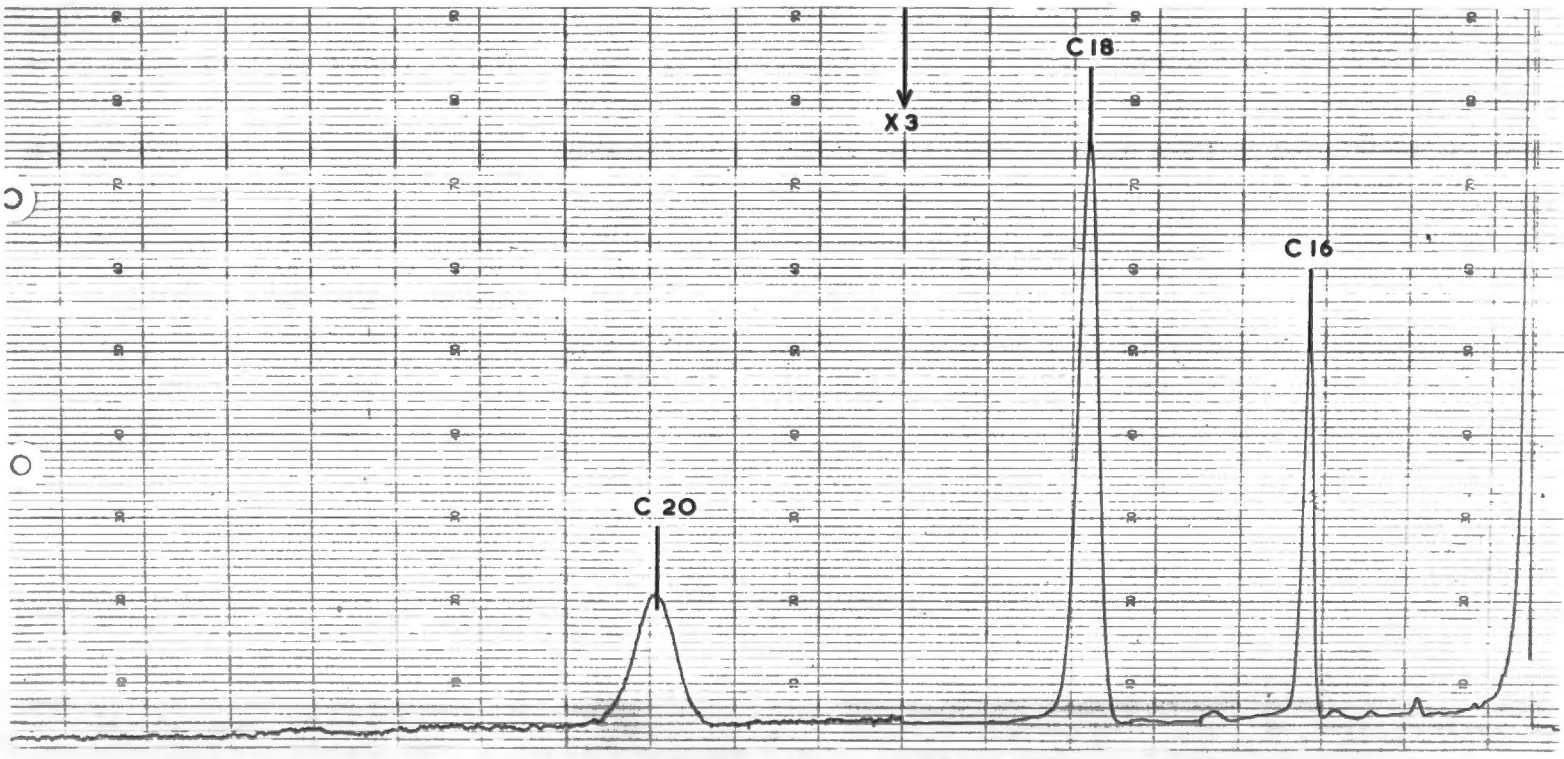
TABLE X

	C14:0	C16:0	C16:1	C18:0	C18:1
Mean	11.7	24.8	6.4	13.1	44.0
S.E. of a single determination	0.32	0.46	0.25	0.51	0.45
C.V. %	2.74	1.84	4.01	3.96	1.03

The percentage composition of a standard fatty acid methyl ester mixture as determined by 10 analyses by G.L.C.



(a)



(b)

Figure 13: Chromatograms of (a) natural and (b) hydrogenated methyl esters of the Bushman serum triglyceride fractions.

It was reported by Farquhar that on repeated analysis of a standard fatty acid methyl ester mixture containing four components, the coefficient of variation for any single component was less than 1.5%. This high degree of reproducibility was not achieved here.

The reproducibility of the whole procedure used in determining the fatty acid composition of the serum triglyceride has been examined. Five 2 ml aliquots of a serum sample were extracted in 2 : 1 chloroform : methanol. The extracted lipids were fractionated by thin-layer chromatography, methylated and the methyl esters analysed by G.L.C. The results are shown in Table XI.

TABLE XI

	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
Mean	1.2	2.3	31.0	5.7	3.7	40.7	15.5
S.E. of a single determination	0.09	0.07	0.55	0.34	0.26	0.46	0.26
C.V.%	7.51	3.26	1.83	5.94	7.02	1.13	1.68

The mean percentage, S.E. of a single determination and C.V. of each serum triglyceride fatty acid as determined on five separate aliquots of a serum sample.

I. XI.

The degree of reproducibility is satisfactory. The coefficient of variation though, is greater for the smaller peaks than the larger ones. This is probably due to the inaccuracy in measuring the area of these smaller peaks.

If the area of a fatty acid peak was found to constitute less than 0.5% of the total, a trace of that particular fatty acid was recorded as being present.

(G) BLOOD SUGAR DETERMINATION

Blood sugar was determined by the method of Hoffman[10], modified for use by the autoanalyzer[27]. The method is based on the reduction of potassium

ferricyanide to potassium ferrocyanide by reducing sugars in the blood and therefore measures all reducing sugars present. Blood for blood sugar estimations was always collected in tubes containing fluoride and kept at -20°C until analysed.

The method is a very precise one and the following data was calculated from the same sample analysed on 25 different occasions, including those on which the samples described in this study were analysed.

Mean blood sugar value:-	125 mg/100ml
Range :-	124 - 128 mg/100ml
S.E. of a single determination:-	1.23
C.V.% :-	0.98%

(H) SERUM INSULIN DETERMINATION

Principle

A modification[31] of the Morgan and Lazarow[21] immunoassay was employed for serum insulin determination. The assay is based on the reaction of a trace amount of I^{125} labelled insulin and anti-insulin antibody, formed in guinea pigs (1st Antibody). This forms a labelled antigen-antibody complex. The labelled insulin is displaced from the available insulin antibody binding sites by any unlabelled insulin (of the standard or unknown serum sample). Competitive inhibition is proportional to the amount of unlabelled insulin present. Therefore, the ratio of antibody-bound labelled insulin (B) to free labelled insulin (F) falls progressively with increasing concentrations of unlabelled insulin. As the insulin first antibody complex is soluble, bound insulin is separated from the free insulin by its reaction with a second antibody directed against the immunoglobulins of the first. The second antiserum contains rabbit antiguinea-pig antibodies. Added guinea pig serum provides a carrier serum to increase the bulk of the precipitate. This precipitate is separated by centrifugation. The insulin concentration of an unknown solution is determined by comparing its B/F ratio with those of standard solutions.

Details

Pork crystalline insulin (NOVO) was used as the external standard. Serial dilutions were made from a solution containing 400 microunits/ml in 0.05 M Veronal buffer (pH. 8.6), with 0.25% bovine serum albumin, so that the standard tubes (pipetted in triplicate) contained 1.25, 2.50, 10.00, 20.00, 40.00 and 80.00 microunits insulin/ml respectively.

0.05 ml of each serum sample was pipetted in duplicate into tubes containing 0.45 ml buffer. 0.1 ml of the first antibody (WELLCOME) was added to each tube. The tubes were shaken and allowed to stand at 4°C for 24 hours, after which time 0.1 ml I^{125} labelled insulin was added (crystalline pork insulin was labelled with carrier-free I^{125} according to the method of Hunter and Greenwood(11).

The tubes were shaken again and allowed to stand for 120 hours at 4°C. 0.1 ml of the second antibody (prepared in rabbits in the Department of Medicine Isotope Unit) was then added together with 0.1 ml guinea-pig serum. After a further 48 hours of incubation at 4°C the precipitate was separated by centrifugation. The radioactivity of the precipitate was counted on a PACKARD 4 WA autogamma spectrophotometer.

B/F ratios of the standards were plotted against the actual insulin concentration on semilogarithmic paper.

The concentration of an unknown sample was determined by comparing its B/F ratio with those of the standard solutions.

All the samples of an individual subject were assayed in the same run. A certain amount of non-specific binding always occurs due to a small amount of iodination damage and formation of some breakdown products of labelled insulin during incubation. The amount of breakdown varies in the sera of each patient. To eliminate inaccuracies due to this, a blank consisting of the subject's serum without the first antibody was run together with the sera from each meal test. The precipitated count indicating the amount of non-specific binding

was subtracted from those of the sera comprising the test.

The mean difference between duplicate analyses expressed as a percentage of mean insulin levels was always less than 5%. In any instance where the error was 5% or greater, the assay was repeated together with a number of other samples of the same run. In most cases, however, there was no, or only 2 microunit/ml difference between duplicate samples.

An internal standard serum was included in duplicate in each of the 10 runs. The mean value obtained was 34 microunits/ml and the range of values 32 to 38 microunits/ml.

I. DETERMINATION OF CALORIFIC VALUE OF FOOD SUBSTANCES

The calorific values of certain food substances and of aliquots of whole meals were determined (after homogenisation and drying of the sample) in a bomb calorimeter[3].

The sample was burnt, in the presence of an adequate supply of oxygen in a heavy steel "bomb" which was surrounded by a water jacket. The temperature rise of the water was used to calculate the heat of combustion.

$$\text{Calorific value expressed in kCal/g} = \frac{(M + E) \times \Delta T}{m}$$

Where M = mass of water in grams

E = water equivalent of calorimeter, in grams

m = mass of sample, in grams

ΔT = temperature rise of water, in degrees centigrade (after correcting for cooling loss in the bomb calorimeter)

All measurements were carried out in duplicate.

J. DETERMINATION OF NITROGEN CONTENT OF FOOD SUBSTANCES

Nitrogen content of certain food substances were determined by a semi-micro Kjeldahl method[28]. All estimations were carried out in duplicate. Protein content was expressed as nitrogen content X 6.25.

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CHAPTER II - ANALYSIS OF DIETARY DATA

A dietary history was obtained from all volunteers in two of the groups described in Part II of this study (Groups C and D). Each subject was provided with a note-book in which he was asked to record everything eaten or drunk for seven days. In addition, three further three-day diet histories were obtained from subjects of Group C during the course of their experimental diets. The subjects were in addition questioned regarding the method of preparation and the amount of fat and oil used in preparing the food. The intake of each foodstuff was estimated on the basis of average portions unless there were obvious deviations.

The dietary analysis of twelve subjects from Group H (African porters at Groote Schuur Hospital) was based on a personal interview of each of the twelve subjects with the dietician who asked each subject to recall all food eaten or drunk for three days.

For each subject the composition of the diet as regards total calories and amounts of protein, fat and carbohydrate (subdivided into sucrose and non-sucrose carbohydrate) was calculated from Table XII which follows. These were culled from several sources[1,2,3,4,5].

From this information, the daily calories and the proportion of total daily calories provided by each dietary constituent were calculated. Mean daily values for each subject are the means for seven or three days.

It is recognised that dietary analysis based on diet histories, gives at best a close approximation for the actual intake and composition of the diet. Statistical analysis of the dietary data was therefore not attempted.

TABLE XII

Refs.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
A						
5	Almonds 30 g		6.5	209	6.2	19.2
5,2	Apple - raw - 100 g	3.1	14.1	58	.2	.3
5	Apple pie 100 g	19.0	38.1	256	2.2	11.1
5,2	Appletizer 9 oz	9.3	35.7	141	.3	
5,2	Apricots, tinned 200 g	22.0	44.0	172	1.2	.2
5	Avocado 200 g		12.6	334	4.2	32.8
B						
+	Babotie 150 g		5.0	300	23.8	20.0
5	Bacon 3 slices 60 g		.2	166	16.5	10.5
3	Bacon and egg pie 200 g		12.5	663	26.0	54.7
5,2	Banana 50 g	5.9	11.1	43	.5	.1
5	Baked beans 100 g		23.0	120	6.3	.5
5,2	Beans, green 100 g	.5	5.4	25	1.6	.2
5	Beans, dried cooked 100 g		23.0	120	6.3	.5
5	Beef and vegetable stew 300 g		18.6	267	19.2	12.9
5	Beef, braised steak 100 g			317	24.9	23.4
5	Beef, corned 100 g			216	25.3	12.0
5	Beef, minced 100 g			286	24.2	20.3
5	Beef, roast 100 g			387	23.0	32.0
4	Beer, 1 pt.	8.2	16.5	173		
4	Beer, 12 oz	5.5	11.0	126		
1++	Beer, Kaffir 1 pt.		36.6	270	4.2	1.2
++++	Biltong 50 g			102	17.2	3.2
5	Biscuits (2) and Cheese 50 g		9.0	232	13.0	16.0
5	Biscuits, cheese (8)		32.5	300	7.0	8.0
5	Biscuits chocolate - one	3.0	7.2	45	.7	1.6
5	Biscuits plain - one		4.0	16		
5	Biscuits sweet - two	7.0	14.0	60	1.2	
5	Blancmange 100 g	7.9	15.9	111	3.5	3.9
++++	Boerewors 100 g			345	15.1	31.1
4	Brandy 1 oz			92		
+	Bredie 100 g		7.8	147	3.8	11.0
5	Bread 1 slice 28 g		16.8	90	2.9	1.0
5	Bread pudding 200 g	28.4	56.8	374	11.2	12.2
5	Brewers yeast 20 g		7.7	56	7.8	.2
+	Brinjal and tomato - fried 100g		8.8	113	2.1	8.5
5	Broccoli 100 g		4.5	26	3.1	.3
5	Brussel sprouts		6.4	36	4.2	.4
5	Butter 10 g			72		8.1
C						
5,2	Cabbage ½ cup 100 g	.3	4.3	20	1.1	.2
5,2	Carrots 100 g	1.7	7.2	32	1.0	.2
5	Cashew nuts 50 g		14.6	280	8.6	22.8
5	Cake, chocolate 100 g	27.9	55.8	369	4.5	16.4
5	Cake, fruit 100 g	29.9	59.7	379	4.8	15.3
5	Cake, pound 100 g	27.4	54.7	411	6.4	18.7
5	Cake, sponge 100 g	27.0	54.1	297	7.6	5.7
5	Cake, cupcakes, no icing 100g	27.9	55.8	350	4.9	12.0

Refs.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
5,2	Cauliflower 100 g	.3	4.1	22	2.3	.2
5	Cervelat 25 g		.4	77	4.6	6.1
5	Cheese, cheddar 50 g		1.0	200	13.0	16.0
3	Champagne 100 g		1.4	74		
5	Chicken, roast 100 g			157	31.6	3.4
5	Chicken, fried 100 g		2.9.	249	30.7	11.8
5	Chicken pie 100 g		18.3	235	10.1	13.5
5	Chocolate 20 g	7.0	13.5	90	1.0	4.2
5	Chocolate drink, 1 10 oz glass	17.8	35.6	279	11.1	11.5
5	Chocolate sauce 100 g	31.3	62.7	245	2.3	2.0
5	Chop Suey 200 g		10.2	240	20.8	13.6
3	Cinzano, 1 glass 3 oz	12.0	12.0	83		
3	Christmas pudding 100 g		45.7	325	4.8	14.4
5	Cocoa 1 cup	12.0	24.5	215	9.0	9.4
4	Coco-cola 230 g	14.0	28.0	105		
5	"Cold drink" 1 10 oz glass	6.0	11.4	44		
+++	Complan 50 g		22.0	225	30.0	2.0
+++	"Copenhagen"	20.0	45.6	422	7.4	23.5
5,2	Corn 100 g	.3	20.5	83	2.5	.5
5,2	Corn cob 200 g	.6	42.0	182	6.6	2.0
5	Cornflakes 30 g		28.4	129	2.6	.1
5	Corn fritters 100 g		39.7	377	7.8	21.5
5	Cornish pasty 200 g		37.6	492	20.2	29.0
5	Cottage cheese 50 g		1.3	43	8.5	.1
	Cottage pie: 50 g minced beef, 200 g mashed potato, 2 tablespoons gravy	.1	32.0	405	16.3	22.2
5	Crayfish 100 g		.3	95	18.7	1.5
5	Cream, heavy 100 g		3.1	352	2.2	37.6
3	Curry 150 g		13.0	252	11.8	16.6
3	Custard 100 g, to accompany fruit	12.0	20.0	114	4.2	2.0
3	Custard baked 100 g		9.9	113	5.2	5.9
5	Custard slice 100 g	25.0	49.9	302	5.0	9.4
D						
5	Dates 50 g		36.5	137	1.1	.3
5	Doughnuts 100 g	25.7	51.4	391	4.6	18.6
5,2	Dried stewed fruit 100 g	7.0	31.4	119	1.0	.3
5	Duck, roast 100 g			313	22.8	23.6
E						
5	Egg, fried, one		.3	108	6.9	8.6
5	Egg, raw, one 50 g		.4	81	6.5	5.8
+++	Egg sandwich filling		.7	184	3.6	18.9
5	Egg scrambled, 2 eggs 100 g		2.4	173	11.2	12.9

Refs.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
F						
5	Fish baked or boiled 200 g			340	57.0	10.6
5	Fishcakes (3) 200 g		18.6	344	29.4	16.0
5	Fish, fried 100 g		5.8	165	19.6	6.4
+++	Fish pie 200 g	.1	26.0	285	32.7	5.4
5	Fishsticks 100 g		6.5	176	16.6	8.9
+	Fruit dainties - one piece	14.0	18.0	72		
5	Fudge - one piece, 20 g	12.0	15.0	80	.5	2.5
G						
5	Garbanzo or chick peas cooked 100g		20.5	120	6.8	1.6
4	Gin 1 oz			83		
5	Ginger ale 8 oz	9.0	18.0	72		
5	Glucose 8 gms, 1 teaspoon		10.0	40		
3	Glucose sweets, one		20.0	80		
+++	Gluten patties 100 g		47.2	378	41.4	1.9
5,2	Grapes 100 g		17.3	67	.6	.3
5	Grapefruit, half 150 g		15.9	60	.7	.1
5	Grapefruit juice 100 g		9.2	39	.5	.1
5,2	Grapefruit segments, tinned 100g	8.9	17.8	70	.6	.1
5	Grape juice 100 g		13.3	53	.2	
5	Gravy, one tablespoon		3.0	66		6.0
5,2	Guavas, raw 100 g	1.9	15.0	62	.8	.6
5,2	Guavas in syrup 200 g	36.0	66.0	262	1.6	1.2
H						
5	Haddock smoked, steamed 100g			103	23.2	.4
5	Ham cold 100 g			289	20.9	22.1
5	Hamburgher (minced beef) 100g			286	24.2	20.3
5	Honey, 1 tablespoon 20 g	.4	16.5	62		
I						
5	Icecream ½ cup 100 g	16.6	20.6	207	4.0	12.5
5	Imperial mints 20 g	7.0	14.1	87	.7	3.4
5	Instant milk pudding 100 g	12.2	24.4	125	3.0	2.5
3	Irish stew 100 g		7.8	147	3.8	11.0
J						
3	Jam roll 100 g	55.0	56.0	327	3.0	10.0
3	Jam tart 100 g	31.4	62.7	394	3.8	15.4
5	Jelly 100 g	35.0	70.6	273		
K						
++++	Koeksister, one, 100 g	41.4	51.4	391	4.6	18.6

Refs.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
L						
5	Lamb chops, broiled 100 g			420	19.5	37.3
5	Lamb roast 200 g			558	50.6	37.8
5	Leeks 100 g		11.2	52	2.2	.3
5	Lentils 100 g		19.3	106	7.8	
5	Liver, calves, fried 200 g		8.0	522	59.0	26.4
5	Liver sausage 50 g		.9	154	8.1	12.8
	Lobster thermidor- 100 g crayfish, 25 g cheese, white sauce		9.6	357	29.1	22.0
M						
5	Macaroni cheese 200 g		40.2	430	16.8	22.2
5	Marmalade Jam 20 g	13.0	14.0	54	.1	
5	Mango 100 g		16.8	66	.7	.4
5	Marshmallow 100 g	40.0	80.0	319	2.0	
5	Mayonnaise, 1 tablespoon 20g		.5	143		16.0
++	"Meat" (Hospital porters) 100g			310	15.4	27.1
	Meat balls,(minced beef) 50g, 2 tablespoons gravy		6.0	275	12.1	22.1
++++	Melktert 100 g	12.0	23.4	218	6.1	11.1
5	Meringue, one	18.0	18.0	69		
1	Mealie meal, cooked, 100 g		10.7	50	1.1	.2
1	Mealie meal, dry 100 g		78.4	364	7.9	1.2
5	Milk, condensed 20 g	5.5	10.9	64	1.6	1.7
5	Milk, evaporated 100 g		9.7	137	7.0	7.9
5	Milk, skim 8 oz		15.3	108	10.8	.3
5	Milk, skim powdered 40 g		20.6	144	14.4	.2
5	Milk, whole 8 oz		11.0	148	8.0	8.3
5	Milk chocolate bar, 50 gms	21.5	28.5	275	3.9	16.2
	Milo, 1 cup 8 oz milk, 1 tsp. sugar, 10 g Milo	6.0	24.2	210	9.3	8.0
5	"Mixed vegetables" 100 g		13.7	65	3.3	.3
	Muesli - raw oats 25 g, Filbert nuts 25 g, Dried apple 25 g, Raisins 25 g (100g)		58.6	398	7.7	17.9
N						
5	Naartjie 50 g		5.8	23	.4	.1
5	Nectarine 100 g		17.1	64	.6	
O						
5	Oatmeal porridge 200 g		19.4	110	4.0	2.0
+	"Oil", Hospital porters 25 g			221		24.6
5	Oil, cooking 100 g			88		10.0
5	Omelette (2 eggs)		3.2	230	14.2	17.2
5	Onions 100 g		6.5	29	1.2	.1
5	Onions fried 100 g		10.1	355	1.8	33.3

Ref.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
5,2	Orange 100 g peeled	6.4	11.3	48	1.0	.1
5	Orange juice 6 oz	6.4	21.4	90	.4	.2
3	Ovaltine 30 g	12.0	24.1	128	4.4	2.1
P						
5	Pancakes (2) 50 g		17.0	116	3.6	3.5
5	Parsnips 100 g		17.5	66	1.5	.5
5	Pawpaw 200 g		20.0	78	1.2	.2
5	Peaches, dried 10 peaches		68.3	262	3.1	.7
5	Peaches, raw 150 g		14.5	57	.9	.1
5	Peaches, tinned 100 g	16.5	20.1	78	.4	.1
5	Peanut butter 10 g		2.0	58	2.6	5.0
5	Peanuts 50 g		9.4	292	13.0	24.9
5,2	Pears, fresh 100 g	1.5	15.3	61	.7	.4
5,2	Pears, tinned 100 g	11.0	19.6	76	.2	.2
5,2	Peas ½ cup 100 g	5.5	9.5	43	2.9	.2
5	Perlemoen 100 g		2.3	80	16.0	.3
5	Pineapple juice 6 oz		27.0	110	.8	.2
5	Pineapple, fresh 200 g		27.4	104	.8	.4
5,2	Pineapple rings, tinned 100 g	10.0	19.4	74	.3	.1
5	Plums, fresh 100 g		19.7	75	.8	.2
5	Plums, tinned 100 g	11.0	21.6	83	.4	.1
5	Polony		.3	113	6.0	9.5
5	Pork chops 100 g			373	22.6	30.6
5	Pork, roast 100 g			362	24.5	28.5
3	Port, 1 glass 3 oz		12.0	152		
5	Potato, baked 200 g		42.2	186	5.2	.2
5,2	Potato, boiled 100 g	.1	17.1	76	2.1	.1
5	Potato chips 1 oz 30 g		16.7	189	1.8	13.3
5,2	Potatoes, french fried, 100 g	.1	36.0	274	4.3	13.2
5,2	Potatoes, mashed ½ cup, 200 g	.1	26.0	130	4.2	.1
5,2	Potatoes, roast 200 g	.2	34.2	224	4.2	8.2
5	Potato salad, hard boiled egg, 100 g		13.4	145	3.0	9.2
5	Potato, sweet, baked 100 g		32.5	141	2.1	.5
5	Prunes 100 g		31.4	119	1.0	.3
	Pro-Nutro mix (Mr. Barberton) Pro-Nutro 30 g Jungle Oats 25 gms Brown sugar 2 teaspoons Sultanas 25 g	12.0	76.8	347	6.8	2.0
5	Provita Biscuits		25.4	115	4.3	.4
5,2	Pumpkin ½ cup 100 g	.6	7.9	33	1.0	.3
Q						
5	Quince, stewed 200 g	33.0	40.2	156	.8	.2
5	Quix toast 5 gms (rusk)		3.6	21	.7	.4
R						
5	Raisins 25 g		19.3	72	.6	
5	Raisin loaf 30 g		17.5	87	2.2	.9

Refs.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
5	Rice ½ cup 100 g		24.2	109	2.0	.1
3	Rice pudding 100 g	7.0	15.7	144	3.6	7.6
5	Roll, plain, one		20.0	104	3.3	1.0
5	Rusk 10 g		7.1	42	1.3	.8
S						
3	Sago pudding 100 g	11.0	20.9	127	3.1	3.7
+++	'Salads'		5.0	20		
5	Salami 25 g		.3	113	6.0	9.5
5	Salmon tinned 100 g			153	20.8	7.1
5	Sardines 100 g			203	24.0	11.0
5	Sauerkraut 200 g		8.0	36	2.0	.4
5	Sausage, pork and beef 100g			336	15.6	29.9
3	Sausage roll 100 g		18.8	246	10.1	14.5
5	Scones 100 g (two)	24.0	56.1	339	8.2	8.7
3	Semolina pudding 100 g	10.1	20.2	131	4.3	3.9
4	Sherry (medium) 3 oz			125		
3	Shortbread, 2 pieces 100 g	32.6	65.1	498	7.2	23.1
+	Snoek 100 g			203	24.0	11.0
5	Sole, fried 100 g			202	30.0	8.2
5	Soup, Asparagus 200 g		13.6	120	5.6	4.8
5	Bean 200 g		17.4	134	6.2	4.6
5	Lentil 200 g		27.1	170	12.0	.9
5	Pea (split) 100 g		8.4	59	3.5	1.3
5	Potato 200 g		15.0	152	6.4	7.8
5	Tomato 200 g		18.0	138	5.2	5.6
5	Vegetable 100 g		3.9	32	2.1	.9
5	Spaghetti Bolognaise 200 g		31.2	268	15.0	9.4
5	Spanish Rice 100 g		16.6	87	1.8	1.7
5	Spinach 200 g		4.2	26	3.4	.4
5,2	Squash ½ cup 100 g	.4	11.6	50	1.8	.4
++	Stamp mielies 100 g		18.8	83	3.2	1.0
3	Steak and kidney pie 100 g		18.8	246	10.1	14.5
5	Steak sirloin 100 g			387	23.0	32.0
++	Stew, Hospital porters 200 g		15.6	294	7.6	22.0
5	Sugar, one teaspoon 6 g	6.0	6.0	23		
5	Sweet melon 100 g		7.7	33	.8	.3
5	Syrup 20 gms or 1 teaspoon	6.2	14.0	53		
T						
5	Tomatoes, raw 100 g		4.7	22	1.1	.2
5	Tomato juice 100 g		4.3	19	.9	.1
+++	Tonic water 8 oz	16.0	16.0	77		
3	Trifle 200 g	22.4	44.8	300	6.6	11.2
5	Tripe 100 g			100	19.1	2.0
5	Tuna Fish 100 g			197	28.8	8.2
V						
5	Veal cutlet 100 g			234	26.4	13.4
	"Vegetables" Mr. Goy - potato 200 g, carrots 100 g, beans 100 g	2.4	46.8	209	6.8	.6

Refs.		Sucrose g	Total CH_2O g	Total Calories kCal	Protein g	Fat g
5	Vienna Sausage (3)		1.6	304	12.4	27.2
5	Venison 300 g			378	63.0	12.0
W						
5	Waffles, two 100 g		37.5	279	9.3	9.8
5	Watermelon 400 g		25.6	104	2.0	.8
5	Wheatabix, a cold cereal, one biscuit		26.0	100	3.3	
4	Whisky, 1 oz			83		
5	White sauce 100 g		8.8	162	3.9	12.5
3	Wine 100 g, 1 glass		4.2	85	.1	
Y						
5	Yoghurt 6 oz		9.8	124	6.0	6.8
3	Yorkshire pudding 100 g		27.0	218	7.1	9.4

Explanatory note on unusual dishes

\+ Traditional foods of the Cape Province

++ Foods eaten by the African people of Southern Africa

+++ Special food mixtures

++++ Traditional South African foods

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CHAPTER III - STATISTICAL METHODS

The following statistical methods have been employed in this study:

(1) Mean $(\bar{x})$ [7]

$$\bar{x} = \frac{\sum x}{n}$$

x = value of each observation

n = number of observations

(2) Standard Deviation (S.D.) [7]

$$S.D. = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

x = value of a particular observation

$\bar{x}$ = mean value

n = number of observations

(3) Standard error of the mean (S.E.M.) [7]

$$S.E.M. = \frac{S.D.}{\sqrt{n}}$$

S.D. = Standard deviation

n = number of observations

(4) Standard error of a single determination (S.E. of a single determination [4]

$$\text{S.E. of a single determination} = \sqrt{\frac{S.S. - G.T^2/n}{(n-1)}}$$

S.S. = Sum of the square of each observation

G.T. = Sum of the values of each observation

n = number of observations

(5) Coefficient of Variation (C.V.) [4]

$$C.V. = \frac{S.E.}{\bar{x}}$$

S.E. = Standard error of a single observation

$\bar{x}$ = Mean value for all observations

(6) Skewness of distribution (g_1)[7]

$$g_1 = \frac{K_3}{K_2 \sqrt{K_2}}$$

$$k_2 = \frac{S_2}{(n-1)} \qquad \text{and} \qquad k_3 = \frac{n\,S_3}{(n-1)(n-2)}$$

$$S_2 = s_2 - \frac{s_1^2}{n} \qquad \text{and} \qquad S = s - \frac{3\,s_1 s_2}{n} + \frac{2\,s_1^3}{n^2}$$

S_1 = Sum of the values of each observation

S_2 = Sum of the squares of the values for each observation

S_3 = Sum of the cubes of the values for each observation

$$\text{S.E. } g_1 = \sqrt{\frac{6n(n-1)}{(n-2)(n+1)(n+3)}}$$

$$t = \frac{g_1}{\text{S.E. } g_1}$$

Significance limits for t with ∞ degrees of freedom were obtained from Documenta Geigy Scientific Tables.

When the distribution of data is asymmetrical or significantly skewed, the mean and the median do not coincide.

(7) Correlation coefficient (r)[7]

$$r = \frac{S x_1 x_2}{\sqrt{(S x_1^2)(S x_2^2)}}$$

X_1 and X_2 are the two sets of observations and x_1 and x_2 are the deviations from the mean of observations X_1 and X_2

$S\,x_1\,x_2$ = The sum of the products of the deviations

$S\,x_1^2$ and $S\,x_2^2$ = The sum of the squares of the deviations from the mean

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

Significance limits for t with (n-2) degrees of freedom were obtained from Documenta Geigy Scientific Tables.

(8) Student's t test, as used for the comparison of paired observations[2]

$$t = \frac{\bar{x} - \mu}{S.D/\sqrt{N}}$$

$\bar{x}$ = Mean of the differences between each pair of observations

μ = 0

S.D. = Standard deviation

n = Number of pairs

The significance limits for t with (n-1) degrees of freedom were obtained from Documenta Geigy Scientific Tables.

Skewness (g_1) was computed on the University of Cape Town Electronic Computer (I.B.M. 1130), by means of a Fortran programme compiled by myself. The remaining calculations were carried out either by means of a desk calculating machine (FACIT) or by means of a desk computer (OLIVETTI DESK PROGRAMMA 203) using programmes compiled by Professor G.H. Menzies of the Land Surveying Department of the University of Cape Town.

(9) Wilcoxon test[6]

The Wilcoxon matched pairs signed ranks test was used for determining the significance of the difference between two related samples. Unlike the parametric t test, the Wilcoxon test does not assume a normal distribution. Significance limits for T (the smaller of the sum of like-signed ranks) were determined from Documenta Geigy Scientific Tables.

(10) Analysis of Variance[3,5].

The significance of differences amongst more than two groups was determined by the Analysis of Variance with a one-way layout. F values were computed on the University of Cape Town Electronic Computer (I.C.T. 1301) by means of a programme compiled in the Department of Mathematical Statistics. The

significance limits for F were determined by the ANOVA test[5] and the use of Documenta Geigy Scientific Tables.

In cases where the null hypothesis was rejected by the ANOVA test, the actual differences were found by the use of Scheffe's "S" method for multiple comparisons[3] as follows:

$$\sum_{1}^{r} c_i \mu_i \in \sum c_i Y_i \pm \left[(r-1) F^{\alpha}_{r-1, N-r}\right]^{1/2} S \left(\sum_{1}^{r} \frac{c_i^2}{n_i}\right)^{1/2}$$

In all statistical tests differences were regarded as significant when significant at the 5% level. Actual significance levels for individual tests are indicated in the text.

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P A R T II

EPIDEMIOLOGICAL FACTORS ASSOCIATED WITH SERUM TRIGLYCERIDE

CHAPTER I - INTRODUCTION

The association between high levels of cholesterol in the blood and the presence of coronary heart disease is well recognised and has been studied frequently for the past twenty years[1,48,75].

More recently, the Framingham prospective study has definitely shown that there is also a direct correlation between the cholesterol concentration and the future development of coronary heart disease[29,45,46]. Similar results have been obtained in other prospective studies[31,49,58,80]. This association has resulted in considerable importance being attached to investigations in which epidemiological and other factors influencing serum cholesterol were studied.

The relationship between elevated serum triglyceride levels and coronary heart disease is not as clear cut and will be reviewed in this study. Epidemiological and other factors affecting serum triglyceride levels have not been nearly as closely studied as the factors influencing serum cholesterol. Southern Africa is populated by a multi-racial community which provided the material for a valuable contribution to the understanding of the role of cholesterol in coronary heart disease and also of some of the factors influencing serum cholesterol levels[13]. It was felt that further study of this community might contribute to the available information on the factors influencing serum triglyceride levels.

CHAPTER II - REVIEW OF THE LITERATURE

(A) EPIDEMIOLOGICAL FACTORS INFLUENCING SERUM TRIGLYCERIDE LEVELS IN NORMAL PEOPLE

There are two principal difficulties associated with surveying serum triglyceride levels in population groups. Firstly, it is essential that triglyceride determinations be carried out on fasting blood samples, since Brown et al have reported that a 300 kCal meal, with as little as 10g of fat may result in an increase of serum triglyceride above the fasting level for 5 hours after it has been ingested[14]. A report has been published by Schilling et al that no change in serum cholesterol or triglyceride occurs for 4 hours after a 384 kCal breakfast containing 17g of fat[63]. Our findings are in keeping with those of Brown; viz. that at least in certain individuals, a very small amount of fat may result in postprandial elevations of serum triglyceride. Secondly, the recognised techniques for measuring serum triglyceride[20,78] and their modifications are laborious laboratory procedures and a satisfactory automated procedure has only recently been devised.

The epidemiological studies of serum triglyceride levels which have appeared in the literature may be divided into two groups. The first includes those in which the volunteers fasted for approximately 12 hours before blood sampling and in which an internationally accepted method[20,78] or a modification thereof was employed for the determination of serum triglyceride[6,7,8,11,14,15,18,23,26,34,59,62,65,79,81]. The second contains those in which the volunteers were not fasting or in which an internationally known method for triglyceride determination had not been employed[3,10,14,24,41,56,64,69,70,74]. Studies in which volunteers had not fasted are only of value if exceptionally low levels have been reported, since higher levels may well be due to the fact that the volunteers had eaten.

Workers in different parts of the world have expressed serum triglyceride concentrations in terms of different units. For the purpose of

comparison all triglyceride results have been expressed as mg triolein/100ml of serum. Values expressed in millimoles of tripalmitin per litre have been converted to milligrams triolein/100ml by means of the factor 88.5, and values expressed in mEq/litre, converted by use of the factor 29.5.

1. Race, Age, Sex and Serum Triglyceride

Inter-study comparisons are extremely difficult to make for two reasons. Firstly, different workers have split up their material into different age groups. Comparisons are complicated, therefore, in view of the great variation with age. Secondly, even identical laboratory techniques produce different results in different laboratories. Bearing these two difficulties in mind, certain generalisations may nevertheless be deduced.

The levels in males will be considered first. Table I has been drawn up using mean values (expressed as mg triolein/100ml) obtained in representative studies from different parts of the world.

TABLE I

DESCRIPTION OF POPULATION GROUP	New York, U.S.A. (10-13 years) (10)	Stockholm, Sweden (23)	Paris, France (45)	French Canadians Montreal, Canada (6)	Denmark (26)	Ireland (41)	N.I. Medical Students (14)	N.I. Older Men (15)	Johannesburg Europeans (7)	Johannesburg Africans (7)	Bolivia Europeans (81)	Bolivia Cape Coloureds (81)	Hawaiian Hawaiians (11)	Hawaiian Japanese (11)	New Zealand Maoris (51)	Rarotongans (59)	Pukapukans (59)	Samburu Tribe, N. Kenya 1st Survey (69)	Samburu Tribe, N. Kenya 2nd Survey (70)	Nomadic Tribe (70)	Turkana Tribe (70)	Bushmen (64)
AGE GROUP 15-19 yrs	75 (632)	71 (3)																	121 (21)	210 (3)		
20-24 yrs		101 (15)	84 (47)	123 (19)	88 (10)	83 (13)	100 (36)		86 (16)	80 (26)	104 (30)	78 (30)						85 (102)	120 (47)	174 (16)	120 (10)	
25-29 yrs		123 (33)		130 (36)																		
30-34 yrs		120 (71)	127 (131)	183 (35)	104 (14)	105 (19)			97 (12)	73 (16)									104 (22)	154 (24)	132 (10)	
35-39 yrs		127 (179)		210 (34)																		
40-44 yrs		140 (289)	132 (110)	189 (56)	117 (22)	109 (16)			174 (7)	66 (9)					251 (26)	119 (35)	82 (44)		115 (42)	136 (25)	137 (16)	49 (9)
45-49 yrs		140 (267)		208 (45)									188 (42)	184 (68)				85 (99)				
50-54 yrs		131 (202)	162 (220)	185 (55)	103 (25)	102 (12)		171 (1711)	174 (8)	68 (6)									119 (32)	129 (19)	144 (17)	
55-59 yrs		126 (126)		161 (47)																		
60-64 yrs		117 (55)	136 (260)	155 (62)					139 (3)										136 (19)	118 (15)		
65-69 yrs		112 (21)		160 (66)		101 (10)																
70-74 yrs		76 (5)																				

Mean serum triglyceride levels in normal males in different age groups throughout the world. Results are expressed in mg triolein/100ml. In studies where triolein has not been used as standard[14,15,23,41,59] the results have been converted using 88.5 as the factor to convert from mMol. tripalmitin/l and 29.5 where the results have been expressed in mEq/l.

The number of subjects studied in each group is given in brackets below the mean value.

II. I.

The pattern in Western urbanised groups throughout the world appears to be fairly constant. In the under-20 year old group a mean value of 75 has been found in a large group of New York school children aged 10-13[10] and a mean value of 71 in a small group of 15-19 year old men in Stockholm[23].

In the 20-29 year old group, mean values have increased and levels varying from 83-130 have been reported. Levels of approximately 85 were recorded in Ireland[41], Denmark[26], Paris[65] and Johannesburg[7].

(47 taxidrivers make up the group studied in Paris but the other three represent

only three small groups of individuals). Larger groups were studied in Stockholm[23], New York[14], Montreal[6], and Bellville, South Africa[81]. In New York and South Africa, the mean values were found to be 100 and 104 respectively. In Stockholm, levels of 101 were found in the 20-24 year old group and 127 in 25-29 year olds. In Montreal, where French-Canadians were studied, a mean of 123 was found in the 20-24 year olds and 130 in the 25-29 year olds.

All the western population groups studied show an increase through the 30-39 age group to the 40-49 age group, though once again there is some variability in the actual values. The exception to this is the Montreal group where the highest value (210) was found in the 35-39 group. This mean value is also higher than the value reported for any other westernised group. It is interesting to note the sudden increase in the Johannesburg group from 97 in the 30-39 year old group to 174 in the 40-49 year old group; but this study unfortunately represents only a small group of people.

Triglyceride levels in the 50-59 year old men started to decrease in Stockholm - 131 and 126, Montreal - 185 and 161, Denmark - 103, Ireland -102; but remained elevated in Johannesburg - 174. In Paris levels were at their highest in this age group - 162. These levels correspond fairly closely to the level of 171 found in 1851 men in this age group surveyed in New York[15].

In the 60-69 year olds mean values had decreased further; in Stockholm - 117 and 112, Montreal - 155 and 160, Ireland - 101 and had also started to decrease in Johannesburg - 139 and Paris -136.

All these studies clearly show the upward trend of serum triglyceride levels with age, reaching a plateau level in most cases between 40-49 years of age and then starting a downward trend. A similar trend was demonstrated in another study carried out by Schaefer in New York[62] but could not be included in Table I since median rather than mean values are given and in view of the skewed distribution of serum triglycerides, these cannot be compared. The

Seventh Day Adventists surveyed in conjunction with this group[79] tended to have somewhat lower serum triglyceride levels than fellow New Yorkers of comparable age and seem to show the age trend but not to the same extent. Seventh Day Adventists tend to eat less meat and animal fat than the average American and do not drink alcohol.

An attempt to compare the few non-Westernised groups which have been surveyed with each other and with the Westernised groups is extremely difficult. African miners in Johannesburg were surveyed and were found to have levels comparable with Johannesburg Europeans in the 15-29 year old group[7]. In the older group of Africans, however, there was no increase above this level with age, whereas a considerable increase was found in the European group. Interpretation of these results is complicated by the fact that the miners had completed an 8-hour shift of work in the mine shafts shortly before the blood samples were collected and vigorous exercise has been clearly shown to result in a fall of serum triglyceride[56].

Three groups of Polynesians living under different conditions have been compared[59]. New Zealand Maoris who are completely adapted to the Western way of life were found to have a mean triglyceride value of 251, whereas Raratogans who have been exposed to Western civilisation for only approximately 10 years, had a mean value of 119. Pukapukans on the other hand, who have not been exposed to any Westernising influence at all have a mean value of 82. The groups are fairly small (26-44 individuals) and a wide range of ages is covered (20-69). The level found in the New Zealand Maoris is very hard to explain since it is considerably higher than any other group which has been surveyed in any part of the world and there are not values for New Zealand Europeans available for comparison.

Three African tribes in Northern Kenya have been surveyed. The Samburu tribe who exist mainly on meat and cows' milk were surveyed for the first time in April 1961[69]. Their diet is high in saturated animal fat,

but their total calorie intake is difficult to assess. In the course of herding their cattle, these people get a great deal of exercise and very often travel for long distances by foot in search of grazing. Not surprisingly, therefore, the mean serum triglyceride levels were in the low range, despite the fact that the subjects surveyed were not fasting - 85 in the 20-29 year old age group, and the same level in the 35-59 year old group. Two fairly large groups were studied. In February 1962, these people were surveyed again, together with two other Northern Kenyan tribes - the Rendille and Turkana who live under similar conditions[70]. The diet of the Rendille is similar to that eaten by the Samburu, except that camels' milk rather than cows' milk is drunk. The Turkana tribe practice some agriculture in addition to keeping cattle, so their diet contains at least some carbohydrate. Once again, the subjects were not fasting. The levels shown in Table I for the Samburu tribe bear little resemblance to those obtained in the first survey of these people. The lowest values on this occasion are 30 mg/100ml higher than on the previous occasion and no explanation is offered by the author as to why this should be the case. There is no variation with age in this group. The values obtained in the Turkana are similar though a smaller number were studied. In the Rendille tribesmen of 20-29 years, a mean value of 174 was recorded which decreased gradually with age. The levels in several age groups of this tribe are considerably higher than those found in most Westernised groups. In view of the fact that these are not fasting levels, no conclusions can be deduced. It is interesting to note that serum cholesterol levels in the Rendille are higher (233) than in the Samburu (190) and Turkana (185). No cases of coronary heart disease were however reported.

On report on serum triglyceride levels in the Bushman appears in the literature[64]. The mean value in 9 Bushmen was found to be 49 mg/100ml. In 7 Bushmen blood was taken some hours after a work capacity test had been performed. This may have resulted in somewhat lower levels than would otherwise

have been found.

In Bellville, South Africa[81] Cape Coloured males were found to have a level of 78 - considerably lower than Europeans of a comparable age. Cape Coloured labourers were studied. These people probably consume a diet which is similar to that eaten by Europeans in the same environment with regard to the proportions of the proximate food constituents. Their standard of living is, however, generally considerably lower.

In Hawaii, healthy Hawaiian and Japanese men were found to have similar serum triglyceride levels - 184 for the whole group aged 35-64 years[11]. Hawaiians exceeded the Japanese in each of several indices for overweight, despite the fact that they engaged in heavier work. No dietary data for the two groups is available.

All these studies indicate a fairly clear pattern in Westernised groups, but there is clearly a need for further studies in which non-Westernised groups are compared with each other and with Westernised groups. The volunteers should be fasting and the serum triglyceride determinations carried out by the same method in the same laboratory.

There is considerably less information available concerning serum triglyceride levels in females. Table II summarises some of the studies which have been conducted.

TABLE II

DESCRIPTION OF POPULATION GROUP	New York, U.S.A. (34)	Ireland (41)	Denmark (26)	Bellville Europeans (81)	Bellville Cape Coloureds (81)	Paris, France (65)	Johannesburg, S. Africa (7)	Stockholm, Sweden (23)
AGE GROUP 15-19 yrs								81 (8)
20-24 yrs	73 (13)		65 (9)	78 (30)	73 (13)	83 (10)		85 (39)
25-29 yrs		82 (22)					69 (18)	89 (37)
30-34 yrs	65 (15)		78 (16)			76 (27)		96 (32)
35-39 yrs							65 (5)	101 (112)
40-44 yrs	111 (19)		87 (18)			98 (22)		95 (269)
45-49 yrs								109 (208)
50-54 yrs	114 (19)		87 (13)			105 (17)		113 (152)
55-59 yrs		85 (20)						112 (69)
60-64 yrs	103 (14)					85 (20)		108 (18)
65-69 yrs								96 (4)
70-74 yrs	124 (13)							122 (1)

TABLE II

Mean serum triglyceride levels in normal females. The table has been compiled in the same way as Table I.

II. II.

Where levels in male and female have been compared in the same group, values for the females have tended to be lower than those found in the males[7,23,26,65,81]

The age trend for triglyceride described above for males is also apparent in

in females[23,65]. Two studies showed the upward trend of age but in these studies there was no downward trend after the plateau had been reached. In only one study was no relationship found between age and triglyceride level[41]. Ovarian function has been offered as an explanation for these lower levels noted in young females[34]. This does not explain however the fact pointed out in the two major studies in which male and female levels have been compared[23,65] that even older and postmenopausal women tend to have lower values than males in comparable age groups.

Two fairly large studies have not been included in the above discussion. In an early study carried out by Albrink et al[3], 508 healthy males and females were investigated. Comparison with other groups is made difficult as no mean values are given. Triglyceride levels have been expressed as mEq/ triglyceride fatty acid per litre and calculated in an indirect way by subtracting the sum of cholesterol ester fatty acid and phospholipid fatty acid from total serum fatty acids.

A group of nearly two thousand males and females were studied in New York by Schilling et al[64]. As the volunteers were not fasting, no definite conclusions can be drawn.

2. Frequency distribution of serum triglyceride

Carlson and Linstedt tested the distribution of serum triglyceride levels by calculating the skewness of the distribution in the different age classes[23]. He found the distribution to be significantly skewed in all classes of males except the 15-19 year old group and the groups between 60-74 years of age. In females the distribution in the below 30 year old group and above 60 year old group were not skewed. The skewness was considerably more pronounced for the triglyceride values than for the cholesterol values. The frequency distribution was normalised in almost all age classes after logarithmic transformation and Carlson therefore concluded that there was no evidence for the presence of two populations.

Schaefer[62] found a noticeable skewness in the frequency distribution of triglyceride concentrations of men aged 30-79 years. A second peak was found between 180 and 200 mg/100ml and this second population comprised 12% of the total number. Logarithmic transformation was not attempted.

Antonis and Bersohn[7] interpreted the considerable standard deviation which they found in their group of older European males to be indicative of the presence of a second population. Their numbers though are really too small for such assumptions to be made. A skewed distribution was also found by Feldman et al in females over the age of 35 years in New York[34]. This distribution was normalised by logarithmic transformation.

3. Normal values for serum triglyceride

The definition of normal values is indeed a difficult task. Hayes and Neill[41] and Albrink and Man[2] have suggested that twice the standard deviation above the mean be regarded as the upper limit of normal for fasting serum triglycerides and quote as their values for males 166 and 159 mg/100ml, respectively. Hayes and Neill suggest a level of 130 mg/100ml for females. Brown et al[14] have established 95% confidence limits for the lipid levels in young adults and suggest 153 mg/100ml as the upper limit of normal for serum triglyceride. These studies have been based on the levels found in 20-29 year old adults and in view of the considerable variation with age shown in nearly all surveys performed on healthy individuals, it is not at all clear whether older sections of the population can be expected to conform to these normal limits. Carlson[19] has established 90% confidence limits for his control population which covers a wider range of ages (46-65 years) and has suggested a level of 185 mg/100ml.

The definition of an upper limit of normal for serum triglycerides is of dubious value since the significance of elevated triglyceride levels is still uncertain. However, awaiting the outcome of studies such as the Stockholm Prospective Study[23] which may clarify the significance of elevated

serum triglyceride levels, normal values may probably be best determined by establishing 95% confidence limits for individual age groups in any population.

Probably the most frequently quoted normal range is that introduced by Fredrickson et al[35], who were also responsible for the introduction of the widely used classification of the hyperlipoproteinaemias.

4. Correlation between triglyceride and cholesterol

A low grade correlation between serum cholesterol and triglyceride has been demonstrated in normal males[18] and in men with coronary heart disease[64]. The fact that only a low grade correlation exists is not surprising since cholesterol and triglyceride are contained chiefly in different lipoprotein classes - the cholesterol chiefly in the beta (low density) and the triglyceride in the pre-beta (very low density) lipoprotein. Gofman et al found that there was no correlation between the concentration of low density (Sf0-12) and very low density lipoproteins (Sf20-400)[38]. Other workers have not been able to demonstrate any correlation between cholesterol and triglyceride[3,41].

5. Physical activity and serum triglyceride

Holloszy[43] has clearly shown that regular physical conditioning reduces the level of serum triglyceride. Six months of training resulted in the lowering of serum triglyceride from a pre-training mean value of 208 to a level of 125 at the end of study. This exercise induced reduction of serum triglyceride appears to be due to an acute effect occurring within 2 to 3 hours after exercise and lasting approximately 2 days. He concluded therefore that serum triglyceride could be kept at a significantly lower level by regularly performing endurance exercises. Similar results, including a fall in the very low density lipoproteins were reported by Hoffman[42].

It has furthermore been shown that physical exercise after a meal reduces the postprandial rise in serum triglyceride[27,57] as well as the level of endogenous plasma triglycerides[21]. Carlson and Linstedt[23] have reported lower triglyceride values in men reporting increased physical activity during time

off work, whereas no significant difference was found between lipid values of subjects reporting various degrees of activity during working hours. There was no correlation between physical activity and serum triglyceride in females.

Allard and Goulet[6] found that physical activity on or off the job as assessed by a questionnaire was not significantly related to serum triglyceride. However, mean values appeared to be lowest in those subjects reporting heavy physical activity during working hours or regular and sustained sporting activity.

6. Relative body weight and serum lipids

There appears to be a significant relationship between relative obesity and serum triglyceride levels. Albrink et al[4] found a definite correlation between triglyceride levels and a weight gain of more than 4.5 kg over the age of 25 years and furthermore suggested[3] that acquired obesity had a stronger association with elevated serum triglyceride than natural obesity. Trunkal obesity characterises acquired obesity whereas inherited obesity is characterised by obesity of the extremeties. Therefore skinfold thickness in the forearm reflecting lifelong obesity is not correlated with elevated serum triglycerides in the way that increased skinfold thickness over the trunk correlates with weight gain after the age of 25 and elevated serum triglyceride levels. Allard and Goulet[6] showed a similar correlation between serum triglyceride and those who had gained more than 4.5 kg after the age of 25 years. They also found that an increase in weight greater than 13.5 kg had no further effect on serum triglyceride. The same phenomenon has been shown in females[34].

Carlson and Linstedt[23] showed that males referred to their obese group had serum triglyceride levels significantly higher than healthy males. Triglyceride levels were not, however, different in obese and non-obese females. "Obesity" was diagnosed when the weight/height index was 1.1 or more for subjects with a height of less than 180 cm, and 1.05 or more for subjects with a height of above 180 cm[36].

Lewis et al[54] found low but significant correlations between concentration of triglyceride-rich lipoproteins (Sf12-20 and 20-100) and weights. A correlation between triglyceride levels and actual weight has also been shown by Allard and Goulet[6].

7. Smoking and serum triglyceride

Carlson and Linstedt[23] noted that younger and middle-aged smoking males had significantly higher triglyceride levels than non-smokers and Gofman et al[39] that very low density lipoprotein was higher in young males smoking more than 20 cigarettes a day. Pozener and Billimaria[60] found significantly increased levels of pre-beta lipoprotein in heavy smokers (more than 50 cigarettes per day) but the triglyceride levels, while higher in this group, were not significantly elevated.

In short term experiments, the smoking of two cigarettes has no effect on the serum triglyceride level[47] but the smoking of several cigarettes after a meal results in smaller postprandial rises in triglyceride after a meal than is found in non-smokers[51].

8. Position at work and serum triglyceride

Men in supervisory capacities up to the age of 50 years were shown to have slightly higher levels of serum triglyceride[23]. It is of interest to note here that emotional stress can increase serum triglyceride levels[22].

9. Seasonal variation and serum triglyceride

Only two studies have been reported in which seasonal variation of serum triglyceride have been studied. In young males in the Antarctica[9] no seasonal variation was found. Carlson and Linstedt[23] have reported lowest triglyceride levels in summer, tending to increase during the autumn.

(B) SERUM TRIGLYCERIDE IN ISCHAEMIC HEART DISEASE

Albrink and Man performed two early studies[2,3] in which fasting serum lipids of normal controls were compared with patients who had suffered from a myocardial infarction one day to 12 years prior to study. 5% of males

between the ages of 20 and 29 years and 36% of healthy men over the age of 32 had triglyceride levels of more than 159 mg/100ml (their suggested upper limit of normal). 82% of patients, however, with coronary artery disease had levels above this value. High serum cholesterol concentration did appear to increase the risk of coronary artery disease in persons with high serum triglyceride levels but by themselves carried little risk except in the small number of individuals suffering from coronary artery disease below 50 years of age. They concluded, therefore, that an abnormality of serum triglyceride was the principal lipid disturbance in coronary artery disease.

Antonis and Bersohn[7] in a very much smaller study, showed elevated serum triglyceride when patients one week after a myocardial infarction were compared with healthy controls.

In 1960 Carlson[18] compared fasting serum lipids in 49 males who had had an infarct 6 months previously with healthy controls and found that in those subjects under the age of 50 years, triglycerides were elevated to a greater extent than cholesterol whereas in older individuals this pattern was reversed.

An invaluable study was carried out in Sweden by Tibblin and Cramer[76] who followed fasting serum lipid patterns during the course of an acute myocardial infarction and one year afterwards. They showed quite clearly that serum triglyceride increased from the second day after the infarct to the twentyfirst day after which time levels started to decrease. Initial levels were reached only 12 months after the infarct.

Hayes and Neill[41] found serum triglycerides were more frequently raised in myocardial infarction patients than was the serum cholesterol level. The reverse was true in those patients with angina pectoris.

Brown[15] studied 1851 males between 50 and 65 years, 140 of whom had suffered from a mild myocardial infarction at least three months previously. He found that the mean serum triglyceride values in the myocardial infarction group were not significantly different from the controls when the whole group was

considered. On the basis of increased triglyceride or cholesterol levels, a high level of either was associated with increased prevalence of ischaemic heart disease, but whereas the condition was highly prevalent in individuals with increased cholesterol and normal triglycerides, an increased triglyceride level with normal cholesterol did not appear to be associated with an increased incidence of coronary heart disease. Unlike other workers, therefore, he concluded that elevated serum triglycerides were not more useful than elevated serum cholesterol when predicting coronary heart disease.

Rifkind et al[61] studied 98 males who had suffered from myocardial infarction two months to several years before their investigation, and 169 controls. They found that an elevated serum cholesterol or triglyceride level alone was found with equal frequency in patients with ischaemic heart disease. The commonest pattern by far was an elevated serum cholesterol and triglyceride.

All these studies in which the lipid pattern of patients who had suffered from myocardial infarction has been studied, should be interpreted in the light of the information supplied by Tibblin and Cramer[76]. Definitive data on the role of elevated serum triglyceride in coronary heart disease can only be expected from prospective studies such as the one being conducted in Stockholm[23] and the results of these are eagerly awaited.

(C) SERUM PRE-BETA LIPOPROTEIN IN HEALTH AND ISCHAEMIC HEART DISEASE

In 1955 Dangerfield and Smith[28] devised a system of paper electrophoresis for fractionation of serum lipoproteins in which, in addition to the alpha and beta lipoprotein, a pre-beta band could sometimes be identified after staining with Sudan black. In 1957, Smith[72] employed this technique to study normal people, patients with fresh and old myocardial infarctions, peripheral arterial disease and hypertension. The pre-beta band was graded subjectively according to the intensity of stain and the extent of the pre-beta band in relation to the serum protein. She found a strong correlation between the occurrence of the pre-beta lipoprotein band and age and sex. Approximately

80% of normal males and females under 30 years had no pre-beta lipoprotein. A moderate amount was present in the remainder of this age group. No females over the age of 30 were studied but the prevalence of the pre-beta lipoprotein in males increased rapidly with age and a moderate band was found in 70% of males over the age of 50 years. A large pre-beta band was found in only one normal male. The prevalence and degree of the abnormality were little greater in a comparable age group with peripheral vascular disease, hypertension or even angina and myocardial infarction three months previously. However, 90% of patients examined within three months after an infarct showed some, and 50% a large amount of pre-beta lipoprotein. The electrophoretic lipoprotein components were correlated with the ultracentrifugal lipoprotein group of Gofman et al[38] and the pre-beta was found to correspond approximately with the Sf20-400 group. Utilising the same technique, other workers[12] have obtained identical results.

These findings are in keeping with the observations of Dodds and Mills who demonstrated an increase in triglyceride-rich lipoprotein (Sf20-100 and 100-400) after recent myocardial infarction[30].

Serum lipoprotein concentrations have been studied in South African Bantu and White subjects with and without ischaemic heart disease[44]. Values for the Sf0-12 and 12-20 lipoproteins are lower in Bantu than White. These findings are not surprising in view of the lower serum cholesterol found in the Bantu[13]. Values of the Sf20-100 fraction were too variable for statistical analysis, but values for the Sf100-400 fraction tended to be higher in the Bantu. However, this is probably explained by the fact that this lipoprotein fraction is particularly affected by not fasting[37] and that while none of the volunteers in this study were instructed to fast before blood sampling, the Europeans who were sampled between 8 a.m. and 9 a.m. are more likely to have been postprandial than the Bantu who were sampled between 3 p.m. and 4 p.m. The Europeans with ischaemic heart disease were found to have higher mean values of Sf0-12 and 12-20

than normal.

Lees and Hatch[53] have achieved a clear separation of the pre-beta lipoprotein band (corresponding to Gofman Sf20-400 group) by using albumin containing buffer, and have demonstrated that with the use of their technique chylomicrons remain at the origin of the strip. Fredrickson et al[35] have utilised this technique to define five different hyperlipoproteinaemic states. Triglycerides are transported in the serum chiefly as chylomicrons (present in the serum of normal people only after the ingestion of a fatty meal and also in individuals with Types I and V hyperlipoproteinaemia of Fredrickson) and in the pre-beta or very low density lipoprotein (Sf20-400). The triglyceride carried in this fraction represents that synthesized in the liver. Smaller amounts of triglyceride are carried in the low density and high density lipoproteins. Types I and V are rather rare and an elevation of fasting serum triglyceride is therefore almost always due to an increase of the pre-beta lipoprotein fraction. This abnormality has been labelled as carbohydrate induced hyperlipoproteinaemia. (Type IV hyperlipoproteinaemia of Fredrickson). There does seem to be a predisposition to coronary heart disease in this condition, but there is surprisingly little information available as to the frequency of its occurrence[32]. It is particularly difficult to decide where the dividing line exists between the normal state and the disease entity, since as Smith has demonstrated at least some healthy people demonstrate the presence of a pre-beta lipoprotein band[72].

Brown and Doyle[16] have utilised the improved technique to show the prevalence of a faint pre-beta lipoprotein band in 30% of healthy American males aged 20 to 25 years. In the same study, 76% of healthy men aged 50 to 65 years and 84% of males in the same age group with coronary heart disease exhibited a pre-beta band of varying intensity. A positive correlation was observed between the intensity of staining of the pre-beta band and serum triglyceride level. There is clearly a need for information regarding the

frequency of occurrence of carbohydrate induced hyperlipoproteinaemia and to determine the confines of this condition.

(D) DIETARY FACTORS INFLUENCING SERUM TRIGLYCERIDE

The role of diet in determining serum triglyceride levels will be discussed in greater detail in Part III. However, certain general remarks are in place in a section considering the epidemiological factors influencing serum triglyceride levels.

The study which sparked off a great deal of interest in the role of diet was not in itself an epidemiological study. In 1960 Antonis and Bersohn[7] studied the long term effects of two different diets on Bantu and European prisoners in Johannesburg. The volunteers (both Bantu and European) were maintained on a "Bantu-type" diet for a period of approximately 9 months. This type of diet comprises of approximately 15% protein, 15% fat and 70% carbohydrate. At the end of the 9 month period, both European and Bantu had triglyceride levels which resembled those found previously in the South African Bantu population (87 mg/100ml)[8].

The volunteers were then placed on a diet which, with regards proportions of proximate food constituents, resembled that eaten by the Europeans in this country (15% protein, 40% fat, 45% carbohydrate). One-third of the volunteers were given fat chiefly in the form of butter, another third received their fat as sunflower seed oil and the final third as partially hydrogenated sunflower seed oil. This diet was given over the course of one year. Those volunteers receiving the sunflower seed oil showed no change in their triglyceride levels during the course of this year. In those consuming the butter and partially hydrogenated sunflower seed oil, however, somewhat different results were noted. No change was present after 8 weeks but after 22 weeks triglyceride levels had shown significant increase and after a year had increased to levels considerably above those suggested as normal by these authors (124 mg/100ml on partially

hydrogenated sunflower seed oil and 127 mg/100ml on butter). There was no difference in the response shown by Bantu and European prisoners. The serum triglyceride fatty acid pattern in the group receiving sunflower seed oil changed by as early as 8 weeks after the institution of the second diet. There was a percentage increase of linoleic acid and a percentage decrease of palmitic, palmitoleic and stearic acids.

After a year on this second diet, the volunteers returned to their original diets. In all cases triglyceride levels showed a transient elevation which returned to the initial levels after variable time intervals. The African volunteers had all returned to their previous level after 14 weeks, whereas the majority of the Europeans had decreased the triglyceride levels by 17 weeks but many only returned to their initial level after 32 weeks. It was suggested that the more rapid adaptation by the Bantu might be due to the fact that they were accustomed to this form of diet.

One might infer from this study that population groups consuming a high carbohydrate (relatively low in sucrose) and low fat diet such as was eaten by the rural Bantu of Southern Africa[73] or alternatively one high in poly-unsaturated fat might have low serum triglyceride levels.

Shaper et al, however, found low levels of serum triglyceride in all age groups in the Samburu tribe of Northern Kenya[69] and these people consume a diet consisting of approximately 60% animal fat. The principal constituents of their diet are cows' milk and meat. They are, however, a pastoral people and get a great deal of exercise in the search of grazing for their cattle. Caloric balance may be the operative factor in maintaining low levels of serum triglyceride. These results are made rather confusing by the fact that he surveyed this tribe a second time a year later[70] at more or less the same time of the year and once again after a very dry winter. This time higher levels were found than had been found the previous year and no explanation was offered for this. The Turkana people inhabiting the same area but who practice

some agriculture as well, were found to have similar triglyceride levels. The Rendille tribe who consume a similar diet except that camels' milk rather than cows' milk is drunk, were found to have still higher triglyceride and cholesterol levels. These people were found to have greater skinfold thicknesses at several sites than the other tribes and the author suggests that caloric balance may be the deciding factor. However, the skinfold thicknesses recorded in the Rendille people are very much lower than those recorded in American students and yet the triglyceride levels found in the Rendille are higher than those reported in many sections of the western world. As the volunteers in this study were not fasting no definite conclusions can be drawn.

Serum triglycerides are known to have a relatively characteristic and constant fatty acid pattern[55] which to a large extent reflects the fatty acid pattern of the diet[8,16,30,35,36,40,50,52,55,66,73,82]. The linoleic acid has been shown to increase from 10-30% of total fatty acids within four days after the institution of a diet high in corn oil[68].

In view of the hypothesis that people consuming diets high in polyunsaturated fat should have lower triglyceride values, it should be of interest to examine the fatty acid pattern of serum triglyceride of people consuming different diets and attempt to relate these to the serum triglyceride levels.

The serum lipid levels and fatty acid patterns of young male Guatemalans and urbanised North American Negroes and Whites have been compared[40]. The Guatemalans were found to have lower serum triglyceride (and cholesterol) levels than Negroes and Whites of North America and the triglyceride fatty acid pattern showed an increased level of linoleic acid in the Guatemalans as compared to the North Americans. There was a slightly increased amount of arachidonic acid in the North Americans.

An interesting study was performed by Scott et al[66] in which they studied 6 groups of people living in Korea, each consuming different quantities and varieties of fat. Serum triglyceride fatty acid patterns were found to

resemble the fatty acids of the diet. In particular, serum triglyceride linoleate seemed to reflect the percentage of polyunsaturated fat in the diet. The authors suggest that serum triglyceride levels were proportional to total fat intake, but do not point out that the groups consuming a greater amount of fat had also the highest caloric intakes. Varying degrees of physical activity and the fact that the volunteers were not fasting, once again makes interpretation of these results difficult.

Shorland et al[71] have published information on the total serum fatty acid patterns of Polynesians living on Pukapuka, Raratoga, and New Zealand (Maoris) and also New Zealand Whites. Dietary fat comprised approximately 40% of total calories in all groups. Coconut oil (rich in lauric and myristic acids) form the principal fat of the Pukapukans and Rarotogans whereas the New Zealanders consume chiefly ruminant fats. Lauric and myristic acids were not found to be elevated in the Polynesians though they showed an elevated palmitic acid. The actual measurement of serum lipid concentration and fatty acid patterns of individual serum lipid fractions may have yielded interesting results.

It seems unlikely that the precise role of diet in determining serum triglyceride levels can ever be determined from epidemiological studies, nevertheless interesting information is to be gained from studies where serum triglyceride and serum triglyceride fatty acids are determined in groups of people consuming different diets.

(E) PRINCIPAL OBJECTIVES FOR THIS PART OF THE STUDY

The principal objective for this part of the study was to clarify further the epidemiological factors which influence serum triglyceride by a study of several of Southern Africa's multi-racial population groups. It was furthermore hoped to obtain some indication of the frequency of occurrence of carbohydrate induced hyperlipoproteinaemia.

CHAPTER III - DESCRIPTION OF THE GROUPS STUDIED

This investigation was confined chiefly to the study of males and representative groups were selected from Southern Africa's multi-racial community. The groups are listed in Table III.

TABLE III

Group	Number in Group	Ages (in years)	Dates	Description of Group
A	62	16 - 18	June, 1969	Schoolboys (Europeans)
B	70	20 - 25	February, March, 1969	4th Year Male Medical Students (European)
C	51	35 - 55	March, April, 1969	Male Office Workers (European)
D	41	35 - 60	December, 1969	Physically active males (European)
E	43	25 - 50	April, 1970	Rural Labourers (African)
F	14	24 - 38	August, 1969	4th Year Male Medical Students (African)
G	19	21 - 26	August, 1969	4th Year Male Medical Students (Indian)
H	50	35 - 55	May, June, 1969	Hospital Porters (African)
I	28	18 - 74(?)	July, 1969	Bushmen
J	12	20 - 23	February, March, 1969	4th Year Female Medical Students (European)
K	5	22 - 24	August, 1969	4th Year Female Medical Students (Indian)

Four groups of South African Europeans were surveyed. Fasting blood samples were collected from 62 schoolboys in June, 1969. They were all boarders at the Diocesan College in Rondebosch and their ages ranged between 16 and 18 years. They were not physically examined by the investigators but had all been pronounced fit by their school doctor, who had examined them during the first half of 1969. They were all physically active, participating in organised sporting activities on at least three afternoons per week. (They constituted Group A).

In February and March, 1969, 70 fourth year medical students (aged 20-25 years) were surveyed at the University of Cape Town. None gave any history of present or past serious illnesses, and in addition to collecting a fasting blood sample from each student, information was obtained as to the degree of extra-mural physical activity of each volunteer. (They constituted Group B).

Groups C and D consisted of older groups of European males. Office workers from the Head Office of the Old Mutual Insurance Company in Pinelands with ages ranging from 35-55 years made up Group C. When questioned as to their activity during leisure hours, only 11 of this group of sedentary workers were found to take regular physical exercise. Enquiries were also made about their smoking habits. The values given for the serum triglyceride and cholesterol of this group represent the mean of three fasting blood samples collected from them during March and April 1969. Group D on the other hand comprised 41 members of the South African Mountain Club in more-or-less the same age group. Most of these volunteers were employed in sedentary occupations but all participated in regular physical exercise during their leisure hours. None of the subjects in Groups C and D reported any serious illnesses.

The way of life of these four European groups is similar to that of any "Westernised community" anywhere in the world. A dietary assessment of Groups C and D was made and is shown in Table IV, and the results illustrated

graphically in Figure 1.

TABLE IV

TABLE IV DIETARY ANALYSIS OF GROUPS C, D, E AND H.

Group	Total Daily Caloric Intake	TOTAL CARBOHYDRATE			SUCROSE			NON-SUCROSE CARBOHYDRATE			FAT			PROTEIN		
		Grams	Cals	% total cals	Grams	Cals	% total cals	Grams	Cals	% total Cals	Grams	Cals	% total cals	Grams	Cals	% total cals
C	2588 (571.0)	297.5	1190	46.0 (7.4)	88.8	355	13.7 (5.3)	209.0	836	32.3 (4.3)	107.9	971	37.5 (4.7)	106.8	427	16.5 (3.2)
D	2655 (493.8)	316.5	1266	47.7 (8.1)	89.0	356	13.4 (4.1)	227.8	911	34.3 (5.2)	109.1	982	37.0 (3.9)	101.5	406	15.3 (4.1)
E	2200	440	1760	80	33	132	6	407	1628	74	20	176	8	55	220	10
H	2447 (611)	266.0	1064	43.5 (5.8)	55.8	223	9.1 (3.2)	210.5	842	34.4 (4.7)	107.4	967	39.5 (5.1)	104.0	416	17.0 (3.5)

Mean daily values are indicated for the total caloric intake (Standard deviation in brackets).

The amount in grams, the caloric content and the percentage of total caloric intake derived from the proximate food constituents are also expressed as mean daily values. (The standard deviation of the percentage of total calories provided by each constituent is indicated in brackets).

II. IV.

Three groups of Africans living in Southern Africa were studied. Fasting blood samples were collected from 43 Pondos aged 25-50 years the day after their arrival in Tongaat (situated on the north coast of Natal). This group of men (who formed Group E), had been recruited to work as sugar-cane cutters in the sugar-cane fields, but in view of their recent arrival from Pondoland, they may be regarded as representative of rural Africans living in the Transkei (one of South Africa's Bantustans). Life for these people is hard as there is little employment and the majority of the group surveyed were employed chiefly in the herding of a limited number of cattle and the cultivation of small plots of maize. The dietary assessment of these people is very difficult, particularly with regards the total caloric intake. The figures

given in Table IV and Figure 1 can at best be regarded as a very rough estimate. One can but state that the diet has an extremely high proportion of complex carbohydrate calories and is low in sucrose and fat. Animals who have died (or been run down by motor cars on the unfenced highways of the Transkei) provide the only source of meat and it is difficult to even guess at the adequacy of the protein content of their diet or the total caloric intake.

Fourteen African medical students from the University of Natal, Durban medical school comprised Group F. All reported regular physical activity during leisure hours. A dietary history was not obtained from these students, but from my personal experience I should say that it does not differ significantly from the ordinary diets consumed by westernised population groups. It should be noted that these students were somewhat older than the European students studied.

Fifty African porters aged 35-55 were studied at Groote Schuur Hospital. These men (Group H) provide an interesting group for study. Some were born in the Transkei but all worked in the city for many years. A reader not personally acquainted with this section of the population might be misled into thinking that their occupation involved a considerable degree of physical activity. Anyone who has observed these people at work for any length of time, however, will be well aware that this is not the case. Trolleys are pushed for relatively short distances at an excessively slow pace and a five minute wait for the hospital lift is always considered preferable to a journey involving two flights of stairs. Dietary assessment on the other hand, is more difficult than the assessment of their physical activity. An attempt was made to obtain by the method of recall, three-day diet histories from 12 volunteers in this group. They seemed most reluctant to provide this information and it is therefore very likely that the values for total caloric intake for this group given in Table IV, err on the low side. It is, however, interesting to note that the percentage of total calories provided by sucrose is lower in this group than in Groups C and D.

Two further groups of males have been studied. Nineteen Indian medical students from the University of Natal form Group C. Only two of them reported regular leisure time physical activity. Dietary details of this group are not available; however, it is a wellknown fact that the Indian diet is high in polyunsaturated fat. Furthermore, since for the greater part, the students studied represent the more privileged section of the Indian community, it is probably reasonable to assume an adequate total daily caloric intake,

A group of !Kung bushmen living in the Kalahari desert comprise Group I. Blood samples were collected by Dr. A.S. Truswell during a visit to the Kalahari in July 1969. This is the only group studied from whom fasting blood samples were not obtained. However, blood was in most cases collected in the morning before the main meal of the day. The Bushmen are very physically active people, often travelling long distances in search of food. Edible wild fruits and plants and the seeds of some of these play an important role in their diet. Ricinodendron rautanenii (Mongongo nuts) and Bauhinia esculenta (Tsi nuts), both usually eaten after roasting, are important dietary constituents and contain a high percentage of polyunsaturated fatty acids[33]. Mongongo nuts in addition, contain approximately 20% of a fatty acid only recently identified as a eleostearic acid[25]. Meat is a comparative luxury and only eaten after a kill which takes place usually less frequently than once a week.

Two very small groups of females were studied, in both cases 4th year medical students, Twelve were studied in Cape Town in conjunction with Group B (Group J) and five in Durban (in conjunction with Group G (Group K).

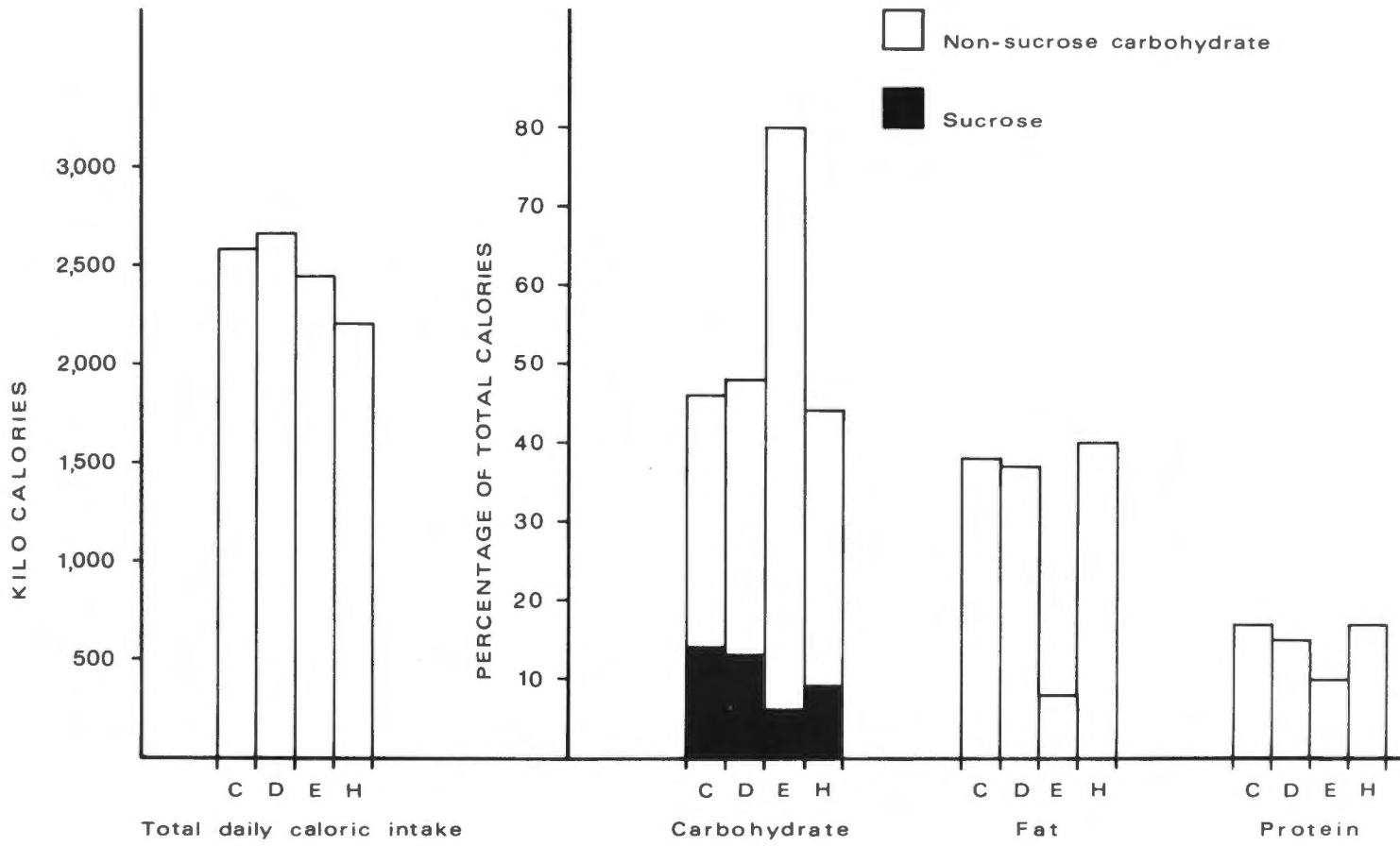


Figure 1: Total daily caloric intake and percentage of total calories derived from each of the proximate food constituents in Groups C, D, E and H.

CHAPTER IV - RESULTS OF THE SURVEYS CONDUCTED

(A) METHODS

Serum triglyceride and cholesterol levels were estimated on each of the 395 blood samples collected. All samples were collected after an overnight (12 hour) fast except in the case of the Bushmen, when samples were collected during the course of the morning, before the main meal of the day. Lipoprotein electrophoresis was carried out on all samples except the 28 samples collected from the Bushmen. Serum phospholipid was measured on 10 serum samples from each of Groups A, C, D, E and H. The fatty acid pattern of the serum triglyceride fraction was determined on twelve serum samples randomly selected from each group. These determinations were carried out as described in Part I of this study.

Volunteers in Group B and C were divided into three groups according to the frequency of physical activity reported during time off work. Regular physical activity was defined as physical activity at least twice weekly, occasional physical activity as physical activity approximately once a week and no regular physical activity where the volunteer was physically active less frequently than every week.

The subjects in Group C were also divided into three groups according to their smoking habits - non-smokers, moderate smokers (less than 20 cigarettes per day) and heavy smokers (more than 20 cigarettes per day).

Body weights are available for those volunteers in Groups D and E. The statistical significance of differences amongst groups was determined by the analysis of variance. The actual differences were found using Scheffe's "S" method for multiple comparisons, as described in the section of Part I dealing with statistical methods. In view of the asymmetrical distribution of serum triglyceride which is reduced after logarithmic transformation, both triglyceride concentration and the logarithms of the triglyceride concentration have been used in the statistical calculations.

Differences were regarded as statistically significant when significance was found at the 5%, or lower level ($p < 0.05$).

(B) FREQUENCY DISTRIBUTION OF VALUES FOR SERUM CHOLESTEROL AND TRIGLYCERIDE IN GROUPS A - K.

The frequency distributions of values for serum cholesterol concentration, serum triglyceride concentration and the logarithms of serum triglyceride concentrations in the different groups are given in Tables V, VI and VII and Figures 2, 3 and 4.

TABLE V

TABLE V FREQUENCY DISTRIBUTION OF CONCENTRATION OF CHOLESTEROL IN EACH GROUP

Cholesterol mg/100ml	A		B		C		D		E		F		G		H		I		J		K	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
000-79	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
80-89	0	0.0							2	4.7	0	0.0			1	2.0	3	10.7				
90-99	0	0.0							1	2.3	0	0.0			0	0.0	2	7.1				
100-109	0	0.0							5	11.6	0	0.0			0	0.0	3	10.7				
110-119	0	0.0							1	2.3	0	0.0			3	6.0	1	3.6				
120-129	3	4.8							6	14.0	0	0.0			3	6.0	6	21.4				
130-139	4	6.5							5	11.6	0	0.0	1	5.3	1	2.0	4	14.3				
140-149	3	4.8	1	1.4			1	2.4	5	11.6	0	0.0	0	0.0	2	4.0	3	10.7				
150-159	1	1.6	6	8.6			0	0.0	6	14.0	0	0.0	1	5.3	5	10.0	3	10.7	1	8.3		
160-169	10	16.1	8	11.4			1	2.4	3	7.0	0	0.0	1	5.3	3	6.0	1	3.6	1	8.3	1	20.0
170-179	9	14.5	11	15.7	2	3.9	1	2.4	4	9.3	2	40.0	0	0.0	3	6.0	2	7.1	1	8.3	0	0.0
180-189	9	14.5	20	28.6	0	0.0	0	0.0	2	4.7	0	0.0	1	5.3	4	8.0			5	41.7	4	80.0
190-199	7	11.3	11	15.7	2	3.9	1	2.4	1	2.3	1	20.0	4	21.1	3	6.0			0	0.0		
200-209	5	8.1	6	8.6	4	7.8	8	19.5	2	4.7	0	0.0	3	15.8	2	4.0			2	16.7		
210-219	1	1.6	5	7.1	3	5.9	2	4.9			1	20.0	0	0.0	4	8.0			1	8.3		
220-229	4	6.5	0	0.0	3	5.9	4	9.8			1	20.0	0	0.0	2	4.0			0	0.0		
230-239	3	4.8	1	1.4	9	17.6	6	14.6					2	10.5	3	6.0			0	0.0		
240-249	1	1.6	0	0.0	6	11.8	2	4.9					3	15.8	2	4.0			1	8.3		
250-259	0	0.0	1	1.4	4	7.8	5	12.2					0	0.0	1	2.0						
260-269	1	1.6			6	11.8	3	7.3					2	10.5	1	2.0						
270-279	0	0.0			5	9.8	0	0.0					1	5.3	3	6.0						
280-289	0	0.0			2	3.9	4	9.8							0	0.0						
290-299	0	0.0			0	0.0	2	4.9							0	0.0						
300-309	0	0.0			1	2.0	0	0.0							2	4.0						
310-319	0	0.0			2	3.9	1	2.4							2	4.0						
320-329	1	1.6			1	2.0																
330-339					0	0.0																
340-349					0	0.0																
350-359					1	2.0																

N = Number of subjects

TABLE VI

TABLE VI

FREQUENCY DISTRIBUTION OF CONCENTRATION OF TRIGLYCERIDE IN EACH GROUP

Triglyceride mg/100ml	A		B		C		D		E		F		G		H		I		J		K	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
000 - 49	1	1.6	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
50 - 59	8	12.9	0	0.0	0	0.0	2	4.9	5	11.6	0	0.0	1	5.3	2	4.0	0	0.0	0.	0.0	1	20.0
60 - 69	15	24.2	4	5.7	3	5.9	3	7.3	6	14.0	1	7.1	2	10.5	4	8.0	4	14.3	0	0.0	2	40.0
70 - 79	9	14.5	3	4.3	3	5.9	5	12.2	3	7.0	6	42.9	4	21.1	5	10.0	5	17.9	4	33.3	0	0.0
80 - 89	10	16.1	16	22.9	1	2.0	5	12.2	8	18.6	0	0.0	1	5.3	4	8.0	7	25.0	3	25.0	1	20.0
90 - 99	3	4.8	10	14.3	4	7.8	4	9.8	6	14.0	3	21.4	3	15.8	3	6.0	2	7.1	0	0.0		
100 - 109	5	8.1	10	14.3	3	5.9	5	12.2	4	9.3	0	0.0	2	10.5	3	6.0	4	14.3	5	41.7		
110 - 119	4	6.5	6	8.6	3	5.9	2	4.9	4	9.3	1	7.1	0	0.0	3	6.0	3	10.7				
120 - 129	3	4.8	7	10.0	6	11.8	4	9.8	4	9.3	0	0.0	1	5.3	6	12.0	2	7.1				
130 - 139	1	1.6	7	10.0	4	7.8	2	4.9	0	0.0	3	21.4	1	5.3	5	10.0	1	3.6				
140 - 149	1	1.6	1	1.4	1	2.0	5	12.2	1	2.3			0	0.0	3	6.0						
150 - 159	1	1.6	3	4.3	5	9.8	3	7.3	1	2.3			1	5.3	2	4.0					1	20.0
160 - 169	0	0.0	3	4.3	1	2.0	0	0.0	0	0.0			0	0.0	3	6.0						
170 - 179	0	0.0			2	3.9	0	0.0	0	0.0			0	0.0	0	0.0						
180 - 189	0	0.0			3	5.9			1	2.3			1	5.3	0	0.0						
190 - 199					2	3.9							0	0.0	2	4.0						
200 - 209					0	0.0							0	0.0	1	2.0						
210 - 219					2	3.9							1	5.3	1	2.0						
220 - 229					1	2.0							0	0.0								
230 - 239					1	2.0							1	5.3								
240 - 249					1	2.0																
250 - 259					0	0.0																
260 - 269	1	1.6			1	2.0																
270 - 279					0	0.0																
280 - 289					0	0.0																
290 - 299					1	2.0																
300 - 309					1	2.0																
310 - 319					0	0.0																
320 - 329					0	0.0																
330 - 339					0	0.0																
340 - 349					0	0.0									1	2.0						
350 - 359					1	2.0																
360 - 369					0	0.0																
370 - 379					0	0.0																
380 - 389					0	0.0																
390 - 399					1	2.0																
400 - 409																						
410 - 419																						
420 - 429																						
430 - 439																						
440 - 449																						
450 - 459															1	2.0						
460 - 469																						

N = Number of subjects

TABLE VII

TABLE VII

FREQUENCY DISTRIBUTION OF LOG CONCENTRATION OF TRIGLYCERIDE IN EACH GROUP

Triglyceride Log mg/100ml	A		B		C		D		E		F		G		H		I		J		K	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
0.0000-1.6499	1	1.6																				
1.6500-1.6999	2	3.2							1	2.3			1	5.3	1	2.0					1	20.0
1.7000-1.7499	3	4.8					2	4.9	3	7.0			0	0.0	1	2.0						
1.7500-1.7999	9	14.5	2	2.9	3	5.9	1	2.4	4	9.3			0	0.0	2	4.0	2	7.1				
1.8000-1.8499	9	14.5	2	2.9	0	0.0	3	7.3	3	7.0	2	14.3	2	10.5	3	6.0	2	7.1			2	40.0
1.8500-1.8999	9	14.5	3	4.3	3	5.9	4	9.8	3	7.0	5	35.7	4	21.1	4	8.0	5	17.9	4	33.3	0	0.0
1.9000-1.9499	10	16.1	16	22.9	1	2.0	5	12.2	8	18.6	0	0.0	1	5.3	4	8.0	7	25.0	3	25.0	1.	10.0
1.9500-1.9999	3	4.8	10	14.3	4	7.8	4	9.8	6	14.0	3	21.4	3	15.8	3	6.0	2	7.1	0	0.0	0	0.0
2.0000-2.0499	7	11.3	11	15.7	4	7.8	5	12.2	6	14.0	0	0.0	2	10.5	3	6.0	5	17.9	5	41.7	0	0.0
2.0500-2.0999	5	8.1	10	14.3	7	13.7	4	9.8	6	14.0	1	7.1	1	5.3	6	12.0	3	10.7			0	0.0
2.1000-2.1499	2	3.2	9	12.9	5	9.8	4	9.8	0	0.0	3	21.4	1	5.3	8	16.0	2	7.1			0	0.0
2.1500-2.1999	1	1.6	4	5.7	6	11.8	8	19.5	2	4.7	0	0.0	1	5.3	5	10.0	-				1	20.0
2.2000-2.2499	0	0.0	3	4.3	2	3.9	0	0.0	0	0.0	0	0.0	0	0.0	3	6.0						
2.2500-2.2999	0	0.0			6	11.8	0	0.0	1	2.3			1	5.3	2	4.0						
2.3000-2.3499	0	0.0			2	3.9	0	0.0					1	5.3	2	4.0						
2.3500-2.3999	0	0.0			3	5.9	0	0.0					1	5.3	0	0.0						
2.4000-2.4499	1	1.6			1	2.0	0	0.0							0	0.0						
2.5000-2.5499					2	3.9	0	0.0							0	0.0						
2.5500-2.5999					1	2.0	1	2.4							2	4.0						
2.6000-2.6499					1	2.0									0	0.0						
2.6500-2.6999															0	0.0						
															1	2.0						

N = Number of subjects

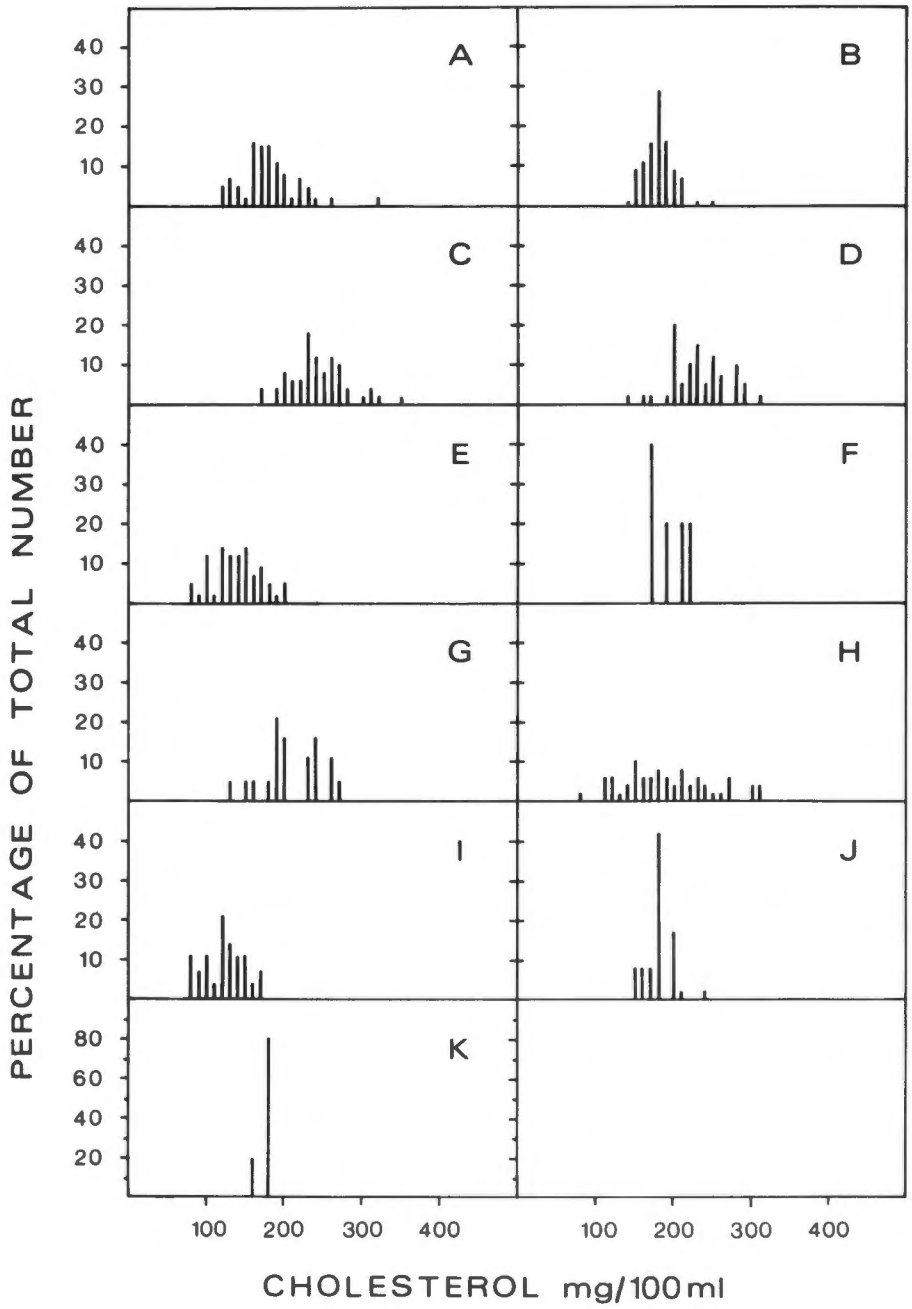


Figure 2: Distribution of values for serum cholesterol concentration in Groups A to K.
Class interval 10 mg/100ml.

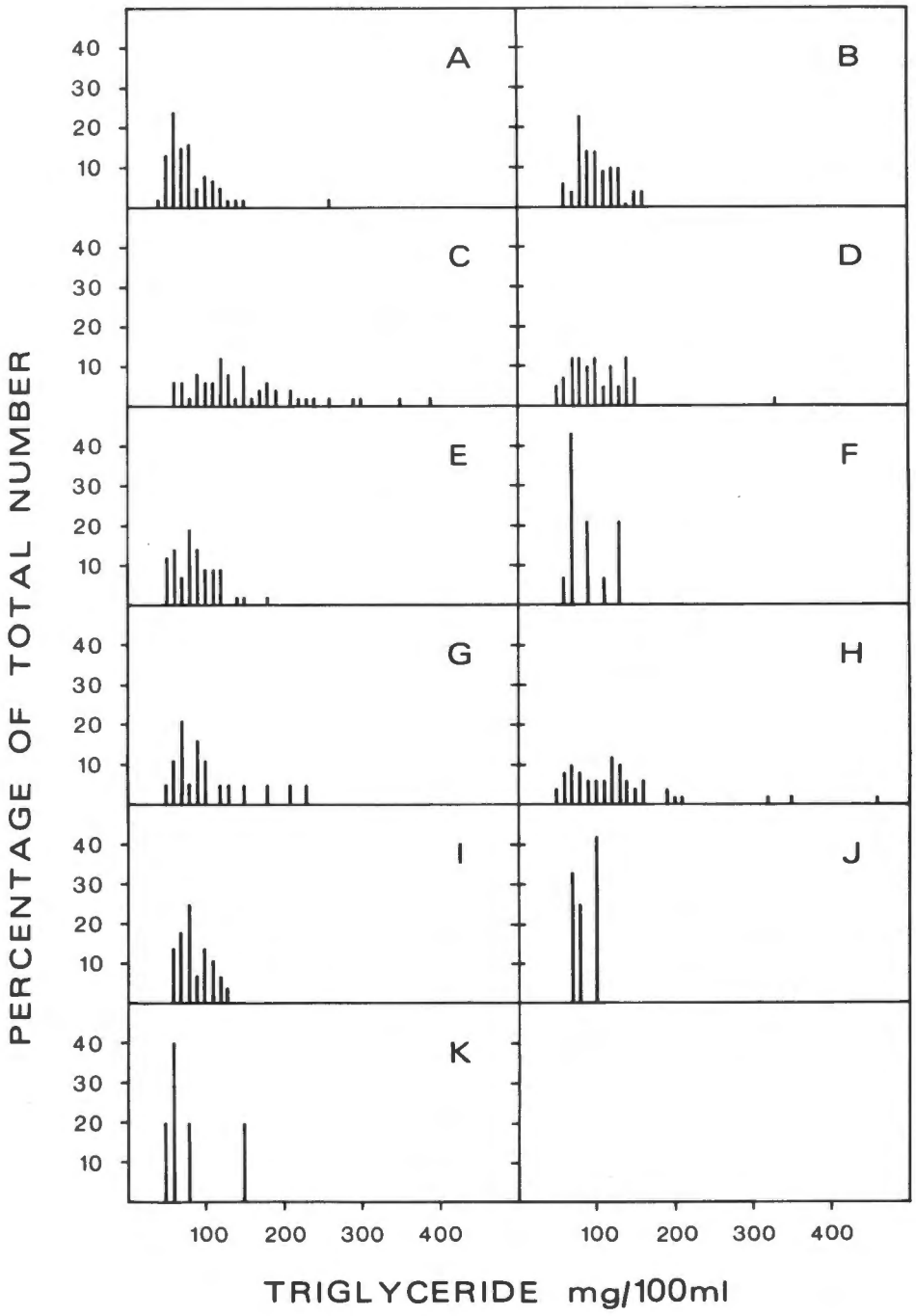


Figure 3: Distribution of values for serum triglyceride concentration in Groups A to K.
Class interval 10 mg/100ml.

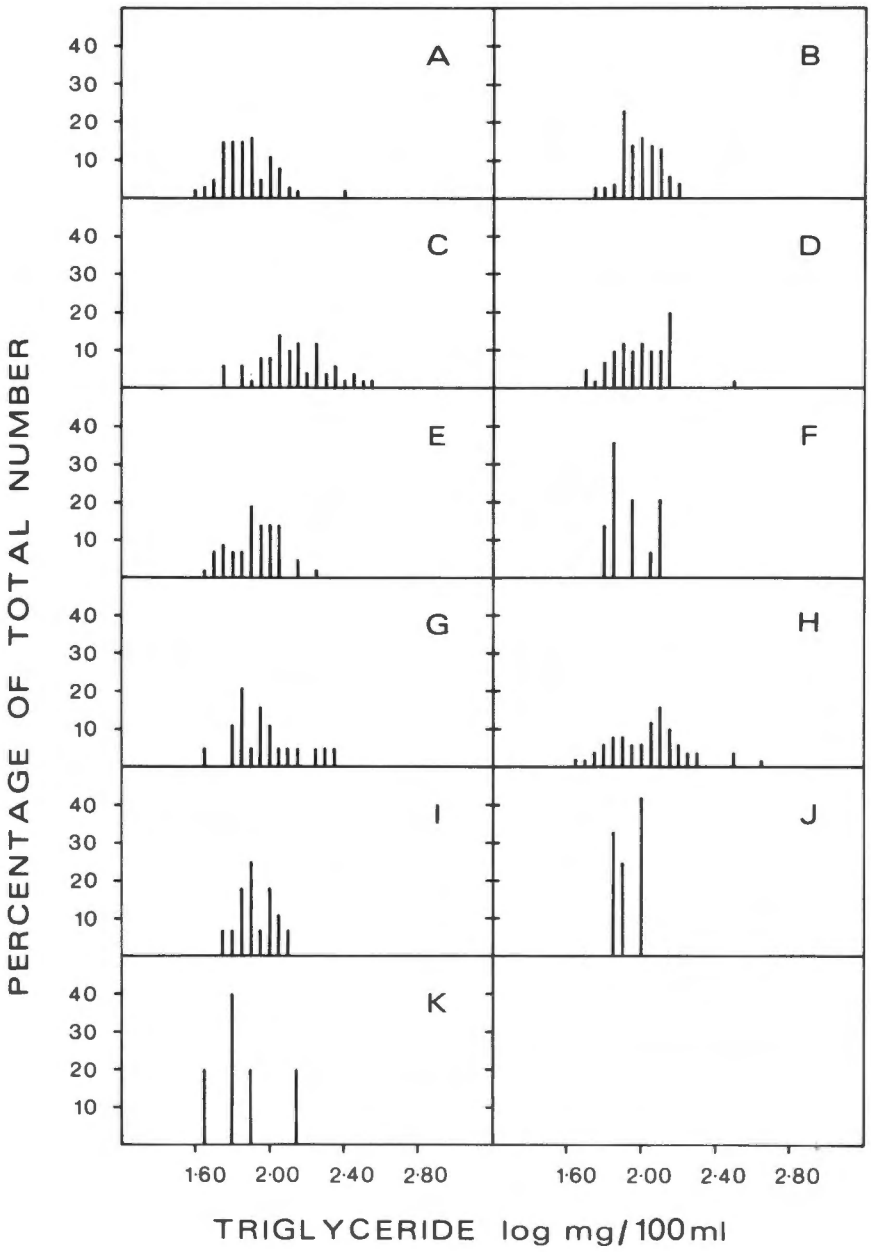


Figure 4: Distribution of value for the logarithm of serum triglyceride concentration in Groups A to K.
Class interval 0.05 log mg/100ml.

The frequency distributions were tested for skewness and the statistical index of skewness, g_1 , its standard error and significance limits are given in Tables VIII, IX and X together with the mean values for each group.

TABLE VIII

TABLE VIII CONCENTRATION OF CHOLESTEROL AND INDEX OF SKEWNESS OF DISTRIBUTION OF VALUES WITHIN EACH GROUP

Group	Mean (mg/100ml)	Median (mg/100ml)	Range (mg/100ml)	Standard Deviation	Standard Error of Mean	Skewness (g_1)	Standard Error of g_1	Significance limits for g_1
A	183.6	180.0	120 - 265 (+ 329)	35.9	4.5	1.14	0.30	$p<0.001$+
B	185.3	184.0	142 - 253	19.6	2.3	0.56	0.29	N.S.
C	247.4	246.0	175 - 351	36.8	5.2	0.46	0.33	N.S.
D	235.7	235.0	145 - 310	37.3	5.8	-0.04	0.37	N.S.
E	143.0	142.0	87 - 209	30.1	4.6	0.18	0.36	N.S.
F	197.4	197.0	170 - 226	23.9	10.8	0.06	0.91	N.S.
G	213.1	205.0	134 - 275	39.2	8.9	-0.13	0.52	N.S.
H	197.4	188.5	87 - 314	57.3	8.1	0.33	0.34	N.S.
I	127.8	129.0	81 - 172	25.4	4.8	-0.12	0.44	N.S.
J	191.3	185.0	155 - 240	23.2	6.7	0.69	0.64	N.S.
K	181.6	184.0	168 - 187	7.9	3.5	-1.85	0.91	$p<0.05$

+ When skewness is calculated omitting the one cholesterol value of 329 mg/100ml, $g_1 = 0.25$ which is not statistically significant.

II. VIII.

The distribution of values for serum cholesterol was significantly skewed in only two groups - Groups A and K. In Group K there are only five subjects, so this information is of little import. In Group A there is only one serum cholesterol value which is considerably higher than the rest. If the skewness of the distribution is recalculated excluding this single high value, the value of g_1 , is reduced from 1.14 - 0.25 which is no longer statistically significant.

TABLE IX

TABLE IX CONCENTRATION OF TRIGLYCERIDE AND INDEX OF SKEWNESS OF DISTRIBUTION OF VALUES WITHIN EACH GROUP

Group	Mean (mg/100ml)	Median (mg/100ml)	Range (mg/100ml)	Standard Deviation	Standard Error of Mean	Skewness (g_1)	Standard Error of g_1	Significance Limits for g_1
A	85.1	77.5	44 - 150 (+ 263)	33.3	4.2	2.79	0.30	$p < 0.001$*
B	105.8	100.0	62 - 168	25.8	3.1	0.57	0.29	$p < 0.05$
C	155.1	139.0	61 - 390	72.2	10.1	1.30	0.33	$p < 0.001$
D	110.0	104.0	56 - 157 (+ 337)	46.8	7.3	2.89	0.37	$p < 0.001$**
E	92.0	85.0	50 - 182	28.6	4.4	0.91	0.36	$p < 0.01$
F	94.5	85.0	64 - 137	25.9	6.9	0.80	0.60	N.S.
G	112.2	97.0	50 - 238	52.9	12.1	1.28	0.52	$p < 0.01$
H	132.2	122.5	56 - 462	75.5	10.7	2.52	0.34	$p < 0.001$
I	91.5	88.0	60 - 130	19.5	3.7	0.36	0.44	N.S.
J	90.9	85.5	74 - 107	13.8	3.9	0.22	0.64	N.S.
K	85.0	67.0	50 - 156	42.0	18.8	1.69	0.91	N.S.

II. IX.

When skewness is calculated omitting the one triglyceride value of 263 mg/100ml, $g_1 = 0.91$, $p < 0.01$.

When skewness is calculated omitting the one triglyceride value of 337 mg/100ml, $g_1 = 0.18$ which is not statistically significant.

A more pronounced skewness was found in the distribution of values for serum triglyceride concentration (g_1 = 0.22 - 2.89) and in all groups except Groups F, I, J and K the skewness was statistically significant. In all cases the mean value was greater than the median. It is of interest to note that in two groups (A and D) there were single isolated triglyceride values far beyond the range for the group. When the value of g_1 for Group A was recalculated omitting the single high value, skewness was reduced from 2.79 to 0.91. The significance limits of g_1 were reduced but the distribution was still significantly skewed ($p < 0.01$). In Group D, when this procedure was carried out, the value of g_1 was reduced from 2.89 - 0.18 and the distribution

was no longer significantly skewed.

TABLE X

TABLE X LOG CONCENTRATION OF TRIGLYCERIDE AND INDEX OF SKEWNESS OF DISTRIBUTION OF VALUES WITHIN EACH GROUP. FOR CLARITY OF PRESENTATION VALUES OBTAINED FOR MEAN, STANDARD DEVIATION AND STANDARD ERROR OF MEAN HAVE BEEN RECONVERTED TO THEIR ANTILOGS. THIS RESULTS IN DIFFERENT VALUES FOR ONE STANDARD DEVIATION AND STANDARD ERROR ABOVE AND BELOW THE MEAN, REFLECTING THE ASYMMETRICAL DISTRIBUTION OF THE ARITHMETIC VALUES

Group	Mean		Standard Deviation		Standard Error of Mean		Skewness (g_1)	Standard Error of g_1	Significance Limits for g_1
	Log (mg/100ml)	Antilog (mg/100)	Log (mg/100ml)	Antilog (mg/100ml)	Log (mg/100ml)	Antilog (mg/100ml)			
A	1.9059	81	0.1374	+ 30 - 21	0.0173	+ 3 - 3	0.97	0.30	$p < 0.01$
B	2.0188	104	0.1039	+ 29 - 22	0.0100	+ 3 - 2	0.03	0.29	N.S.
C	2.1490	141	0.1900	+ 77 - 50	0.0264	+ 9 - 8	0.16	0.33	N.S.
D	2.0130	103	0.1509	+ 43 - 30	0.0223	+ 6 - 5	0.70	0.37	N.S.
E	1.9441	88	0.1307	+ 31 - 23	0.0173	+ 4 - 3	0.14	0.36	N.S.
F	1.9613	92	0.1122	+ 26 - 21	0.0300	+ 7 - 6	0.56	0.60	N.S.
G	2.0106	102	0.1841	+ 55 - 35	0.0412	+ 11 - 9	0.58	0.52	N.S.
H	2.0718	118	0.1977	+ 68 - 43	0.0264	+ 7 - 7	0.70	0.34	$p < 0.05$
I	1.9517	90	0.0921	+ 21 - 16	0.0173	+ 3 - 3	0.02	0.44	N.S.
J	1.9540	90	0.0648	+ 14 - 13	0.1730	+ 4 - 4	0.01	0.64	N.S.
K	1.8937	78	0.1884	+ 43 - 27	0.0842	+ 17 - 13	0.89	0.91	N.S.

The skewness was reduced in all groups after the transformation of the triglyceride concentration values to their logarithms; therefore both the triglyceride concentrations and the logarithms of the triglyceride concentrations have been used in the statistical calculations in this work.

(C) SERUM TRIGLYCERIDE CONCENTRATION

1. Mean levels in different groups

Mean serum triglyceride levels for the groups studied are given together

with the median, range, standard deviation and standard error of the mean in Table IX. The results are illustrated graphically in Figure 5. Mean log concentrations of the serum triglyceride with standard deviation and standard error of the mean are given in Table X. For clarity of presentation, the values for the antilogarithms are also indicated. This results in different values for one standard deviation and standard error above and below the mean, reflecting the asymmetrical distribution of the arithmetic values.

The mean triglyceride value of Group C is significantly higher than all other groups and the mean level in Group H, while being significantly lower than Group C, is significantly higher than the remaining groups. While there is no statistically significant difference between serum triglyceride in all other groups, it is of interest to note that the lowest levels are found in Group A, with slightly higher values in Groups E, I and F. The values for Groups B, G and D are almost identical.

2. Within group variation of serum triglyceride with age

Within group variation with age in Groups C, D, E and H is shown in Tables XI and XII.

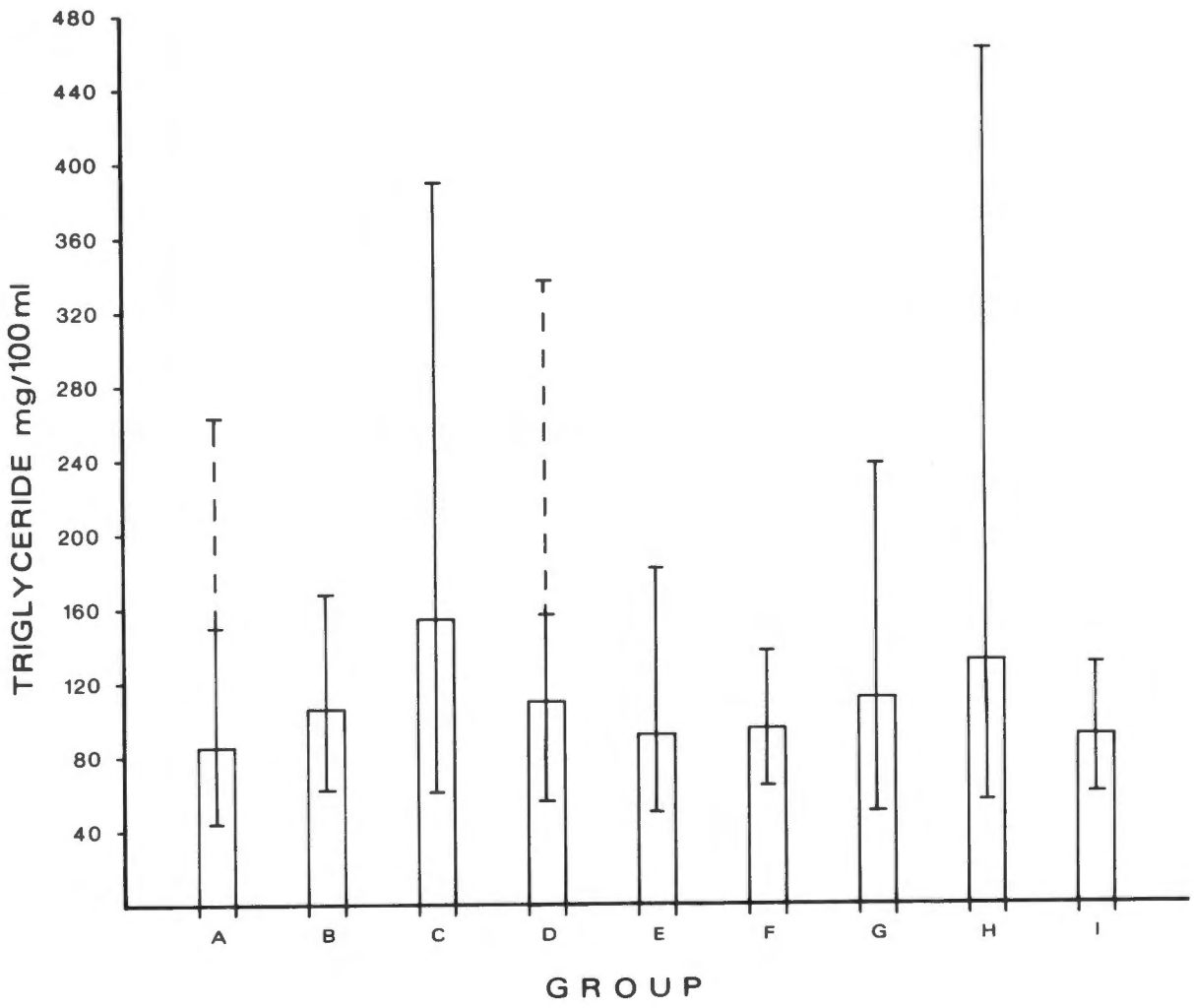


Figure 5: Serum triglyceride concentration in Groups A to I. The top line of each bar indicates the mean value. The range is also indicated. The broken line indicates that there is a single value at the top end of the range.

TABLE XI

CONCENTRATION OF TRIGLYCERIDE WITHIN EACH AGE CLASS OF GROUPS C, D, E AND H

Group and Age Class (years)	No.	Mean (mg/100ml)	Range (mg/100ml)	Standard Deviation	Standard Error of Mean
C 35-39	20	148.8	90 - 264	49.2	11.0
C 40-44	22	161.6	61 - 390	88.0	18.8
C 45-49	5	162.8	77 - 306	100.4	44.9
C 50-55	4	141.0	77 - 210	55.0	27.5
D 35-39	9	85.7	62 - 144	24.9	8.3
D 40-44	8	97.5	56 - 127	26.0	9.2
D 45-49	8	119.5	74 - 157	28.7	10.1
D 50-54	8	141.8	86 - 144 + 337	82.5	29.2
D 55-60	8	108.6	56 - 150	36.1	12.8
E 25-29	18	85.0	61 - 136	24.3	4.7
E 30-34	16	85.2	50 - 145	26.1	5.9
E 35-50	9	117.7	85 - 182	30.6	10.2
H 35-39	18	100.7	56 - 194	41.1	9.7
H 40-44	14	131.4	68 - 462	98.8	26.4
H 45-49	10	156.8	76 - 325	67.5	21.3
H 50-55	8	155.8	88 - 350	89.2	31.5

TABLE XII

LOG CONCENTRATION OF TRIGLYCERIDE WITHIN EACH AGE CLASS OF GROUPS C, D, E AND H

Group and Age Class (years)	No.	Mean Log (mg/100ml)	Mean Antilog (mg/100ml)	Standard Deviation Log (mg/100ml)	Standard Deviation Antilog (mg/100ml)	Standard Error of Mean Log (mg/100ml)	Standard Error of Mean Antilog (mg/100ml)
C 35-39	20	2.1493	141.9	0.1483	+ 37 - 31	0.0331	+ 7 - 6
C 40-44	22	2.1847	153.0	0.1969	+ 51 - 44	0.0412	+ 12 - 9
C 45-49	5	2.1843	154.2	0.2167	+ 57 - 46	0.1170	+ 14 - 11
C 50-55	4	2.4700	140.3	0.1808	+ 39 - 35	0.0900	+ 11 - 10
D 35-39	9	1.9193	83.1	0.1090	+ 22 - 19	0.0360	+ 6 - 6
D 40-44	8	1.9733	94.1	0.1260	+ 24 - 22	0.0435	+ 6 - 6
D 45-49	8	2.0657	116.1	0.1086	+ 33 - 24	0.0374	+ 9 - 8
D 50-54	8	2.1065	127.8	0.1939	+ 64 - 51	0.0685	+ 17 - 15
D 55-60	8	2.0119	102.8	0.1593	+ 36 - 25	0.0556	+ 9 - 8
E 25-29	18	1.9136	82	0.1208	+ 26 - 20	0.0198	+ 4 - 4
E 30-34	16	1.9137	82	0.1208	+ 26 - 20	0.0200	+ 4 - 4
E35-50	9	2.0590	115	0.1304	+ 34 - 24	0.0331	+ 9 - 9
H 35-39	18	1.9699	93.3	0.1752	+ 47 - 32	0.0412	+ 10 - 8
H 40-44	14	2.0584	114.4	0.2051	+ 70 - 42	0.0547	+ 16 - 13
H 45-49	10	2.1655	146.4	0.1630	+ 54 - 43	0.0509	+ 19 - 15
H 50-55	8	2.1415	138.6	0.2133	+ 87 - 53	0.0748	+ 27 - 19

Group C shows an increase from the 35-39 year age group to the 40-44 and 45-49 year age group with a tendency to decrease in the 50-55 year age groups. These changes with age are not statistically significant.

A similar age trend is shown in Group D though at lower levels. The value indicated for the 50-54 year group may possibly err on the high side, since in this group of 8 individuals, there is one value of 337 mg/100ml, considerably above the range. Once again these changes with age are not statistically significant.

In Group E there is no difference between levels in the 25-29 and 30-34 year age groups, but levels are significantly higher in the 35-50 year group.

Group H shows an increase from the 35-39 age group, through the 40-44 age group to 45-49 group. These changes with age are statistically significant. The levels do not decrease in the 50-55 age group.

3. Within group variation of serum triglyceride level with degree of physical activity

Within group variation with degree of physical activity reported in leisure hours by the volunteers has been considered in Groups B and C and the results are shown in Tables XIII and XIV and Figure 6.

TABLE XIII

SERUM TRIGLYCERIDE CONCENTRATION FOR INDIVIDUALS IN GROUPS B AND C REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY IN LEISURE HOURS

Group and Degree of Activity	Number	Mean (mg/100ml)	Range	Standard Deviation	Standard Error of Mean
B None	14	131.9	82 - 168	25.8	6.9
Occasional	27	109.6	80 - 138	18.6	3.6
Regular	29	89.6	61 - 124	19.4	3.6
C None	19	207.0	109 - 390	76.8	17.6
Occasional	21	143.0	62 - 290	47.9	10.5
Regular	11	88.5	61 - 149	24.1	7.3

TABLE XIV

TABLE XIV LOG TRIGLYCERIDE CONCENTRATION FOR INDIVIDUALS IN GROUP B AND C REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY

Group and Degree of Activity	Number	Mean		Standard Deviation		Standard Error of Mean	
		Log (mg/100ml)	Antilog (mg/100ml)	Log (mg/100ml)	Antilog (mg/100ml)	Log (mg/100ml)	Antilog (mg/100ml)
B None	14	2.1116	129.3	0.0905	+ 30 - 24	0.0223	+ 7 - 6
Occasional	27	2.0340	108.1	0.0721	+ 20 - 15	0.0100	+ 3 - 2
Regular	29	1.9435	87.8	0.0871	+ 19 - 16	0.0141	+ 2 - 2
C None	19	2.2685	185.6	0.1356	+ 68 - 49	0.0300	+ 12 - 11
Occasional	21	2.1341	136.1	0.1382	+ 51 - 35	0.0300	+ 14 - 9
Regular	11	1.9343	86.0	0.1072	+ 24 - 19	0.0316	+ 6 - 6

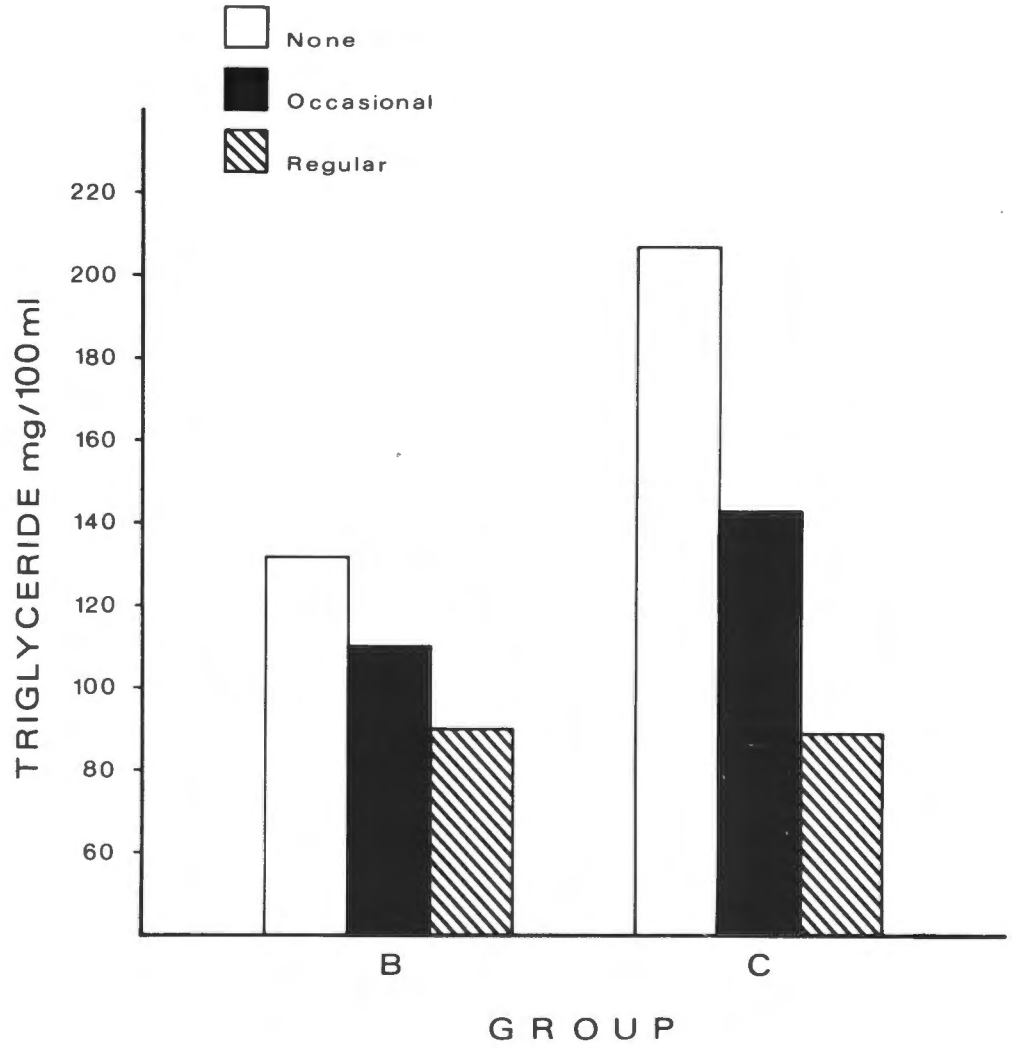


Figure 6: Serum triglyceride concentration for individuals in Groups B and C reporting different degrees of physical activity in leisure. The top line of each bar indicates the mean value.

In both groups, significantly lower levels of serum triglyceride are apparent with increasing degrees of physical activity. Triglyceride levels are significantly lower in volunteers reporting occasional exercise than those reporting no physical exercise and in the regular exercisers significantly lower still than the occasional exercisers.

It is of particular interest to note that despite the age discrepancy between the two groups, the subjects reporting regular physical exercise have almost identical serum triglyceride levels.

Serum triglyceride concentration for different age classes in Group C reporting different degrees of physical activity is shown in Table XV and Table XVI.

TABLE XV

SERUM TRIGLYCERIDE CONCENTRATIONS FOR DIFFERENT AGE CLASSES IN GROUP C, REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY IN LEISURE HOURS

Degree of Activity	Age (in years)	Number	Mean	Standard Deviation	Standard Error of Mean
None	35 - 39	7	175.7	53.4	20.2
	40 - 44	8	231.0	90.9	32.1
	45 - 49	3	215.0	99.4	57.4
	50 - 55	1	210.0	-	-
Occasional	35 - 39	10	148.8	35.1	11.1
	40 - 44	10	138.7	61.5	19.5
	45 - 49	0	-	-	-
	50 - 55	1	128.0	-	-
Regular	35 - 39	3	86.0	21.0	12.1
	40 - 44	4	80.1	15.2	7.6
	45 - 49	2	85.5	10.6	7.5
	50 - 55	2	113.0	50.9	36.0

TABLE XVI

TABLE XVI LOG SERUM TRIGLYCERIDE CONCENTRATION FOR DIFFERENT AGE CLASSES IN GROUP C, REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY

Degree of Activity	Age (years)	No.	Mean		Standard Deviation		Standard Error of Mean	
			Log mg/100ml	Antilog mg/100ml	Log mg/100ml	Antilog mg/100ml	Log mg/100ml	Antilog mg/100ml
None	35 - 39	7	2.2284	169.2	0.1268	+ 58 - 42	0.0479	+ 20 - 17
	40 - 44	8	2.2869	193.6	0.1248	+ 65 - 44	0.0435	+ 20 - 18
	45 - 49	3	2.2949	196.8	0.2313	+128 - 80	0.1334	+ 69 - 47
	50 - 55	1	2.3222	210.8	-	-	-	-
Occasional	35 - 39	10	2.1613	145.0	0.1048	+ 44 - 30	0.0331	+ 11 - 10
	40 - 44	10	2.1095	125.8	0.1729	+ 65 - 41	0.0538	+ 21 - 13
	45 - 49	0	-	-	-	-	-	-
	50 - 59	1	2.1072	128.0	-	-	-	-
Regular	35 - 39	3	1.9248	84.5	0.1148	+ 26 - 21	0.0663	+ 14 - 13
	40 - 44	4	1.8983	79.1	0.0848	+ 17 - 14	0.0424	+ 11 - 7
	45 - 49	2	1.9251	84.4	0.0538	+ 11 - 10	0.0374	+ 8 - 7
	50 - 55	2	2.0298	107.1	0.2024	+ 63 - 39	0.1431	+ 42 - 30

II. XVI.

A significant decrease with exercise is apparent in the 35-39 and 40-44 year subgroups. It is of interest to note that similar low values are found in the regular exercisers in all four age subgroups. The numbers in each are of course too small for statistical analysis.

4. Variation of serum triglyceride with smoking habits.

Within group variation with smoking habits reported by the volunteers in Group C is shown in Table XVII and Figure 7.

TABLE XVII

TABLE XVII SERUM CHOLESTEROL AND SERUM TRIGLYCERIDE CONCENTRATION AND LOG-TRIGLYCERIDE CONCENTRATION IN GROUP B, REPORTING DIFFERENT SMOKING HABITS *

		Number	Mean	Standard Deviation	Standard Error of Mean
NON-SMOKING	Cholesterol	26	242.4	32.1	6.3
	Triglyceride		138.4	58.2	11.4
	Log Triglyceride		2.1109 (129.1)	0.1600 (+ 58 - 39)	0.0300 (+ 10 - 8)
MODERATE	Cholesterol	11	246.4	43.8	13.2
	Triglyceride		182.2	96.8	29.2
	Log Triglyceride		2.2053 (160.4)	0.2340 (+115 - 99)	0.0700 (+ 29 - 23)
HEAVY	Cholesterol	14	258.0	39.3	10.5
	Triglyceride		164.7	71.1	19.0
	Log Triglyceride		2.1753 (149.7)	0.2027 (+ 88 - 55)	0.0538 (+ 20 - 16)

* The values for serum cholesterol and triglyceride are expressed as mg/100ml and for log triglyceride as log mg/100ml. The antilogarithms of these log values are also indicated.

II. XVII.

Serum triglyceride levels are significantly higher in smokers than non-smokers with no significant difference being apparent between moderate and heavy smokers.

There is however a significant inverse correlation between degree of smoking and degree of physical activity ($r = 0.29$, $p < 0.05$).

Tables XVIII and XIX indicate that there is no significant difference between smokers and non-smokers within the subgroups reporting different degrees of physical activity.

TABLE XVIII

SERUM TRIGLYCERIDE LEVELS IN VOLUNTEERS IN GROUP C REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY, DIFFERENTIATING BETWEEN SMOKERS AND NON-SMOKERS

		Number	Mean (mg/100ml)	Standard Deviation	Standard Error of Mean
No Exercise	Smokers	13	202	32.9	9.3
	Non-Smokers	6	208	44.3	18.5
Occasional Exercise	Smokers	9	155	61.0	20.3
	Non-Smokers	12	140	28.8	8.3
Regular Exercise	Smokers	4	78	11.3	5.7
	Non-smokers	7	89	15.8	6.1

II. XVIII.

TABLE XIX

TABLE XIX

LOG SERUM TRIGLYCERIDE LEVELS IN VOLUNTEERS IN GROUP C REPORTING DIFFERENT DEGREES OF PHYSICAL EXERCISE DIFFERENTIATING BETWEEN SMOKERS AND NON-SMOKERS

		Number	Mean		Standard Deviation		Standard Error of Mean	
			Log mg/100ml	Antilog mg/100ml	Log mg/100ml	Antilog mg/100ml	Log mg/100ml	Antilog mg/100ml
NO EXERCISE	Smokers	13	2.2774	189.4	0.0619	+ 29 - 25	0.0171	+ 8 - 7
	Non-smokers	6	2.2849	192.7	0.0763	+ 37 - 31	0.0311	+ 17 - 12
OCCASIONAL EXERCISE	Smokers	9	2.1712	148.3	0.1233	+ 49 - 37	0.0411	+ 14 - 13
	Non-Smokers	12	2.1349	136.5	0.0692	+ 24 - 20	0.0197	+ 7 - 6
REGULAR EXERCISE	Smokers	4	1.8698	74.1	0.0333	+ 6 - 5	0.0166	+ 2 - 2
	Non-Smokers	7	1.9360	86.3	0.0371	+ 8 - 6	0.0142	+ 3 - 2

II. XIX.

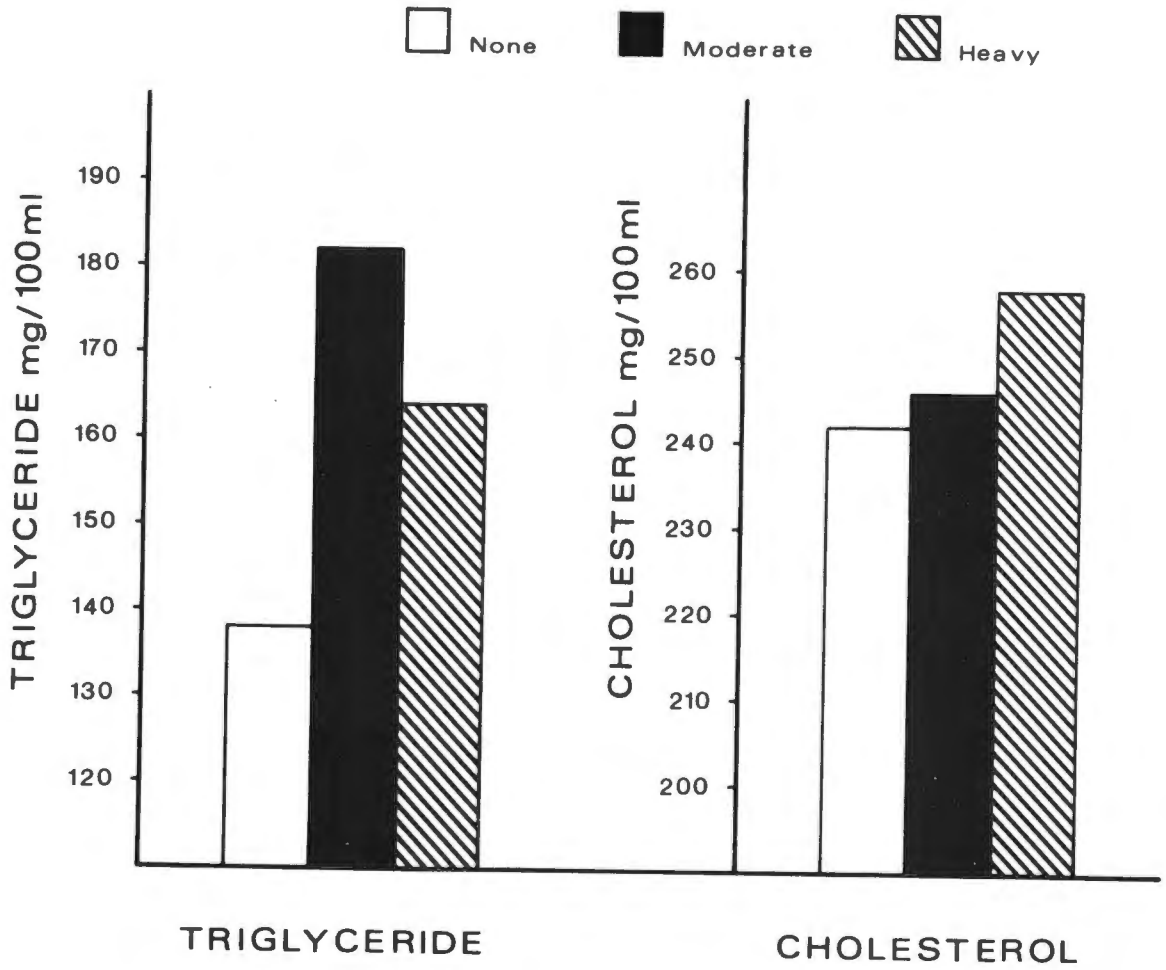


Figure 7: Mean serum cholesterol and triglyceride levels for individuals in Group C reporting different smoking habits.

Furthermore, Tables XX and XXI show that when smokers and non-smokers are divided up into those who report some degree of physical exercise and those who do not, there is no difference between smokers and non-smokers.

TABLE XX

SERUM TRIGLYCERIDE LEVELS IN VOLUNTEERS IN GROUP C REPORTING DIFFERENT SMOKING HABITS, DIFFERENTIATING BETWEEN THOSE WHO REPORT TAKING EXERCISE AND THOSE WHO DO NOT

		Number	Mean (mg/100ml)	Standard Deviation	Standard Error of Mean
NON-SMOKERS	Exercise	20	120	33.1	7.4
	No Exercise	6	202	72.3	29.0
SMOKERS	Exercise	12	133	63.3	18.1
	No Exercise	13	209	73.5	20.4

TABLE XXI LOG SERUM TRIGLYCERIDE LEVELS IN VOLUNTEERS IN GROUP C REPORTING DIFFERENT SMOKING HABITS DIFFERENTIATING BETWEEN THOSE WHO REPORT TAKING EXERCISE AND THOSE WHO DO NOT

		Number	Mean (log mg/100ml)	Mean (Antilog mg/100ml)	Standard Deviation (Log mg/100ml)	Standard Deviation Antilog mg/100ml)	Standard Error of Mean (Log mg/100ml)	Standard Error of Mean Antilog mg/100m
NON-SMOKERS	Exercise	20	2.0601	114.8	0.0922	+ 27 - 20	0.0206	+ 6 - 5
	No Exercise	6	2.2815	191.2	0.1164	+ 60 - 48	0.0465	+ 22 - 19
SMOKERS	Exercise	12	2.0844	121.4	0.1660	+ 57 - 39	0.0474	+ 14 - 12
	No Exercise	13	2.2939	196.7	0.1211	+ 63 - 48	0.0336	+ 16 - 14

II. XXI.

This information tends to suggest that the relationship between smoking and serum triglyceride is due rather to the fact that smokers tend to have less physical exercise.

(C) SERUM CHOLESTEROL CONCENTRATION

1. Mean levels in different groups

Mean serum cholesterol levels for the groups studied are given together with the median, range, standard deviation and standard error of the mean in Table VIII and illustrated graphically in Figure 8. Group C and D have mean values which are significantly higher than the remaining groups but not significantly different from each other. Groups E and I have mean values significantly lower than all other groups, but not significantly different from each other. Groups A, B, F, G and H have mean values not significantly different from each other.

2. Within group variation of serum cholesterol with age

Within group variation with age for Groups C, D, E and H is shown in Table XXII.

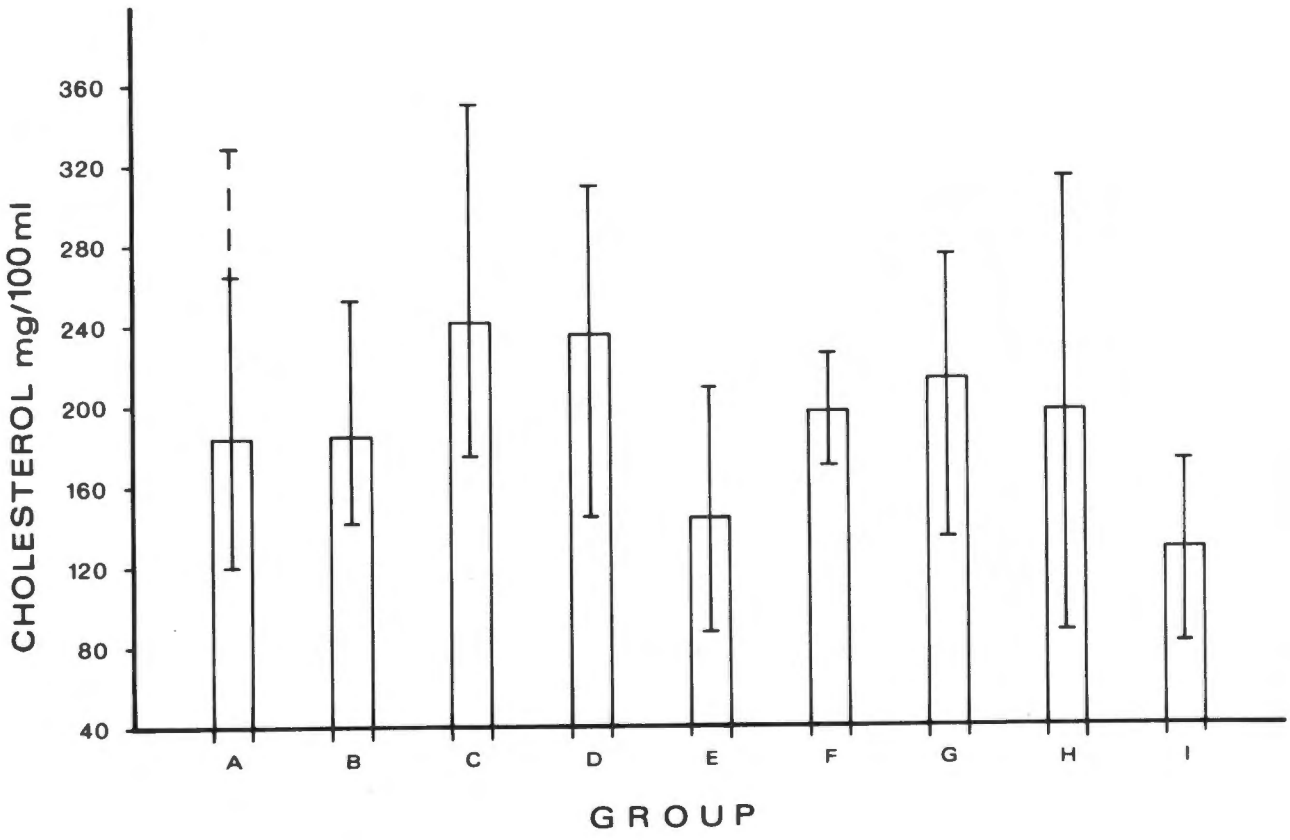


Figure 8: Serum cholesterol concentration in Groups A to I. The top line of each bar indicates the mean value. The range is also indicated. The broken line in Group A indicates that there is a single value at the top end of the range.

TABLE XXII

CONCENTRATION OF CHOLESTEROL WITHIN EACH AGE CLASS OF GROUPS C, D, E AND H

Group and Age Class years	Number	Mean (mg/100ml)	Range (mg/100ml)	Standard Deviation	Standard Error of Mean
C 35 - 39	20	242.0	195 - 275	25.7	5.8
C 40 - 44	22	245.9	175 - 317	44.4	9.5
C 45 - 49	5	266.2	234 - 351	48.4	21.7
C 50 - 55	4	259.3	247 - 282	20.3	10.1
D 35 - 39	9	224.0	145 - 298	42.0	14.0
D 40 - 44	8	220.3	179 - 245	22.8	8.1
D 45 - 49	8	230.6	163 - 286	40.1	14.2
D 50 - 54	8	253.3	202 - 310	46.9	16.6
D 55 - 60	8	251.8	214 - 289	21.4	7.6
E 25 - 29	18	140.3	87 - 209	30.4	6.3
E 30 - 34	16	139.7	98 - 203	26.1	7.1
E 35 - 50	9	153.4	109 - 201	28.4	9.5
H 35 - 39	18	166.3	87 - 261	49.7	11.7
H 40 - 44	14	216.0	122 - 308	61.5	16.4
H 45 - 49	10	210.5	117 - 309	58.8	18.6
H 50 - 55	8	229.5	156 - 274	38.9	13.8

Levels in Group C tend to increase from the 35-39 year age group through the 40-44 year group to the 45-49 year group and decrease slightly in the 50-55 group. In Group D levels remained fairly constant in the 35-39 and 40-44 year groups, but increase from here to the 50-54 year age group. They do not appear to change between 55-60. In Group E similar levels are found in the 25-29 and 30-34 year group, appearing to increase in the 35-50 year group. However, none of the changes mentioned above reach statistical significance.

In Group H levels between 35 and 39 years are significantly lower than the remaining age groups, amongst whom levels are not significantly different.

3. Within group variation of serum cholesterol with degree of physical activity in leisure hours

Within group variation with degree of physical activity reported by the volunteers in Groups B and C is shown in Table XXIII and Figure 9.

TABLE XXIII

SERUM CHOLESTEROL CONCENTRATION FOR INDIVIDUALS IN GROUPS B AND C REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY IN LEISURE HOURS

Group and Degree of Activity	Number	Mean (mg/100ml)	Range	Standard Deviation	Standard Error of Mean
B None	14	197.9	178 - 253	19.7	5.3
Occasional	27	184.8	154 - 218	17.0	3.3
Regular	29	179.7	151 - 235	19.6	3.6
C None	19	262.2	207 - 351	38.3	8.8
Occasional	21	240.9	175 - 315	36.7	8.2
Regular	11	234.5	178 - 278	27.1	8.2

In both groups lower levels of serum cholesterol appear to be associated with increasing degrees of physical activity and in both cases levels are significantly higher in volunteers reporting no physical exercise than in those reporting physical exercise. However, the difference between occasional and regular exercisers is not statistically significant.

Serum cholesterol concentration for different age classes in Group C reporting different degrees of physical activity is shown in Table XXIV.

TABLE XXIV

SERUM CHOLESTEROL CONCENTRATION FOR DIFFERENT AGE CLASSES IN GROUP C, REPORTING DIFFERENT DEGREES OF PHYSICAL ACTIVITY IN LEISURE HOURS

Degree of Activity	Age (in years)	Number	Mean	Standard Deviation	Standard Error of Mean
None	35 - 39	7	250.1	22.2	8.3
	40 - 44	8	262.5	45.4	16.0
	45 - 49	3	286.7	55.8	32.2
	50 - 55	1	270.0	-	-
Occasional	35 - 39	10	237.0	27.9	8.8
	40 - 44	10	240.2	44.8	14.17
	45 - 49	0	-	-	-
	50 - 55	1	282.0	-	-
Regular	35 - 39	3	238.3	30.6	17.6
	40 - 44	4	227.0	41.0	20.5
	45 - 49	2	235.5	2.1	1.5
	50 - 55	2	242.5	6.4	4.5

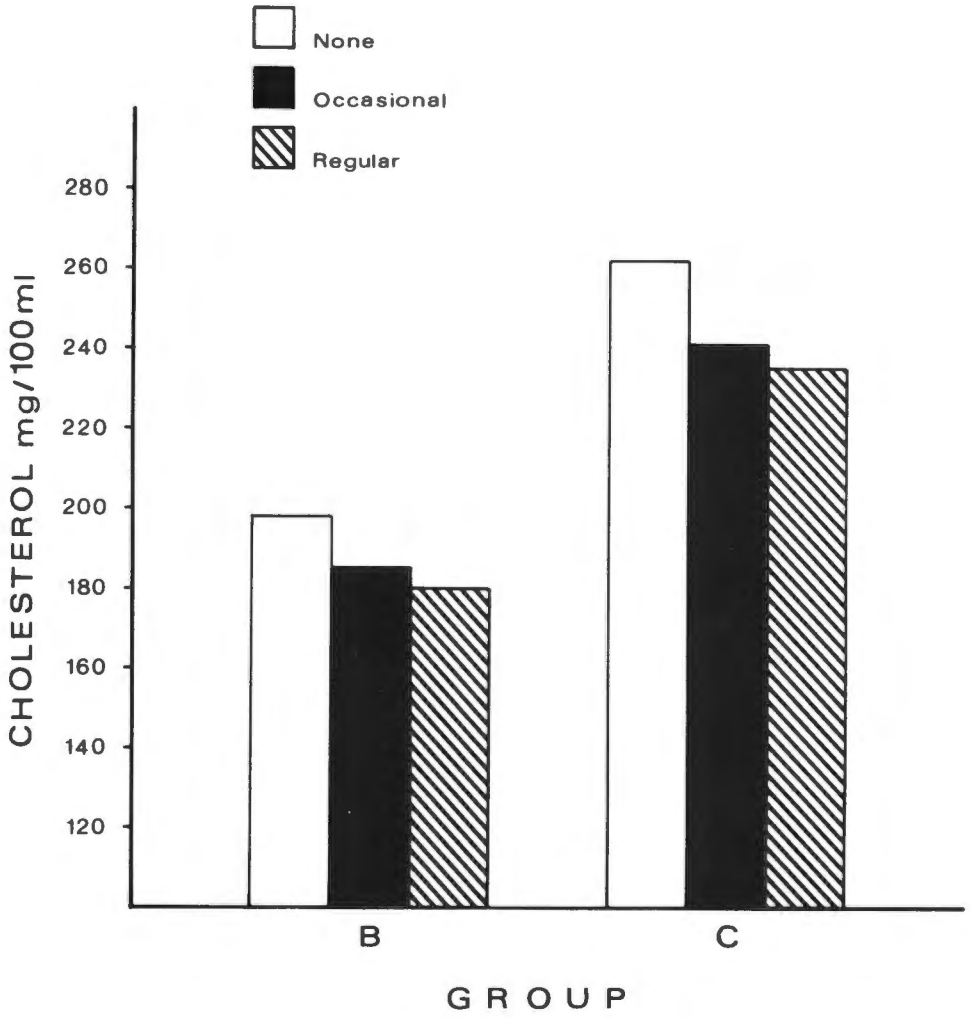


Figure 9: Mean serum cholesterol concentration for individuals in Groups B and C reporting different degrees of physical activity.

In the 35-39 and 40-44 year subgroups, regular and occasional exercisers appear to have a lower cholesterol concentration than those reporting no exercise. The differences are not statistically significant.

4. Variation of serum cholesterol with smoking habits

Serum cholesterol levels in volunteers in Group C reporting different smoking habits is shown in Table XVII and Figure 7. Mean values appear to be slightly higher in smokers than non-smokers, but the difference is not statistically significant.

(E) SERUM PHOSPHOLIPID CONCENTRATIONS

The mean serum phospholipid concentration was determined for ten samples from each of Groups A, C, D, E, H and I and is given together with the range in Table XXV.

TABLE XXV

MEAN SERUM PHOSPHOLIPID CONCENTRATIONS IN TEN SAMPLES FROM GROUPS A, C, D, E and H

Group	Mean (mg/100ml)	Range (mg/100ml)
A	182	153 - 240
C	230	194 - 307
D	238	200 - 302
E	166	131 - 209
H	198	100 - 242

The level in Group C and D is significantly higher than in Groups A, E and H amongst whom there is no significant difference.

(F) SERUM PRE-BETA LIPOPROTEIN

In view of the fact that serum triglyceride in the fasting state is present chiefly in the pre-beta lipoprotein, the close correlation between the pre-beta lipoprotein grading and fasting serum triglyceride level which has already been described is not surprising.

The frequency distribution of the pre-beta lipoprotein grading in each group is shown in Table XXVI and Figure 10.

TABLE XXVI

TABLE XXVI FREQUENCY DISTRIBUTION OF PRE B LIPOPROTEIN GRADING IN EACH GROUP

Pre B Grading	0		Trace		+		++		+++		++++	
Group	Number of Subjects	%	Number of Subjects	%	Number of Subjects	%	Number of Subjects	%	Number of Subjects	%	Number of Subjects	%
A	25	40.3	28	45.2	8	12.9	1	1.6	0	0.0	0	0.0
B	20	28.6	34	48.6	12	17.1	4	5.7	0	0.0	0	0.0
C	2	3.9	12	23.5	14	27.5	20	39.2	3	5.9	0	0.0
D	4	9.8	17	41.5	17	41.5	2	4.9	0	0.0	0	0.0
E	10	23.3	16	37.2	16	37.2	1	2.3	0	0.0	0	0.0
F	9	64.3	5	35.7	0	0.0	0	0	0	0.0	0	0.0
G	1	5.3	11	57.9	4	21.1	3	15.8	0	0.0	0	0.0
H	1	2.0	20	40.0	16	32.0	9	18.0	4	8.0	0	0.0
I												
J	4	33.3	7	58.3	1	8.3	0	0.0	0	0.0	0	0.0
K	+	20.0	3	60.0	1	20.0	0	0.0	0	0.0	0	0.0

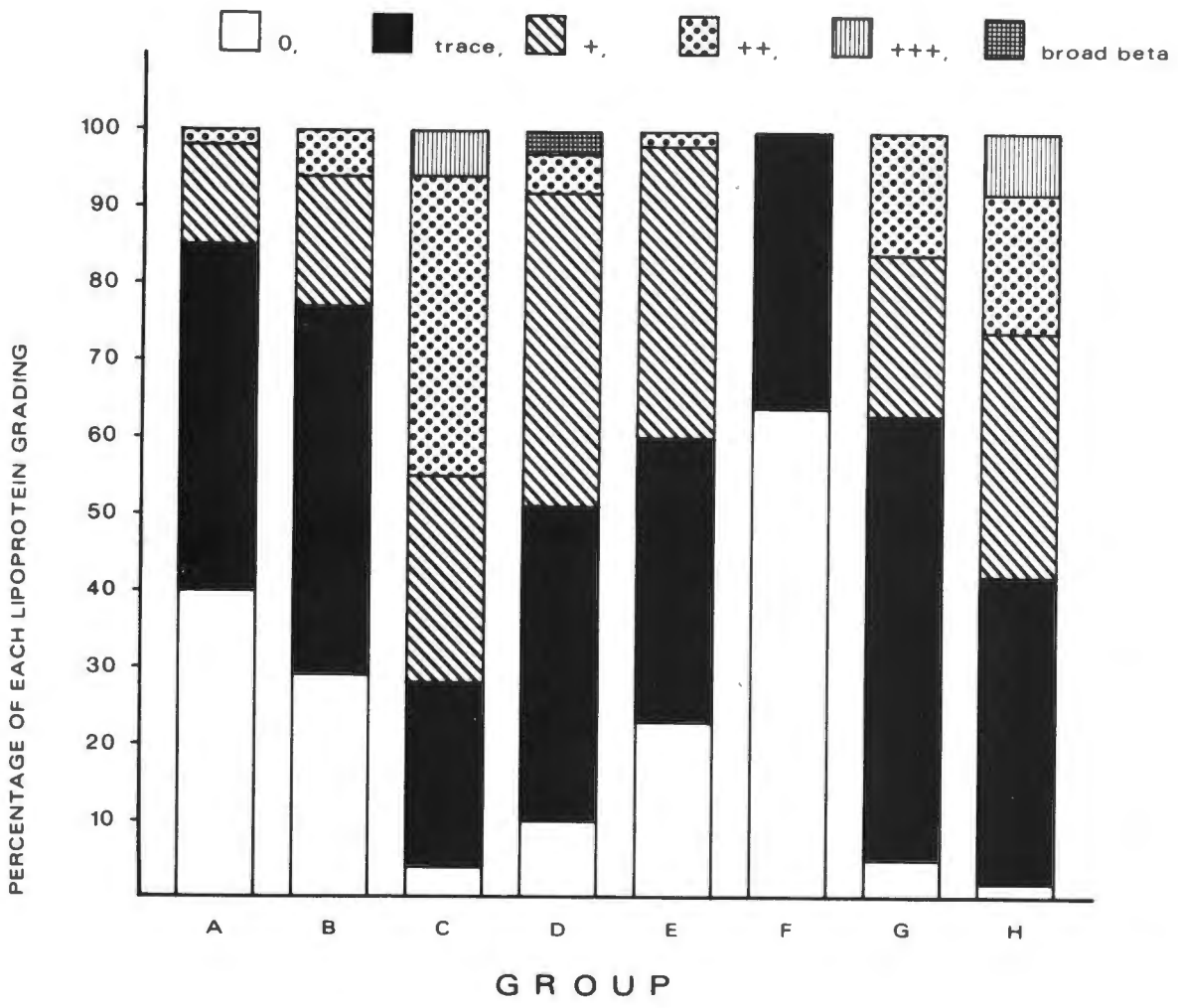


Figure 10: Frequency distribution of pre-beta lipoprotein grading in Groups A to H.

Groups C and H which have significantly higher serum triglyceride levels than the other groups are the only two groups in which some of the volunteers have been graded as +++ pre-beta lipoprotein (5.9% in Group C and 8.0% in Group H). In these two groups an appreciable number of subjects have also been graded in the ++ category (39.2% in Group C and 15.8% in Group H). In the remaining groups the majority of the volunteers have been graded as 0, trace and +.

(G) SERUM TRIGLYCERIDE FATTY ACID PATTERNS

The mean percentage of fatty acid present in the serum triglyceride fraction of each group is indicated in Table XXVII and Figure 11. Triglyceride fatty acid pattern reflects the nature of dietary fat[8,40,50,52,66,82].

TABLE XXVII

TABLE XXVII MEAN PERCENTAGE OF FATTY ACIDS PRESENT IN THE SERUM TRIGLYCERIDE FRACTION. THE STANDARD DEVIATION IS GIVEN IN BRACKETS.
THE FOLLOWING FATTY ACIDS WERE PRESENT IN EACH OF THE GROUPS IN TRACE AMOUNTS:- C13:0, C14:1, C15:0, C15:1, C17:0, C17:1.

Fatty Acid	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	F	C18:3	X	C20:1	C20:4	Total saturated	Total unsaturated	Mono-unsaturated	Poly unsaturated
Group A		3.2 (0.74)	31.7 (2.62)	5.8 (1.20)	4.7 (0.82)	43.0 (2.63)	11.6 (2.00)	-	-	-	-	tr	39.6 (3.45)	60.4 (3.41)	48.8 (3.39)	11.6 (2.00)
B	0.1 (0.22)	2.7 (1.49)	31.4 (3.13)	5.7 (1.24)	3.7 (0.68)	41.2 (3.96)	15.2 (5.16)	-	-	-	-	tr	37.9 (4.25)	62.1 (4.28)	46.9 (4.30)	15.2 (5.16)
C	0.7 (0.88)	3.6 (1.21)	34.5 (2.65)	6.7 (1.21)	4.8 (1.33)	40.4 (4.77)	9.3 (5.00)	-	-	-	-	tr	43.6 (3.63)	56.4 (3.63)	47.1 (3.81)	9.3 (5.00)
D	0.1 (0.30)	2.8 (1.10)	32.6 (2.86)	6.0 (0.94)	4.1 (1.24)	48.4 (3.82)	12.0 (3.64)	-	-	-	-	tr	39.6 (4.41)	60.4 (4.36)	48.4 (4.21)	12.0 (3.64)
E	-	1.6 (0.44)	29.5 (5.81)	7.2 (1.65)	4.1 (1.12)	43.4 (5.16)	12.1 (3.54)	0.4 (0.39)	0.2 (0.28)	-	-	1.3 (0.51)	35.2 (6.67)	64.8 (6.44)	50.6 (6.58)	14.2 (3.32)
F	0.3 (0.41)	3.0 (1.01)	31.4 (2.92)	6.6 (1.34)	5.6 (0.97)	42.1 (4.10)	11.0 (2.79)	-	-	-	-	tr	40.3 (3.46)	59.7 (3.37)	48.7 (3.54)	11.0 (2.79)
G	0.1 (0.22)	3.1 (0.88)	28.7 (2.75)	4.3 (1.42)	4.3 (0.61)	35.4 (4.31)	24.1 (7.21)	-	-	-	-	tr	36.1 (3.87)	63.9 (3.89)	39.8 (3.90)	24.1 (7.21)
H	-	2.9 (0.65)	32.2 (3.05)	5.8 (1.14)	5.8 (2.39)	43.2 (4.44)	10.1 (5.46)	-	-	-	-	tr	40.9 (4.31)	59.1 (4.32)	49.0 (4.36)	10.1 (5.46)
I	2.0 (1.64)	1.0 (0.45)	25.1 (4.67)	2.6 (0.90)	6.1 (1.30)	34.2 (9.24)	25.9 (10.63)	tr	-	1.8 (1.07)	0.7 (0.61)	0.5 (0.73)	34.2 (4.47)	65.8 (3.49)	37.5 (4.14)	28.3 (9.98)

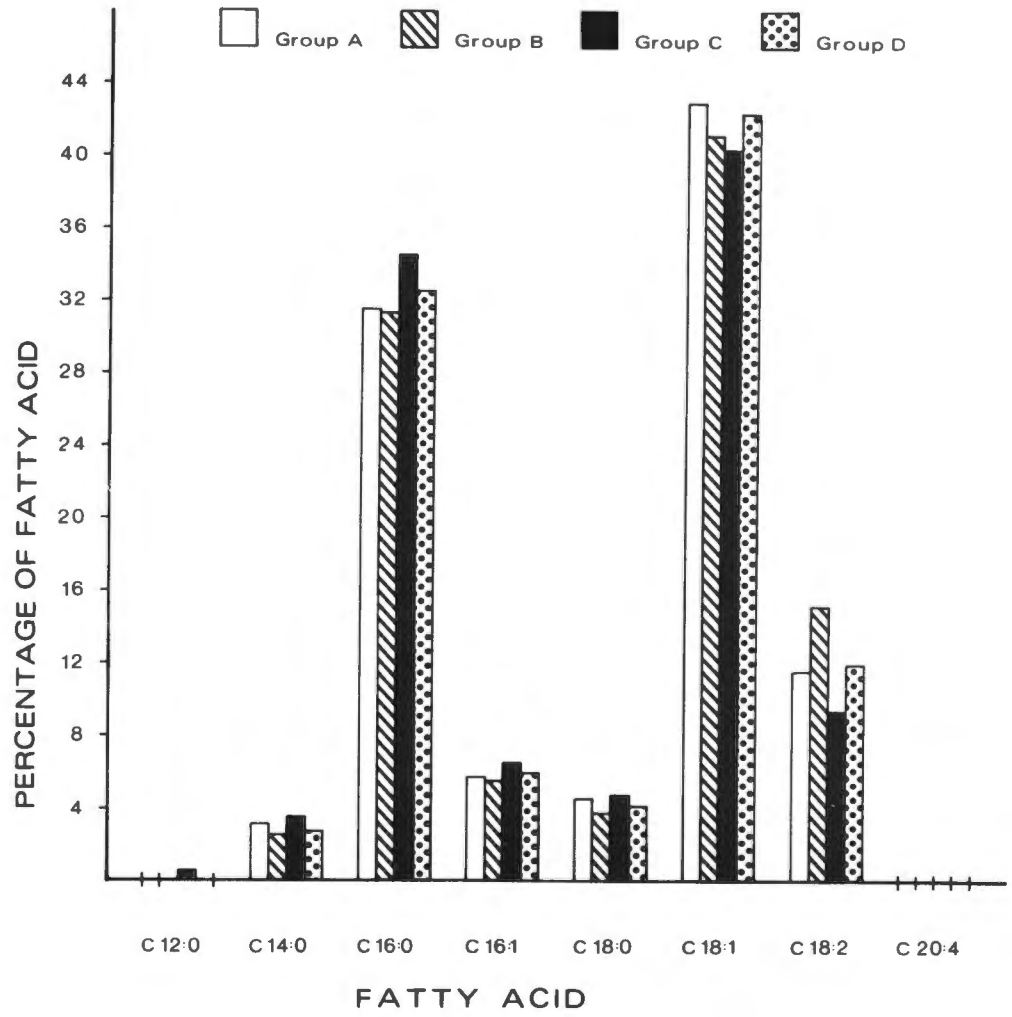


Figure 11(a): The mean percentage of fatty acids present in the serum triglyceride fractions of Groups A, B, C and D - (the "Westernised" population groups).

When considering westernised population groups (A,B,C and D), the African medical students (F) and African porters (H), the only significant differences amongst these groups was a significantly lower percentage of C18:2 in Group C, which appears to have resulted from increased amount of C12:0, C14:0, C16:0 and C16:1; though these fatty acids are not significantly higher in this group than the others. This pattern is reflected in percentage of total saturated and unsaturated fatty acids in this group, which are respectively significantly higher and lower than the other groups. The percentage of polyunsaturated fatty acids is also significantly lower than in other groups. Measurable amounts of C12:0 were not present in Group A.

More differences were noted when comparing Groups E, G and I with each other and with the above groups and examples of chromatograms from Groups B, E and G are shown in Figure 12. An example of a chromatogram from Group I is shown in Part I, Figure 11(b).

Group E had significantly lower levels of C14:0 than all other groups except Group I (from which it did not differ significantly). Measurable amounts of two fatty acids not present in other groups were present in this group - 0.4% of fatty acid F identified as an unsaturated fatty acid with 18 carbon atoms and 0,2% C18:3. In addition C20:4 comprised 1.3% total fatty acids, significantly more than was present in all other groups. Total saturated fatty acids were significantly lower than all other groups except Groups G and I. The mean value for percentage of total unsaturated fatty acids tended to be higher than in the westernised groups but the difference was not statistically significant.

Group G had significantly lower C16:1 and C18:1 and significantly higher C18:2 than all other groups except Group I. Total saturated fatty acids were significantly lower in this group than all other groups except Groups E and I. The mean value for percentage of total unsaturated fatty acid tended to be higher than in the westernised groups but the difference was not statistically significant. The percentage of polyunsaturated fatty acids was significantly higher than all other groups except Group I.

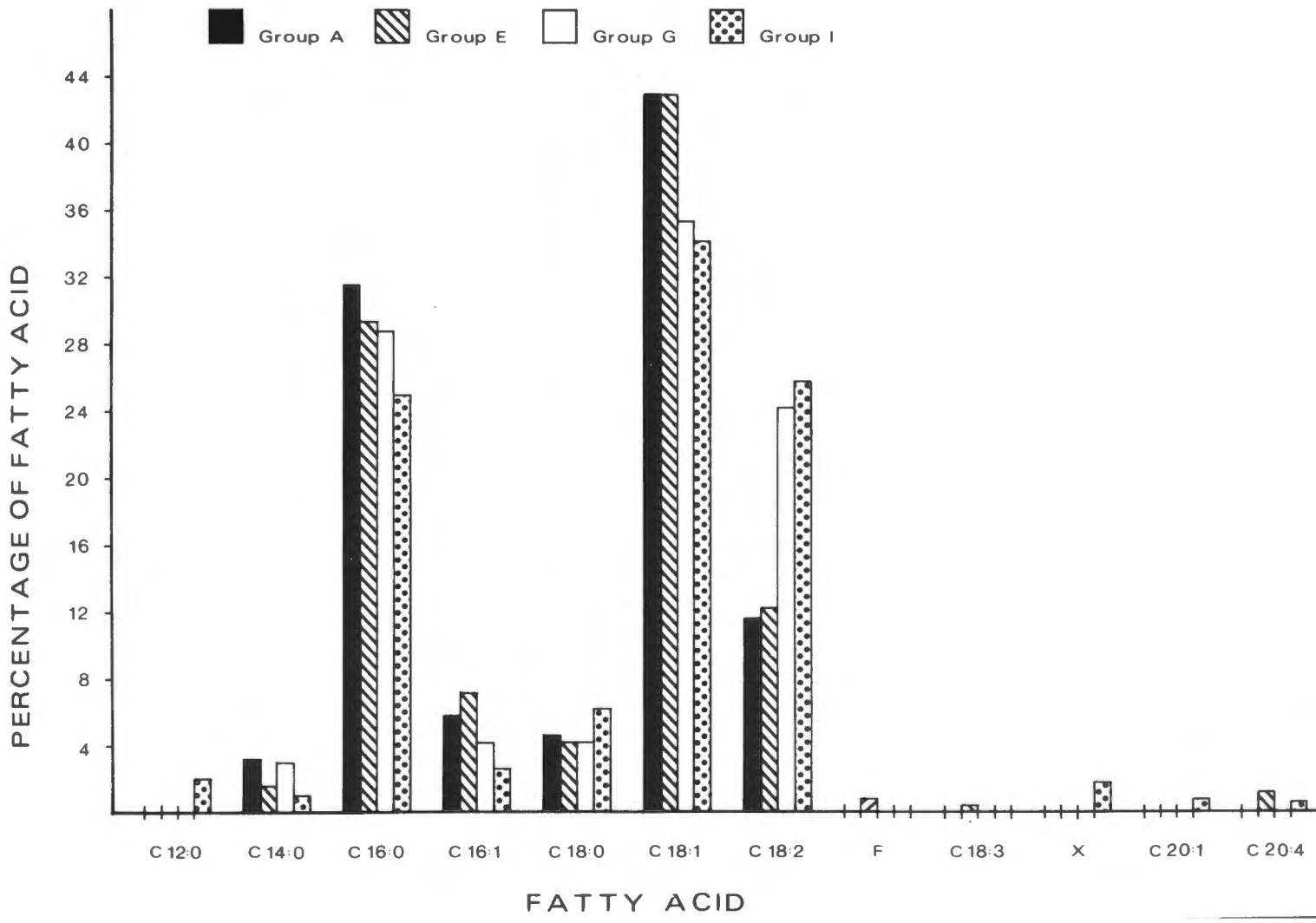


Figure 11(b): The mean percentage of fatty acids present in the serum triglyceride of Group A (representing the "Westernised" population groups), E, G and I.

Group I has significantly more C12:0 than all other groups and significantly less C14:0 than all groups except E. C16:1 and C18:1 are present in significantly smaller smounts and C18:2 in significantly greater amounts than in all other groups except Group G. C16:0 is present in significantly smaller amounts than all other groups. Trace amounts of fatty acid F, 0.7% of C20:1 and 0.5% of C20:4 are present. 1.8% of fatty acid X identified as unsaturated fatty acid with 18 carbon atoms is also present. Significantly less saturated fatty acid is present than in all other groups except Groups E and G, and significantly more total saturated fatty acid is present than in all other groups. The percentage of polyunsaturated fatty acid is significantly higher than in all groups except Group G.

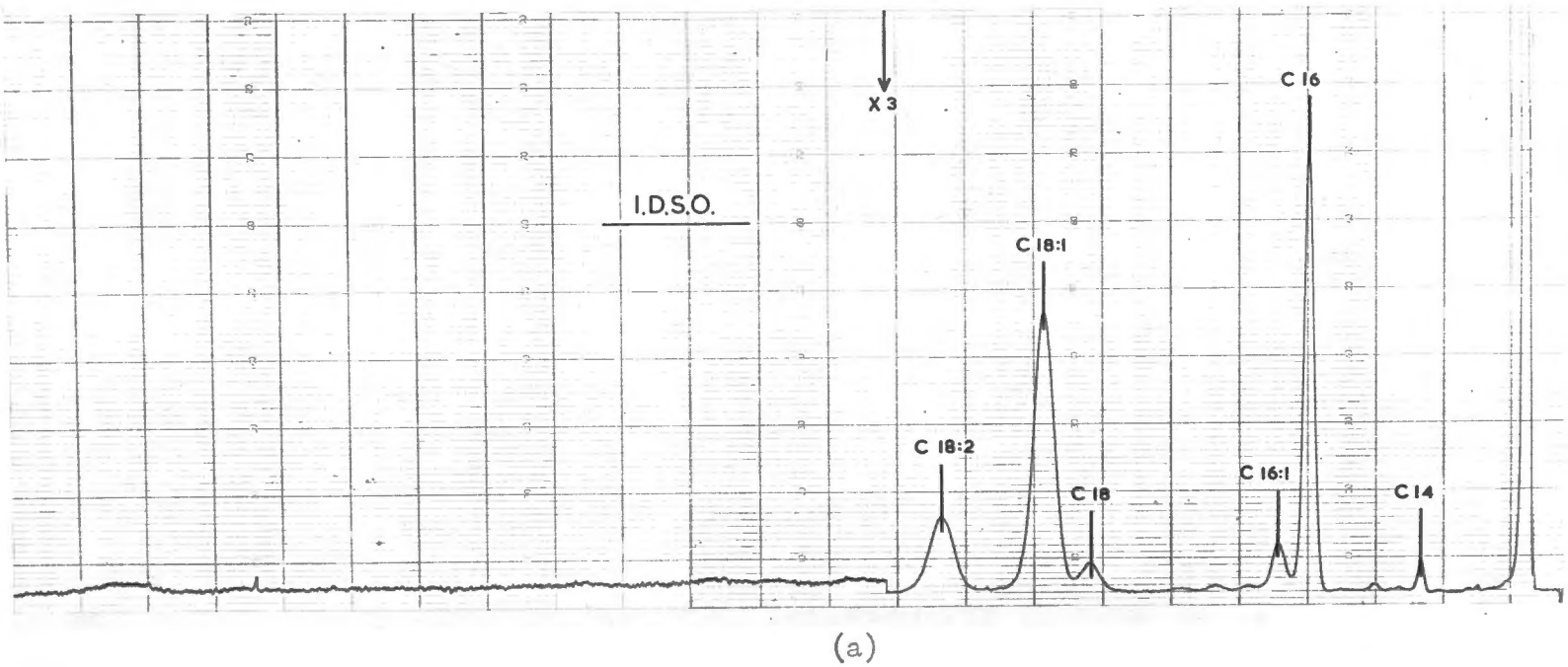
Correlation between percentage of linoleic acid and serum cholesterol and triglyceride levels

Serum cholesterol and triglyceride levels have been correlated with linoleic acid expressed as a percentage of total serum fatty acids.

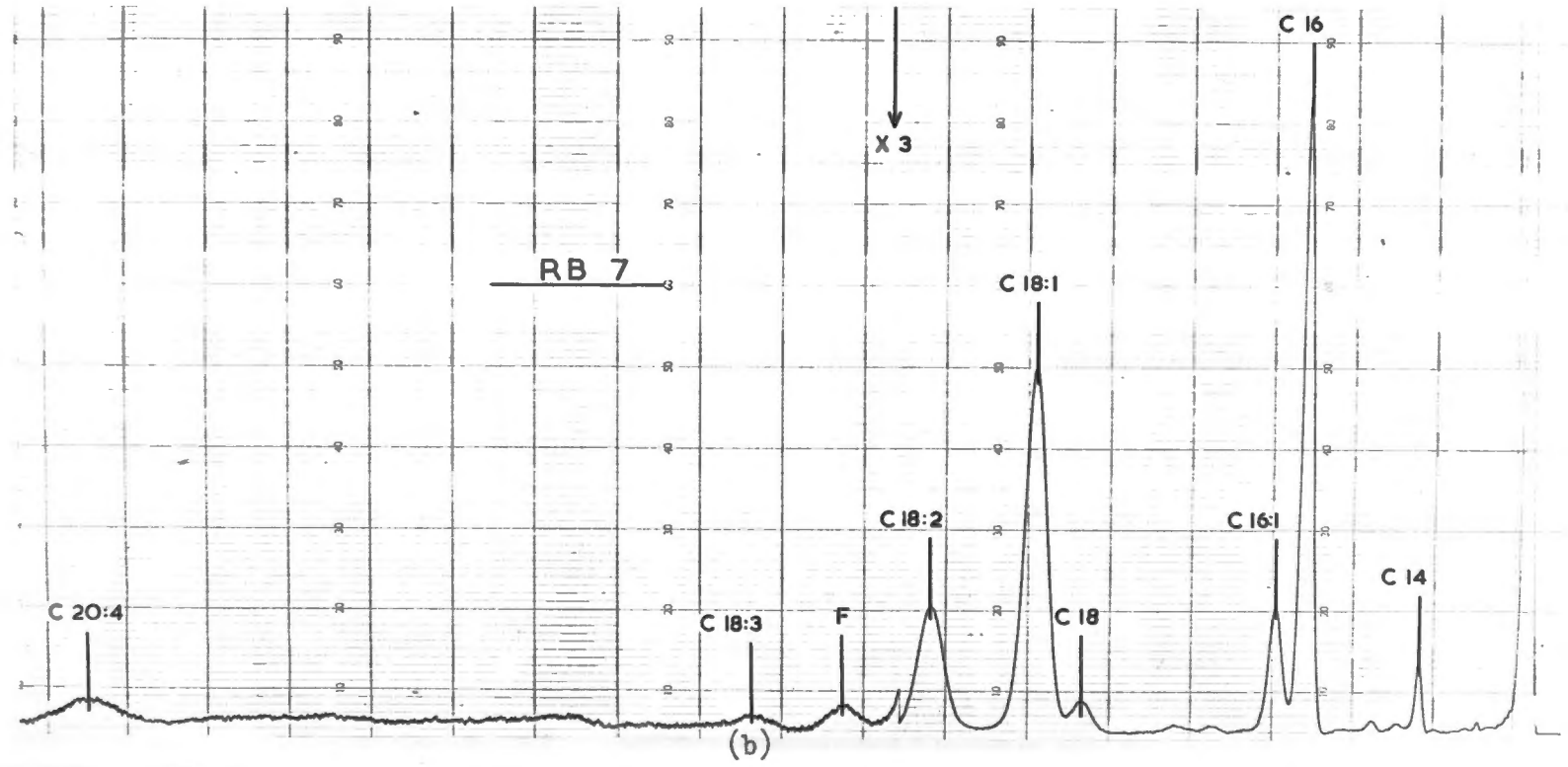
The negative (inverse) correlation between linoleic acid and serum cholesterol and triglyceride levels was statistically significant at the 1% level. (r for cholesterol and linoleic acid = -0.34; and r for triglyceride and linoleic acid = -0.32).

The above correlation coefficients have been calculated from 84 samples representing 12 samples from each of groups A, C, D, E, G, H and I.

The correlation between serum cholesterol and triglyceride and linoleic acid for each group (12 samples) is shown in Table XXVIII.



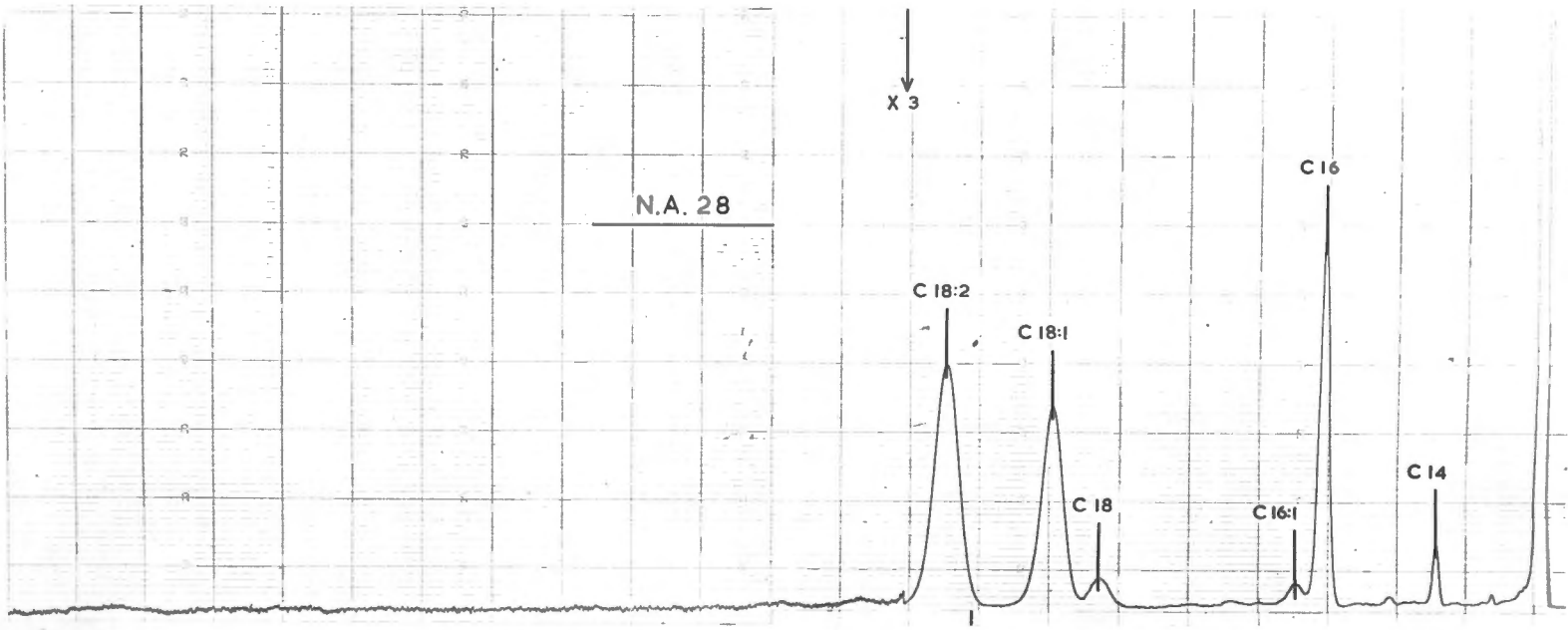
(a)



(b)

Figure 12(a): The serum triglyceride fatty acid pattern of Group B.

(b): The serum triglyceride fatty acid pattern of Group E.



(c)

Figure 12(c): The Serum triglyceride fatty acid pattern of group G.

TABLE XXVIII

THE CORRELATION BETWEEN SERUM CHOLESTEROL AND TRIGLYCERIDE AND PERCENTAGE OF LINOLEIC ACID FOR EACH GROUP (12 SAMPLES) WITHIN EACH GROUP

Group	Serum Cholesterol versus Linoleic Acid		Serum Triglyceride versus Linoleic Acid	
A	$r = -0.20$	N.S.	$r = +0.40$	N.S.
C	$r = -0.39$	N.S.	$r = -0.13$	N.S.
D	$r = +0.32$	N.S.	$r = -0.19$	N.S.
E	$r = +0.37$	N.S.	$r = -0.33$	N.S.
G	$r = -0.60$	$p < 0.05$	$r = -0.22$	N.S.
H	$r = -0.44$	N.S.	$r = -0.66$	$p < 0.05$
I	$r = -0.61$	$p < 0.05$	$r = -0.70$	$p < 0.05$

II. XXVIII.

(H) CLASSIFICATION OF THOSE INDIVIDUALS WITH HIGH LIPIDS

In order to give a rough estimate of the frequency of elevated serum lipid levels, Table XXX and Figure 13 have been drawn up. Individuals in each group with elevated lipid levels (regarding the upper limit of Fredrickson's range[35] shown in Table XXIX as the upper limit of normal values) have been classified according to the system described by Fredrickson[35].

TABLE XXIX

THE NORMAL RANGE (MG/100ML) FOR SERUM CHOLESTEROL AND TRIGLYCERIDE, ACCORDING TO FREDRICKSON ET AL [35]

Age (Years)	Cholesterol	Triglyceride
0 - 19	120 - 230	10 - 140
20 - 29	120 - 240	10 - 140
30 - 39	140 - 270	10 - 150
40 - 49	150 - 310	10 - 160
50 - 59	160 - 330	10 - 190

II. XXIX.

This system of typing has been briefly discussed in Part I. An additional group has been included as type II(b) and this includes individuals with both elevated serum triglyceride and cholesterol levels who did not clearly fit into one of Fredrickson's types.

TABLE XXX

TABLE XXX CLASSIFICATION OF VOLUNTEERS IN EACH GROUP WITH ELEVATED SERUM CHOLESTEROL AND TRIGLYCERIDE LEVELS ACCORDING TO THE CLASSIFICATION OF FREDRICKSON

Group	TYPE II						TYPE IV						TYPE II(b)						Total % in group with elevated lipid levels
	N	%	CHOLESTEROL		TRIGLYCERIDE		N	%	CHOLESTEROL		TRIGLYCERIDE		N	%	CHOLESTEROL		TRIGLYCERIDE		
			Mean	Range	Mean	Range			Mean	Range	Mean	Range			Mean	Range	Mean	Range	
A	3	4.8	248.3	237-265	57.3	50-62	1	1.6	189.0		150		1	1.6	329.0		263.0		8.0
B							6	8.5	192.5	175-214	156.7	144-168	1	1.4	253.0		162.0		9.9
C	1	2.0	315.0		114.0		16	31.4	242.5	204-288	222.0	154-390	4	7.8	316.0	275-357	217.5	154-306	41.2
D +																			2.5
E							2	4.6	181.5	178-185	163.5	145-182							4.6
G	2	10.5	256.0	243-269	66.5	65-68							4	21.0	258.5	243-275	200.0	157-238	31.5
H							5	10.0	226.0	149-304	224.4	163-350	2	4.0	315.0	314-316	393.5	325-462	14.0

No cases conforming to Types I and V of Fredrickson were detected in the surveys.
No individuals in Groups F and I were found to have elevated serum lipid levels.

\+ Group D contained only one individual with elevated serum lipid levels.

Cholesterol:- 310 mg/100ml
Triglyceride:- 337 mg/100ml
Lipoprotein electrophoresis:- A "broad beta" band.
This was therefore diagnosed as Type III Hyperlipoproteinaemia.

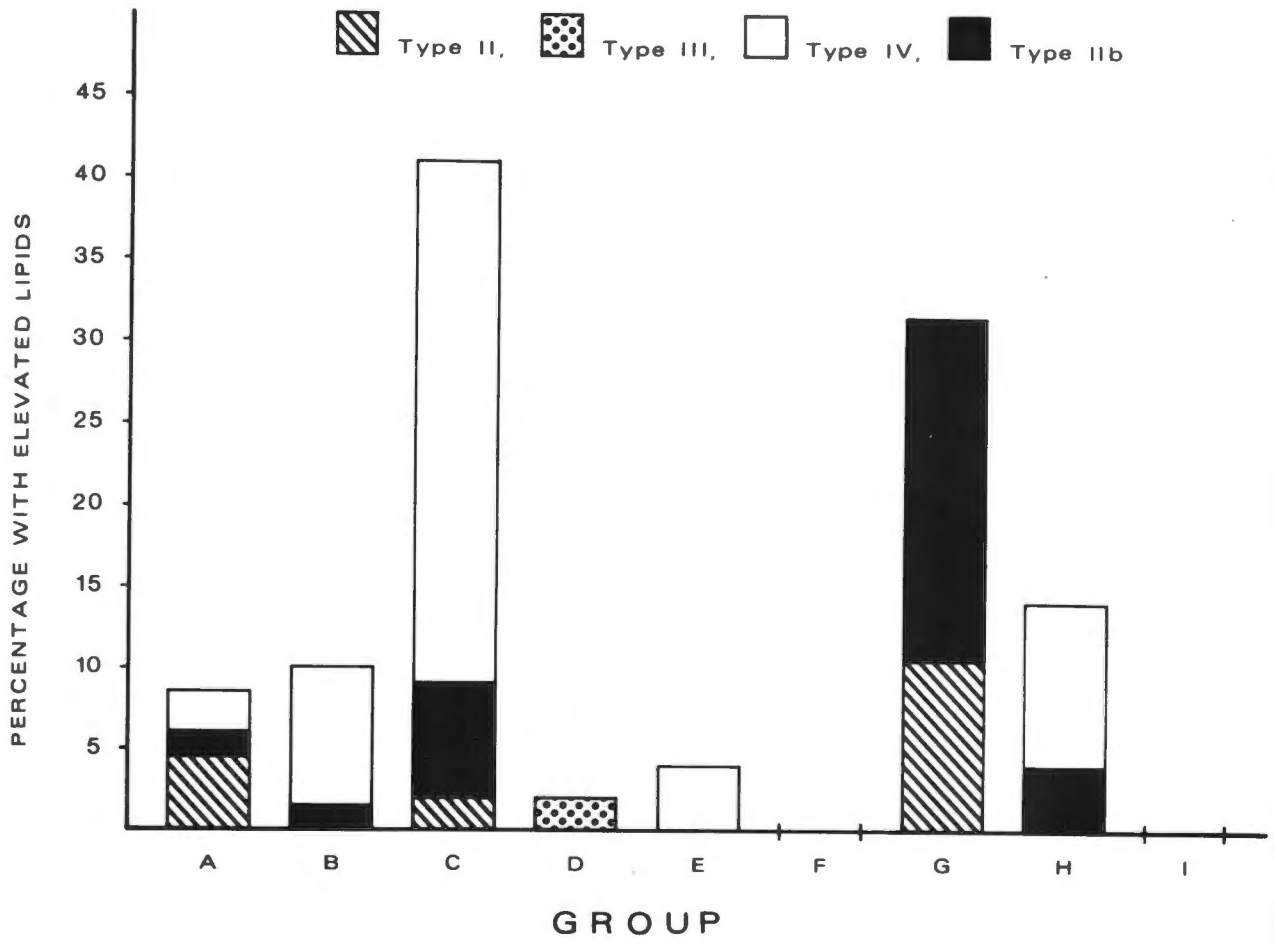


Figure 13: The percentage of individuals in each group with elevated serum lipid levels according to Fredrickson (35). The extent of each type of hyperlipoproteinaemia is indicated within the bar for each group.

In Group C, 41.2% of individuals had elevated serum lipids, in Group G 31.5% and in Group H 14.0%. In the remaining groups, less than 10% had elevated serum lipids and none of the individuals in Groups F and I had elevated lipids.

The most common abnormality was type IV hyperlipoproteinaemia (the carbohydrate-induced variety of hyperlipoproteinaemia) which was present in 31.4% of individuals in Group C, 10% of Group H, 8.5% of Group B, 46% of Group E and 1.6% of Group A. No individuals in Groups D and G were classified as type IV. The next most common abnormality was the type II(b) abnormality which included elevation of both cholesterol and triglyceride. This was present in 21% of Group G, 7.8% of Group C, 4% of Group H, 1.6% of Group A and 1.4% of Group B. The abnormality was not present in Groups D and E.

Type II was only present in 10.5% of Group G, 4.8% of Group A and 2% of Group C with no cases to be found in Groups B, D, E and H.

One case of type III hyperlipoproteinaemia was found in Group D and represented 2.4% of the individuals in this group. No cases conforming to types I and V of Fredrickson were detected in these surveys.

(I) CORRELATION BETWEEN SERUM CHOLESTEROL AND SERUM TRIGLYCERIDE

The relationship between serum cholesterol and serum triglyceride was examined in Groups B and C. A low grade but significant correlation was found between serum cholesterol and serum triglyceride levels in both groups. (The correlation coefficient r, was 0.34 and 0.37 in Groups B and C, respectively). Both values were significant at the 5% level).

(J) CORRELATION BETWEEN SERUM LIPIDS AND BODY WEIGHT

Body weights were available for the individuals in Groups C and E. In group C the correlation coefficient (r) was 0.30 and this was significant at 5% level. There was no significant correlation in Group E.

(K) SERUM LIPID LEVELS IN FEMALES

The values for serum lipids of Groups J and K, the two small groups of females studied, are given in Tables VIII, IX and X. The serum cholesterol levels are not significantly different from their comparable male groups (B and G respectively). The serum triglyceride levels on the other hand were lower in Groups J and K than in Groups B and G.

(L) A COMPARISON OF THE SUGAR CANE CUTTERS AFTER 3 MONTHS OF WORK IN THE CANE FIELDS

By the courtesy of the Tongaat Sugar Company we were able to collect fasting blood samples from 28 of the subjects in Group E after 3 months work in the cane fields. The differences between the two diets consumed by the group in Pondoland (Homelands) and Tongaat are shown in Table XXXI.

TABLE XXXI

TABLE XXXI DIETARY ANALYSIS OF GROUP E, COMPARING THE DIET CONSUMED IN THE HOMELANDS WITH THE DIET CONSUMED WHILE CUTTING SUGAR CANE IN TONGAAT

Place	Total Daily Caloric intake	Total Carbohydrate			Sucrose			Non-Sucrose Carbohydrate			Fat			Protein		
		Grams	Cals	% total cals.	Grams	Cals	% total cals	Grams	Cals	% total cals	Grams	Cals	% total cals	Grams	Cals	% total cals
Pondoland	2200	440	1760	80	33	132	6	407	1628	74	20	176	8	55	220	10
Tongaat	5672	1149	4594	81	284	1134	20	865	3459	61	50	454	8	142	567	10-

II. XXXI.

The difficulties experienced with the analysis of the Pondoland diet have already been described. Figures for the analysis of the diet in Tongaat were provided by the Tongaat Sugar Company. The source of inaccuracy is the amount of raw sugar cane consumed by the cane cutters while cutting sugar cane during the day. Campbell and Goldberg[17] have estimated that the amount consumed per day contains a half to three-quarters pound of sucrose, and the value therefore of half a pound per day has been used. In addition each labourer is provided with a weekly ration of two pounds of refined sugar. This results in an estimated daily sucrose intake of 284 g. In view of the very large total caloric intake, fat and protein appear percentage-wise to comprise only a fairly small proportion of total calories. However, in terms of actual quantities, adequate amounts are consumed.

It is difficult to estimate the total energy expenditure of this group of people. Campbell and Goldberg[17] have estimated that a very good worker is able to cut and move by hand approximately 7 tons of sugar cane per day. Enormous amounts of energy must be expended daily.

Mean values for body weight, serum cholesterol and serum triglyceride at the two sampling times are given in Table XXXII.

TABLE XXXII

BODY WEIGHT, SERUM CHOLESTEROL AND SERUM TRIGLYCERIDE CONCENTRATION OF GROUP E (28 SUBJECTS) IN APRIL AND JULY 1970

Time of Visit	No.	Body Weight			Serum Cholesterol			Serum Triglyceride		
		Mean (kg)	S.D.	S.E.M.	Mean (mg/100ml)	S.D.	S.E.M.	Mean (mg/100ml)	S.D.	S.E.M.
April 1970	28	59.0	11.6	2.2	45.0	37.8	7.2	92.4	28.3	5.4
July 1970	28	61.1	12.1	2.3	142.3	27.9	5.3	94.1	31.8	6.2
July 1970 (those who gained less than 2.3 kg in body weight)	14	58.3	15.3	4.1	145.4	23.1	3.9	96.0	31.8	5.1
July 1970 (those who gained more than 2.3kg in body weight)	14	63.5	16.8	4.5	140.1	32.6	4.9	92.8	24.2	4.6

II. XXXII.

There has been a mean increase of 2.1 kg in body weight. (The increase is significant at the 1% level using the Wilcoxon test for pair differences). There is, however, no significant change in cholesterol or triglyceride values when considering either the whole group or when the group had been subdivided according to how much weight change had occurred.

CHAPTER V - DISCUSSION OF RESULTS

These studies concerning chiefly the lipid levels in males confirm certain information already available. In addition, certain new aspects have come to light. These will be discussed in this chapter.

Serum triglyceride values show a pronounced skewness in frequency distribution in nearly all the groups studied and this skewness was reduced by logarithmic transformation. Serum cholesterol was significantly skewed in only one of the male groups studied - the schoolboys. In this group, however, the skewness was no longer significant when the value for g_1 was recalculated omitting the one cholesterol value at the top end of the range. In general these findings are in agreement with those of Carlson and Linstedt[23] who have carefully studied the distribution curves of these two major serum lipids. They did not however, study in detail subjects under the age of 20 years (only 3 males in this age group were studied by them) and found that in this age group the distribution of serum triglyceride was not significantly skewed. We have studied a fairly large group of 16-18 year old healthy males (62 subjects) and have in fact found a significantly skewed distribution for serum triglyceride even in this age group.

From our studies it appears that the single most important epidemiological factor influencing serum triglyceride levels is the frequency of physical activity. This is turn presumably reflects satisfactory caloric balance. European sedentary office workers (Group C) have significantly higher serum triglyceride levels than a comparable group who in their leisure hours participate in regular physical exercise (Group D). The two groups report almost identical diet histories and apart from the frequency of physical activity are subjected to a similar "westernised way of life". Group D in turn have serum triglyceride levels not significantly different from the groups which have the lowest mean values (Table IX). Further evidence for this suggestion is provided by the subdivision of the individuals in Groups B

and C according to the frequency of physical activity reported in their leisure hours. The significant decrease of serum triglyceride levels with an increased amount of leisure time physical activity is clearly apparent from Tables XIII and XIV. It is furthermore interesting to note that there is no significant difference between the serum triglyceride levels of the regular exercisers in the two groups despite the wide age discrepancy between them.

The serum triglyceride levels of the relatively sedentary African porters at Groote Schuur Hospital (Group H) are significantly higher than rural African labourers (Group E), but as their diets differ considerably, no definite conclusions can be drawn. It is however important to note that this group of rural Africans showed no increase of their serum lipids after 3 months of labour in the sugar-cane fields, despite a tremendous increase of their total daily caloric intake and quantities of each food constituent. It seems very likely that the reason for this is their great energy expenditure while cutting and moving sugar cane. The increase in weight is probably due to an increase in the lean body mass.

From the mean levels reported in Table IX, certain interracial differences are apparent. Serum triglyceride levels in the Bushmen (Group I) are lower in all other groups except the 16-18 year old European schoolboys. They consume a diet which is different from all other groups and in addition must expend a great deal of energy in their wanderings across the Kalahari Desert.

It is interesting to consider the 3 groups of medical students - African, European and Indian. The Africans and Europeans probably consume similar diets whereas the diet of the Indian differs considerably, being higher in polyunsaturated oils. The mean triglyceride level in the Africans is lower than in the Europeans. The group of African students, however, all reported regular physical activity during leisure hours and when compared with

the regular physical exercisers of Group B (the European students), the mean values are almost identical (see Tables IX and XIII). Mean values in the Indian students (who on the whole reported sedentary pastimes during leisure hours) and European students are remarkably similar. This finding tends to conflict with the view of Antonis and Bersohn[8]. Diets high in poly-unsaturated fats do not necessarily appear to result in low serum triglyceride levels. Caloric balance appears to be a more important factor.

Increasing levels of serum triglyceride with increasing age, reaching a plateau between 40 and 50 years and then slowly decreasing have been described by Carlson and Linstedt[23] and other workers[6,65]. This observation is also apparent from the mean values given in Table IX. The European schoolboys have lower serum triglyceride levels than the medical students who in turn had lower levels than sedentary office workers. These three groups may be regarded as having a fairly similar environment and diet. The fact that students do not have levels significantly different from physically active Europeans in an older age group does though tend to suggest that this age trend may be influenced by physical activity or caloric balance.

The age trend is further illustrated by the sub-division into age sub-classes of those groups covering a fairly wide range of ages (Table IX). Once again though, by examining the age trend in individuals reporting different degrees of physical activity in Group C (Table XV), it appears that physical activity plays an important role. A significant increase of serum triglyceride with age is evident in the African labourers. This is in conflict with the observations of Antonis and Bersohn[7].

Mean serum triglyceride values in this study for the four Westernised groups studied (A,B,C and D) correspond closely to levels reported in comparable groups in the literature[10,14,23,65,81].

It also appears that the relationship between smoking and serum triglyceride levels which has been reported by some workers[23] may be explained

on the basis of the relationship between smoking and physical exercise (Tables XXVIII and XX).

Serum cholesterol levels do not appear to be influenced by physical exercise to nearly the same extent as serum triglyceride levels. The mean serum cholesterol level is slightly lower in Group D than Group C, but the difference is not statistically significant (Table VIII). When sub-dividing Groups B and C according to the degree of physical activity reported, a significant difference is noted between exercisers and non-exercisers; however, the difference is not as striking as when serum triglyceride values are considered (Table XXIII).

As has been reported by other workers[13], it appears that diet is the principal factor influencing serum cholesterol levels - the westernised diet being associated with the highest levels of serum cholesterol. Lowest serum cholesterol levels were found in the Bushmen and the African labourers, whose diet is very low in animal fat and sucrose, high in complex carbohydrate and dubious as to the adequacy of protein intake and daily total caloric intake. The diet of the Bushmen is in addition high in polyunsaturated fat. A diet high in polyunsaturated fat does not per se appear to result in low levels of serum cholesterol. This is suggested by the fact that the Indian medical students do not have a serum cholesterol which is significantly different from that of the African and European students. It is in fact slightly higher than the other two groups. This observation in addition suggests that there is no racial difference in serum cholesterol levels.

The African porters at Groote Schuur Hospital (Group H) have a serum cholesterol level which is significantly higher than African labourers, but still significantly lower than a comparable age group of Europeans. Their diet is clearly intermediate between these two groups.

Increasing levels of serum cholesterol with age are also evident in this study. Lower levels of cholesterol are seen in the European school-

boys and medical students than Europeans in the 35-55 age group. This is also evident in the age sub-groups of Groups C, D, E and H shown in Table XXII.

Only a limited amount of information is available on the phospholipid concentration in serum. However, it appears that phospholipid levels parallel levels of serum cholesterol.

Interesting results have been obtained from the study of the serum triglyceride fatty acid patterns which, as has already been established,[8,40,50,52,66,82], reflect the nature and type of dietary fat. Increased amounts of myristic acid have been found in individuals with coronary heart disease[66] and it is worthy of mention that only in the two groups in this study in which coronary heart disease has not been reported (the Bushmen[77] and the rural African labourers[67]) was myristic acid present in significantly lower amounts than all other groups. Coronary heart disease is beginning to emerge in the urbanised African[67] and in the group of African hospital porters, studied here, mean percentage of myristic acid did not differ from comparable European groups. Linoleic acid is present in a greater percentage in the Bushmen and Indian students than in all other groups, reflecting a greater dietary intake of this fatty acid. However, despite the significant inverse correlation between amounts of this fatty acid in the serum triglyceride fraction and actual levels of serum triglyceride and cholesterol, it is apparent that this alone is not the most important factor influencing the levels of serum lipids. This observation is based on the fact that levels of serum lipids are not significantly lower in the Indian students than in the African and European students.

The fatty acid pattern of the serum triglyceride is fairly constant but several fatty acids not present in the other groups were found in the Bushmen and rural Africans.

The classification of the individuals with elevated serum lipid levels according to Fredrickson[35] has also provided interesting results, particularly

with regards the type IV or carbohydrate-induced variety of Hyperlipoproteinaemia. Fredrickson has suspected that this abnormality may well be the commonest type of hyperlipoproteinaemia, but as has already been stated, there is no information as to the frequency of this condition. In our surveys it was found to be the most common single abnormality of serum lipids occurring in 31.4% of Group C, 10% of the Group H and 8.5% of Group B. A striking contrast is the fact that this abnormality was not present in any individuals in Group D which differed from Group C only with regard to degree of physical activity. It is suggested therefore that the apparently common condition of primary Type IV Hyperlipoproteinaemia may in fact consist of two sub-groups - the more common variety being due to caloric excess and a far smaller group of true primary familial Type IV Hyperlipoproteinaemia, the genetics of which have not yet been clearly worked out[35]. It is also interesting to note that this abnormality was actually present in two of the African labourers studied - one under and one over 35 years.

Individuals who had elevated levels of both cholesterol and triglyceride and did not clearly fit into any of Fredrickson's five groups, comprised the next most common group.

Only a relatively few individuals were found to have Type II Hyperlipoproteinaemia (essential familial hypercholesterolaemia) and these tended to have only moderately elevated serum cholesterol levels. Only one case of Type III was detected (in Group D) and no cases were found of Types I and V.

The finding of low grade correlation between serum cholesterol and serum triglyceride and between serum triglyceride and body weight confirm the findings of other workers[6,23].

SUMMARY

It appears from this study that caloric balance is probably the single most important epidemiological factor influencing serum triglyceride levels in males. Variation with age does occur but appears to be modified by degree of physical activity. No evidence has been found to support the idea that race or diet per se may influence serum triglyceride levels.

Serum cholesterol on the other hand seems to be influenced to a very great extent by diet - a "westernised diet" being associated with the highest levels of serum cholesterol. Variation of serum cholesterol with age does occur and increased physical activity appears to be associated with lower levels of serum cholesterol. Physical exercise does not however influence serum cholesterol to the same extent as serum triglyceride. There is no evidence from this study that race per se influences serum cholesterol levels.

The following information was gained by studying the individuals in each group with elevated serum lipids. In the group of sedentary European office workers 41.2% of individuals had elevated serum lipids, in the group of Indian students 31.5% and in the group of African porters 14.0%. In the remaining groups less than 10% had elevated serum lipids and none of the African students and Bushmen had elevated lipids. The most common abnormality was Type IV Hyperlipoproteinaemia of Fredrickson. It was present in 31.4% of individuals in the group of sedentary office workers, 10% of the Groote Schuur Hospital African porters, 8.5% of European medical students and two individuals in the group of rural African labourers studied. No cases of this condition were found in the group of physically fit European males of comparable age and environment to the sedentary office workers. It is therefore suggested that the apparently common condition of primary Type IV Hyperlipoproteinaemia may consist of two sub-groups - the more common variety being due to caloric excess and a far smaller group of true primary familial Type IV Hyperlipo-proteinaemia.

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PART III

THE RELATIONSHIP BETWEEN DIETARY CARBOHYDRATE AND SERUM LIPIDS IN MAN

CHAPTER I - REVIEW OF THE LITERATURE

(A) INTRODUCTION

The influence of dietary factors on serum lipids has aroused considerable interest. Attention has primarily been focused on the effect of dietary fat on the concentration and composition of lipids. Both the quantity and the type of fat present in the diet appear to be relevant. In several epidemiological studies it was found that in groups where the percentage of dietary calories consumed as fat was about 40%, serum cholesterol levels were high and rose with age. Conversely, in groups eating little fat, serum cholesterol levels were considerably lower and the rise with age insignificant[17,62,63,64,65,66,125,129]. In addition it was noticed that serum cholesterol trends could be correlated with the consumption of animal fat but not with that of vegetable fat[18,44].

Arising from these observations it was shown in numerous control feeding experiments that serum cholesterol, triglyceride and phospholipid were increased by the consumption of saturated fats and decreased by the consumption of unsaturated fat, particularly those rich in linoleic acid[1,18,69].

The relationship between dietary carbohydrate and serum lipids has not been studied to the same extent. Carbohydrate appears to influence chiefly the serum triglyceride fraction of the serum lipids[89]. In view of the crucial role which caloric balance plays in determining serum triglyceride levels, epidemiological studies can provide only a limited amount of information and more carefully controlled feeding experiments are necessary to determine the precise effect of dietary carbohydrate on serum lipids.

The control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin is the subject of an excellent review article by Nikkilä[100]. Certain aspects of this review are, however, very relevant to the series of experiments conducted as part of this study and will therefore be briefly discussed here.

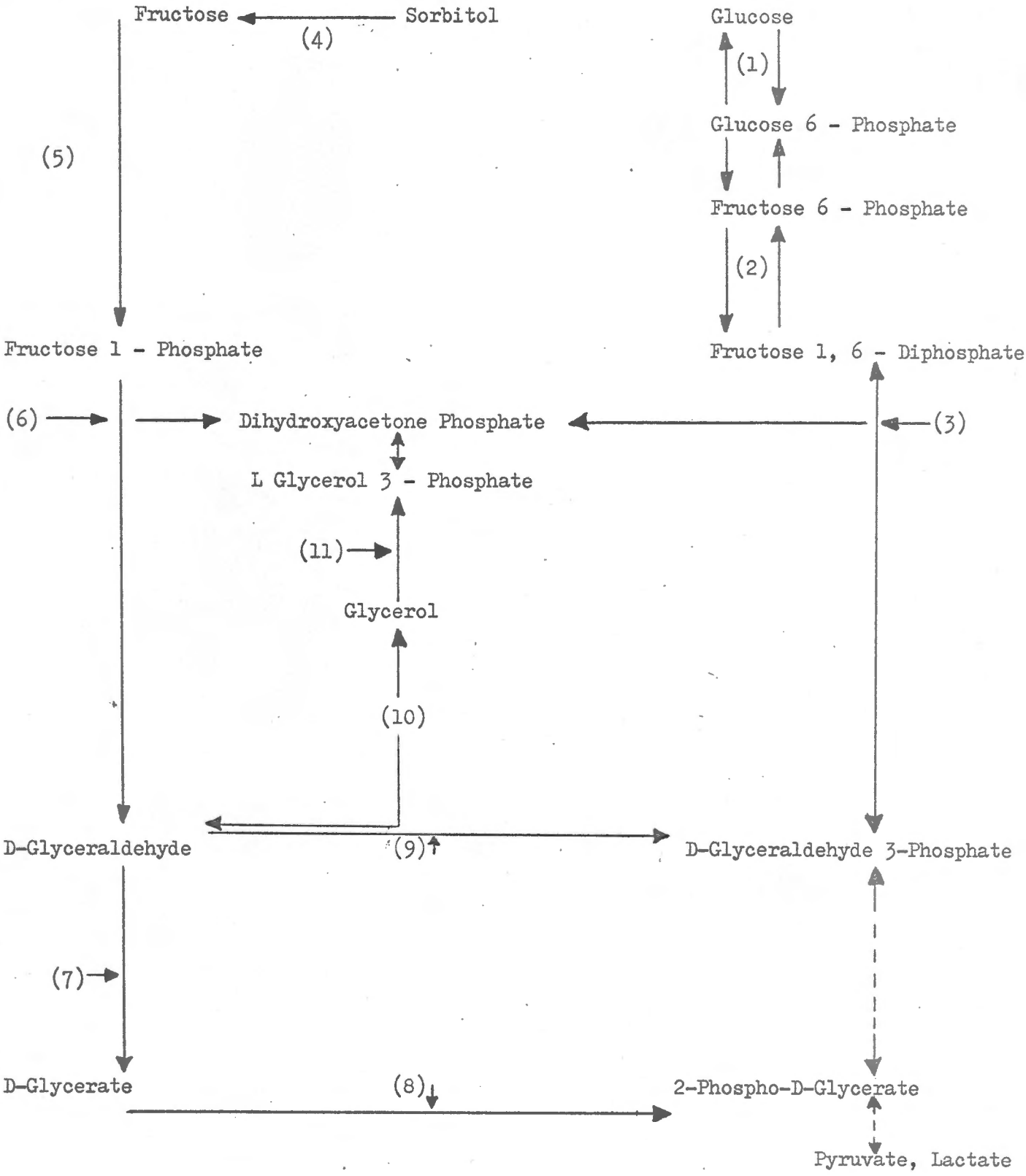
(B) THE ROLE OF CARBOHYDRATE IN THE SYNTHESIS OF TRIGLYCERIDE BY THE LIVER

It has been clearly established that fatty acids are synthesized in the liver by both a mitochondrial and extramitochondrial system, chiefly from acetyl CoA, which is derived from carbohydrate via the oxidation of pyruvate within the mitochondria[127]. An additional important source of fatty acid substrate for glyceride synthesis is the plasma free fatty acid[133]. The flux of free fatty acid into liver is determined by the rate of release from adipose tissue and any factor that increases lipolysis or decreases glycerol esterification in the adipose tissue causes outpouring of free fatty acid[119]. Both fatty acids and glycerol must be activated by A.T.P. before they become incorporated into glyceride. In the liver, the enzyme glycerokinase will catalyse the activation, by phosphorolation of glycerol to glycerol phosphate[59]. However, glycerol phosphate may also be derived from an intermediate of the glycolytic system, dihydroxy-acetone-phosphate, by reduction with NADH. This reaction is catalysed by glycero-phosphate dehydrogenase[93].

There is evidence to show that different dietary carbohydrates may influence this hepatic triglyceride synthesis in different ways. Zakim and Herman[139] gave intravenous loads of either glucose (200 mg) or fructose (200 mg) to rats and measured the concentration of glycerol phosphate in the liver at 5 and 20 minutes after injection. Five minutes after the fructose load, the concentration of glycerol phosphate had increased fourfold above the controls at zero time, but only increased 1.1 fold when glucose was used. By 20 minutes values had levelled at 1 to 2 times control values with glucose and fructose, suggesting that the excess glycerol phosphate produced from fructose had now been converted to something else. Unfortunately hepatic glycerides were not measured at the same time. These experiments do however indicate that fructose is somehow converted to glycerol phosphate, the precursor (and in rats the rate limiting step[126]) for glyceride synthesis more quickly than glucose.

The steps involved in the glycolytic pathway have been clearly established[90] and more recently a more potent enzyme system for fructose metabolism in the rat liver has been described[46,48]. This is illustrated diagramatically on the next page :

The Metabolism of Glucose and Fructose in Liver (47)



1. Glucokinase, Hexokinase
2. Hexose-diphosphatase
3. Aldolase
4. Sorbitol Dehydrogenases
5. Ketohexokinase
6. Aldolase
7. Aldehyde Dehydrogenase
8. Glycerate kinase
9. Triokinase
10. Alcohol Dehydrogenases
11. Glycerolkinase

Heinz and his co-workers[47] have compared the activity of enzymes concerned in the metabolism of glucose and fructose in biopsy specimens from the human liver. The enzyme responsible for the phosphorolation of fructose (ketahexokinase) has an activity of 1.23 units per gram of tissue compared with glucokinase, the enzyme responsible for the phosphorolation of glucose which has an activity of only 0.28 units per gram of tissue. Furthermore, the fructose 1-phosphate thus formed is able to bypass the second phosphorolation of glucose and break down directly to di-hydroxyacetone phosphate (the precursor of glycerol phosphate) and D-glyceraldehyde[48]. The enzyme responsible is known as Aldolase 1-B[115] but is the same enzyme that catalyzes the conversion of fructose 1,6-diphosphate to dehydroxyacelenephosphate and D-glyceraldehyde-3-phosphate[47]. Thus fructose is converted to glycerol phosphate, the precursor for glyceride synthesis more rapidly than glucose.

This more rapid metabolism of fructose than glucose might also explain the increased rate of absorption of labelled fructose as compared with labelled glucose into the free fatty acids of the liver in both the rat[137] and man[140].

Insulin plays a crucial role in triglyceride metabolism and affects serum triglyceride concentration by its effect on hepatic triglyceride synthesis and on serum triglyceride outflow. Most of the experimental studies have been carried out using experimental animals and the whole subject has been discussed in the previously mentioned review article by Nikkilä[100].

Before being secreted into the blood stream glycerides synthesized in the liver form complexes with proteins known as lipoproteins. Glyceride is transported chiefly as pre-beta lipoprotein[90].

(C) STUDIES IN WHICH FORMULA FEEDING WAS USED TO EXPLAIN THE EFFECTS OF DIFFERENT CARBOHYDRATES ON SERUM LIPIDS

A number of studies have been carried out in which semipurified formula diets have been used to study the effects of carbohydrates on serum lipids.

Winitz et al[133] fed 18 subjects a formula-type diet consisting of

aminoacids, essential vitamins and salts, glucose and 2 grams per day of ethyl linoleate as the only source of dietary fat. The total daily caloric content was adjusted to suit each individual and ranged from 2100 to 3700 kCal per day. After the diet had been administered for 4 weeks the mean serum cholesterol had fallen from 227 to 160 g/100ml. 25% of the glucose in this formula was then replaced by an equal weight of sucrose and after 3 weeks on this high sucrose diet, the cholesterol had risen to 208 mg/100ml. A return to the glucose formula was followed by a decrease in cholesterol to 175 mg/100ml after 1 week and 151 mg/100ml 11 weeks later. Within 4 weeks after returning to their original self selected diets, serum cholesterol had risen to 233 mg/100ml.

Carbohydrate induction of hyperlipaemia has been clearly demonstrated in normal men by Lees and Fredrickson[77]. In 13 volunteers they were able to demonstrate a mean increase of 224 mg/100ml in the serum triglyceride fraction within 3-7 days after commencement of a very high carbohydrate diet. The increase in serum triglyceride was due to the increase in the serum pre-beta lipoprotein and occurred independently of caloric balance. The hyper-triglyceridaemia started to diminish after 7-14 days despite continuation of the high carbohydrate diet. This increase in serum triglyceride after changing from a diet high in fat and comparatively low in carbohydrate to one high in carbohydrate and low in fat has also been demonstrated in a field study[10]. Here too, serum triglyceride tended to return to the original levels when the high carbohydrate diet was continued for several months.

The increments in triglycerides observed during carbohydrate induction are chiefly due to elevation of triglyceride carried by the very low density lipoprotein produced by the liver. There is evidence to show that each very low density lipoprotein molecule formed is loaded with an abnormally large amount of triglyceride[118].

An important study was undertaken by Farquhar et al[30] in an attempt to determine the mechanism of hypertriglyceridaemia during carbohydrate induction. Fifteen volunteers were in turn fed different formula diets for

variable time periods (approximately 1 month). One diet contained 68% of calories from fat and the other 85% from carbohydrate, both diets contained 15% of total calories from protein. The fat was derived chiefly from corn-oil and the carbohydrate from lactose and dextrins. A wide spectrum of plasma triglyceride responses was obtained. The triglyceride was in all cases elevated on the high carbohydrate diet as compared with the high fat diet. A high degree of correlation existed between the fasting plasma triglyceride level and the average levels of plasma glucose and immunoreactive insulin reached during 9 hours of a representative day of ingestion of either the high fat or the high carbohydrate diet. However, despite the presence of hyperinsulinism, a high carbohydrate diet did not produce hypertriglyceridaemia in a patient with hypoglycaemia secondary to an islet cell adenoma. The authors suggest, therefore, that hyperinsulinaemia in the presence of normal to moderately elevated levels of plasma glucose may be an important cause of enhanced hepatic triglyceride production, that underlies endogenous hypertriglyceridaemia.

MacDonald has conducted a series of experiments in which formula feeding has been employed to determine the effects on serum lipids of different dietary carbohydrates.

In 1964 he and Braithwaite[79] studied the changes in blood lipids in 7 males who were maintained on a high carbohydrate diet for two 25-day dietary periods. 500 grams of sucrose or corn starch provided the carbohydrate source. The remainder of the diet consisted of lean meat, fish, green vegetables and fruit. Fat comprised 10-13% of total daily calories. The two experimental dietary periods were separated by 25 days on self selected diets. While on the high sucrose diet, there was an increase in serum triglyceride; whereas on the high starch diet there was a fall of serum phospholipid and total cholesterol and no change in triglyceride. Changes also occurred in the fatty acid patterns of adipose tissue and serum lipid. Both high carbohydrate diets were associated with an increased percentage of palmitic and oleic acid and a decrease of linoleic acid in serum lipid fatty acids. This finding is not surprising as it has been clearly shown that

animal tissue cells convert carbohydrates primarily into palmitic, palmitoleic and oleic acids, but not linoleic acid[49,72,112]. Differences in the response to the starch and sucrose diets were seen in the mean percentage of myristic acid which was higher while on the sucrose diet and stearic acid which is lower while on the starch diet. An average decrease of 1.1 kg in body weight occurred on the starch diet.

The following year[80] he reported a further study in which 7 men consumed a fat-free diet consisting of 50 grams of calcium caseinate and 7.5 grams/kg carbohydrate. There were five 5-day dietary periods each separated by a week on an ad lib diet and in each diet period maize starch, glucose, sucrose, maltose and liquid glucose in turn provided the carbohydrate source. A weight loss occurred in each dietary period and was greatest during the starch period during which a mean of 1.2 kg was lost in five days. Serum cholesterol levels fell significantly in all dietary periods except during the sucrose period. Serum triglycerides were increased by sucrose, fell while on maltose and glucose and remained the same on starch and liquid glucose. Serum phospholipid remained unchanged.

A similar study to the above was carried out on five young women[81]. In them sucrose actually resulted in a slight decrease in serum triglyceride. Finally, using similar proportions of the proximate food constituents but three mixtures as the carbohydrate source (40% fructose and 60% corn starch, 40% glucose and 60% corn starch and 40% fructose and 60% glucose), young men and women were compared with post-menopausal women[83]. Once again three 5-day diet periods were employed, each separated by a week of ad lib diet. In the men and in the post-menopausal females serum triglyceride increased with both diets containing fructose, but there was no change in the young women. MacDonald concludes that oestrogens or progesterones must prevent the rise in fasting serum triglyceride levels associated with dietary fructose.

When considering the results of the above studies it should be borne

in mind that all the experimental diets described thusfar contained an abnormally high proportion of carbohydrate calories and were low in fat.

MacDonald has also examined the interrelationship between the influences of dietary carbohydrate and fat on serum lipids[84]. Four diets were given, each for five days with a nine-day interval between diets. Approximately 10% of total daily calories were derived from calcium caseinate, 60% from carbohydrate (either glucose or sucrose) and 30% from fat. The fat was provided either as cream or as sunflower seed oil. Serum triglyceride levels were elevated on the sucrose/cream diet but remained unchanged on the other diet. Serum cholesterol levels fell on all diets containing sunflower seed oil.

Two workers in the field[11,77] feel that the different effect of different carbohydrates discussed above may all be explained on the basis of inadequate attention to calorie balance. Changes in weight which are not statistically significant may nevertheless well be of biological significance. Lees[77] has suggested that the different effects of raw maize starch and sucrose demonstrated by MacDonald[79] may be due to the fact that the uncooked starch is poorly absorbed. He has shown no differences in serum triglyceride levels when purified diets containing 10% of total calories as protein and 90% as carbohydrate regardless of whether the carbohydrate was cooked starch, wheat and rice or sucrose. Each of 7 subjects were fed both diets for 4-14 days, the periods being separated by 3-7 days of normal diets. Serum triglyceride increased by 61-221 mg/100ml on the high starch diet and by 30-231 on the high sugar diet. Cholesterol response too was identical. Body weight remained absolutely constant.

Antonis et al[11] fed formula diets to 14 subjects. They were first given a diet containing 40% fat (soya-oil), 45% carbohydrate and 15% protein which was supplied as fat-free skim milk powder which also contained lactose which accounted for 20% of total daily calories. The skim milk was given

throughout the experiment. After stable lipids had been achieved, the fat was isocalorically replaced by glucose, lactose, sucrose, cooked starch and uncooked maize starch in different combinations. When carbohydrate was isocalorically substituted for fat, serum triglyceride levels rose but there was no difference in the effect produced by the individual carbohydrate.

The findings of all these experiments may be summed up thus:- Serum triglyceride levels are elevated on high carbohydrate diet and furthermore, sucrose appears to result in higher serum lipid levels than complex carbohydrates. However, the practical significance of these experiments is limited in view of the fact that the formula diets rather than ordinary food substances, were provided and also because carbohydrate in these studies provided an abnormally high proportion of total calories.

(D) STUDIES IN WHICH NORMAL FOOD SUBSTANCES WERE USED TO TEST THE EFFECTS OF CARBOHYDRATE ON SERUM LIPIDS

The transient increase in serum triglyceride associated with the change from a high fat-low carbohydrate diet to a low fat-high carbohydrate diet has already been discussed[10]. A series of experiments which have been conducted paying attention to the type and level of dietary carbohydrate will now be discussed.

Keys et al[67] studied institutionalised subjects. Using dietary periods of 6 weeks' duration, they compared the response of blood lipids to the type of dietary carbohydrate at two levels of fat (protein kept constant at 13% of total calories). At both levels of fat (16 and 30% of total calories) two types of dietary carbohydrate were fed - one high in complex carbohydrate and the other high in sugar. At both levels of fat serum cholesterol was found to be 18 mg/100ml lower in the diet high in complex carbohydrate, but there was no difference in serum triglyceride levels. (Sucrose provided 17% of total calories). Intake of dietary fibre was higher in diets high in complex carbohydrate and these authors, therefore, carried out further studies[68].

The relative proportions of simple and total carbohydrates was varied even further with fat providing 40% of total calories. The differences in serum cholesterol was similar to the findings of their previous study. The addition of 15 grams of cellulose per day to diets containing both simple and complex carbohydrates, produced no further change, but the addition of 15 grams of pectin produced a fall of about 10 mg/100ml in serum cholesterol in both types of diet. The authors did not, however, consider these substances of any practical significance in the regulation of serum lipids.

In 1963 Anderson et al[6] studied 23 psychiatric patients in who the normal hospital diet containing 35% of total calories as fat, was replaced by diets containing only 13% of total daily calories as fat. Carbohydrate was provided in turn as glucose, sucrose or a mixture of lactose and glucose. Diet periods were of three weeks duration. Serum cholesterol levels fell and serum triglyceride levels rose significantly when the high carbohydrate diet was introduced. However, no significant differences were noted when the different carbohydrates were fed. Triglyceride levels did, however, tend to be higher on sucrose than glucose and approached statistical significance ($p = 0.04$).

The same group of workers[37] once again using experimental diets of three weeks duration with fat providing 40% of total calories, showed no significant difference in serum cholesterol when bread and potato were substituted for dietary sucrose with dietary protein kept constant. However, a small but significant decrease in cholesterol occurred when the carbohydrate source came from a mixture of leguminous seed. The small decrease was attributed to either the carbohydrate or a specific substance in the legumes. (Sucrose comprised 20% of total calories).

Using the 25-day feeding periods and a cross-over design, Irwin et al[53] showed a slight but not significant lower serum cholesterol when rice replaced sucrose as the carbohydrate source in 6 young male students. Fat

provided 35% and carbohydrate 55% of total calories (sucrose supplied 30% of total calories). Unfortunately, serum triglyceride levels were not estimated in this study.

Using diets in which proportions of the proximate food constituents resembled that normally consumed (fat 40%, carbohydrate 44% and protein 16%) Antar and Ohlson studied eight young adults[7]. A cross-over experimental design was employed in which either 20% or 80% of total carbohydrate calories were derived from simple sugars, the remainder being provided by starch from cereal and potatoes. Dietary periods were each of 4 weeks duration. All serum lipid fractions were significantly reduced with the high cereal diet and increased with the high sucrose diet in which sucrose comprised 35% of total calories.

In the same year Hodges and Krehl[51] published their study on eight married couples. In four diet periods each of 4 weeks duration, protein, fat and carbohydrate were fed in normal proportions. In diet periods 1 and 3, carbohydrates were essentially simple sugars and in periods 2 and 4 essentially complex. Similar results were found in periods 1 and 3 and 2 and 4, namely that all serum lipid fractions were significantly higher on the simple carbohydrates. It must once again be pointed out that while total carbohydrate was fed in normal proportions, sucrose comprised 35% of total daily calories in diets 1 and 3.

McGandy et al[88] fed mixed diets made of ordinary foodstuffs prepared in their usual American way, to 18 healthy schizophrenic males. Fat provided 38% and carbohydrate 45% of total daily calories. The diets were designed so that any one of three fats could be used in combination with either high sugar or low sugar carbohydrate. The sugar starch difference amounted to 23% of total caloric intake and when the sugar was fed it was in proportions normally eaten[97,103,136]. The subjects were divided into two groups of nine each and the high sugar and low sugar diets fed in a criss-cross pattern. At the end of experimental periods of 4 weeks, levels of cholesterol were

slightly but significantly lower (approximately 10 mg/100ml) on periods with low sugar. The effect induced by changes in dietary fat were much larger and apparently independent of the type of carbohydrate given. Serum triglyceride levels were unfortunately not reported.

Groen et al[40] had fed volunteers a low fat diet with either bread or sucrose providing the bulk of the calories. Serum cholesterol fell on both diets which were low in fat but was significantly higher on the diet high in sucrose. Fifteen volunteers took part in the study in which a crossover experimental design was employed.

Szanto and Yudkin[122] have studied the effects of high and low sucrose intake for periods of 14 days in a group of 19 apparently healthy men. The high sucrose diet produced a significant increase in triglyceride levels in all 19 males but no change in cholesterol and phospholipid. In 6 of these subjects there was in addition, a rise of serum immunoreactive insulin with respect to both the fasting levels and the levels observed during a glucose tolerance test. There was no impairment of glucose tolerance. The same 6 subjects showed a considerable increase in body weight and a significant increase in platelet adhesiveness. The changes produced by sucrose had disappeared after 14 days on a normal diet. Sucrose in the high sucrose diet provided approximately 50% of total daily calories - an amount far in excess of that normally consumed[97,103,136]. This confirms previous results obtained by the same group of workers[4].

The same author[134,136] has suggested a closer association between intake of sugar and mortality from coronary heart disease than the better described association between intake of dietary fat and mortality from this disease[17,54].

This hypothesis has been supported by some[23] and rejected by others[52,104] and is virtually impossible to verify in view of the very close correlation between consumption of sugar and saturated fat ($r = 0.92$). This association is higher even than that between heart disease mortality and

sugar (r = 0.80) or saturated fats (r = 0.82)[54].

Nevertheless, it is this suggestion which has sparked off much of the work described above, since if intake of sucrose does play an important role in coronary heart disease it is likely to be via the elevation of one or more serum lipids.

There appears to be little doubt that serum triglyceride levels become elevated when a diet high in fat is replaced by one low in fat and high in carbohydrate[10,11,30,77]. This elevation of serum triglyceride though tends to return towards normal if the new diet is maintained for a period of some months[10]. However, the different effects which different carbohydrates have on serum lipids is not as clear cut.

In summary, it would seem that in males serum triglyceride levels become elevated on diets containing abnormally high proportions of dietary sucrose[4,7,51,79,80,83,122] particularly when the fat component of the diet is saturated[84]. In two studies[11,77] no elevation of serum triglyceride was detected when sucrose replaced starch as the principal carbohydrate source, even though the amount of sucrose was excessively high. However, in these studies fat was either not given at all[77] or in an unsaturated form[11].

I have in my study of the literature not been able to find any evidence which satisfactorily explains the mechanism of the synergistic hyperlipidaemic effect between dietary sucrose and saturated fat. The fact that higher fasting serum triglyceride levels are seen when saturated rather than unsaturated fat is the chief dietary fat has already been mentioned[1]. In the rat lipoprotein lipase activity in adipose tissue is lower when saturated rather than polyunsaturated fat is fed[105]. This suggests that in these animals, impaired clearance of triglyceride might occur on a diet high in saturated fat. It has been shown in several studies that in westernised population groups dietary sucrose comprises approximately 15% of total daily calories[97,103,136] and only rarely exceeds 25%. When sucrose,

given in physiological amounts (i.e. 25% of total daily calories or less) is compared with starch, little[6] or no[37,67] differences in serum triglycerides have been detected. Serum cholesterol levels appear to be slightly reduced when dietary sugar is replaced by complex carbohydrates[7,40,51,53,67,68,79,88,133].

It is highly relevant to point out here that all dietary periods in the above described studies were relatively short, the longest being 5 weeks and the evidence at present is certainly inadequate for suggesting that the population at large change the nature of its dietary carbohydrate.

(E) PRINCIPAL OBJECTIVES FOR THIS PART OF THE STUDY

It seemed, therefore, that a most relevant study at the present time would be a longterm field experiment in which a section of the population not suffering from any disease, was asked to cut out the sugar from their diet and the effects of this manoeuvre on the serum lipids compared with what happened when a similar group of people cut down on the complex carbohydrate of their diet without changing their weight by compensating with other foodstuffs. The longest time that the effects of restricting dietary sugar have been measured in man is 10 weeks[114]. In that experiment 11 men who had returned to work after a mild myocardial infarct showed a reduction of serum triglycerides which was significant at the beginning of sugar restriction but had become not significant by the 10th week. Their serum cholesterol fell slightly and the patients lost an average of 3 lbs in weight at the beginning of sugar restriction.

Furthermore, in view of Yudkin's[122] finding that serum insulin levels both in the fasting state and after a glucose load were elevated in certain individuals on high sucrose diet, it seemed important to examine possible changes in carbohydrate metabolism on diets containing different carbohydrates but with sucrose constituting a normal, rather than a very high proportion of total calories. Such a study would have to be carried out under metabolic ward conditions and this would therefore also afford the opportunity of comparing the effects of complex carbohydrates with sucrose in normal amounts

under conditions of strictly accurate caloric balance since caloric imbalance has been implicated as the cause for many of the findings in previous studies[11].

Finally, the mechanism of elevated fasting serum triglyceride on high sucrose diets remains unclear. The studies which have been performed in an attempt to clarify this mechanism have been carefully reviewed by Nikkilä[100], and may be very briefly summarised thus: An increase in concentration of fasting serum triglyceride must occur either through accelerated influx into or decreased efflux from the serum triglyceride pool or both mechanisms may operate. There is no doubt that in both man and experimental animals, the fructose of sucrose is more rapidly converted to the precursors of triglyceride than glucose, but the conversion of fructose and glucose into hepatic and plasma esterified lipids forms only a very minor fraction (1-3%) of the metabolism of these two hexoses. Other factors must therefore be sought. It has been suggested that impaired removal from the serum triglyceride pool may be an important mechanism. This suggestion however is based on indirect evidence in experimental animals[12]. Some further evidence that this mechanism may in addition operate in man will also be presented in this thesis.

(F) THE EFFECTS OF DIFFERENT DIETARY CARBOHYDRATES IN INDIVIDUALS WITH HYPERLIPIDAEMIA

Hyperlipaemia was initially divided into carbohydrate and fat induced varieties by Ahrens et al[2] and later by Fredrickson[32] into five different types. Fredrickson's type IV corresponds to the carbohydrate induced variety and it is in this type that different carbohydrates might be expected to have different effects.

Kuo and Bassett[73] compared the effects of diets high in sucrose or starch on the plasma lipids of patients with types II and IV hyperlipoproteinaemia of Fredrickson. The diets contained normal proportions of the proximate food constituents but the diet high in sucrose contained sucrose in rather higher than normal proportions (approximately 40% of total daily calories). Dietary

periods were of six weeks duration. In the two subjects with type II hyperlipoproteinaemia serum cholesterol and phospholipids fell on both experimental diets as compared with the self-selected diets. On the high sucrose diet serum triglyceride levels were elevated. The patients with type IV hyperlipoproteinaemia all showed elevation of serum triglyceride levels on the diet high in sucrose as compared with self-selected diets. Serum cholesterol phospholipids remained unchanged. All lipid fractions were lower on the diets high in starch. Body weight remained fairly constant throughout these studies. These workers in addition studied fatty acid patterns of the serum lipids of their subjects and found no significant differences when comparing subjects with hyperlipidaemia with a group of normal controls when both were on self-selected diets. However on high sucrose diets significant changes were evident in all lipid fractions of the hyperlipidaemic patients studied. Proportions of palmitic, palmitoleic and stearic acids were increased and linoleic acid decreased. Similar changes were observed on the high starch diets, but did not occur to the same extent.

Similar results were obtained by Kaufman et al[57] who used highly abnormal amounts of both sucrose and complex carbohydrates (60% of total daily calories) in his diets and only 5% fat, and later again by Kuo et al[74].

Porte et al[111] compared the effects of dietary starch and dextrose on plasma triglycerides in two lipaemic subjects. Plasma triglycerides were elevated when these two subjects were on a virtually fat-free diet. No change in triglyceride was produced when starch replaced the high dextrose formula previously administered. Caloric restriction, however, produced a marked decrease in serum triglyceride.

The interrelationships between kinds of dietary carbohydrate and fat in hyperlipoproteinaemic patients have been clarified recently by a series of articles by Little and co-workers[8,14,78] and may be summarised as follows:

When sucrose and starch (each comprising 20% of total calories) were compared in diets containing 65% of calories as corn-oil, no differences in

serum triglyceride were noticed in type II, and only slightly higher levels on sucrose were noticed in types III and IV. Feeding normal proportions of the proximate food constituents, but with the fat component being chiefly poly-unsaturated and low in cholesterol, once again no significant differences were noted in all types of hyperlipoproteinaemia when sucrose was exchanged for 40% of calories as starch. However, when the fat component of the diet was chiefly saturated and contained cholesterol (310 mg/1000 kCal), substitution of 40% of calories of sucrose for starch resulted in significantly higher serum cholesterol phospholipid and triglyceride. Similar results were obtained by Antar et al[8].

The effects of interchange of starch and sucrose with sucrose comprising the usual 25% or less of total daily calories, in individuals with hyperlipo-proteinaemia remains to be demonstrated.

CHAPTER II - THE EFFECTS ON SERUM LIPIDS IN NORMAL MEN OF REDUCING DIETARY SUCROSE OR STARCH FOR FIVE MONTHS

(A) INTRODUCTION

Many studies have appeared in the literature in which the effects of different carbohydrates on serum lipids have been studied and these have been reviewed in the previous chapter. There is, in addition, great uncertainty whether what can be done in a metabolic ward will be carried out by free-living people. There is uncertainty whether any changes in serum lipids, resulting from altering the quantity or quality of dietary carbohydrate will last after subjects adapt to the new diet.

A fairly longterm field experiment would answer at least some of these questions and this was an area in which very little research had been done. The longest period during which the effects of sucrose restriction had been studied was 10 weeks and this was in patients who had suffered from myocardial infarction 4-24 months previously[114]. During this period, serum triglyceride which rises immediately after an infarct, tends to return to normal[124]. There was no control group in this experiment which makes interpretation of the significant fall of triglyceride in these 11 patients who gave up sugar, very difficult. Interpretation is further complicated by the fact that approximately 3 lbs of weight was lost at the beginning of the sucrose restriction. The fall in triglyceride which occurred had become not significant by the tenth week. This finding was confirmed by Dunnigan et al[28] who studied a further 9 patients who had suffered from myocardial infarction. These authors suggest that the reduction of triglyceride can be explained by the moderate weight loss.

No studies of sucrose restriction had been carried out in healthy volunteers and for this reason it was decided to study the effects on serum lipids in normal men of reducing dietary sucrose or starch for five months.

(B) DESIGN OF THE EXPERIMENT

1. Subjects

An approach was made to the Chief Medical Officer and management of a large insurance company, the head office of the Old Mutual at Pinelands, Cape Town. These offices are conveniently situated a short drive from the Medical School. As a result of a detailed circular letter, 54 volunteers were recruited. Three withdrew before the start of the experiment and only one withdrew during the course of the study. All volunteers were male sedentary office workers aged 36-55 years. Their positions in the company varied and included amongst them clerks, lawyers, accountants, department heads and an actuary.

2. The Control Month

The subjects were interviewed individually (by the investigator and dietician) and the nature of the experiment was explained to them at length. They were each asked to complete a questionnaire. Their personal doctors were consulted about previous illnesses where necessary. All were in apparent good health. Only one had had clinical coronary disease (an uncomplicated myocardial infarct) three years previously.

The subjects were asked to continue their usual work and recreational activities (including smoking habits) throughout the experiment.

During the control month, in which the interviews were conducted, blood was taken after an overnight fast on three consecutive weeks to provide satisfactory baseline readings in view of the well-described daily variation in serum lipids[24]. On each occasion the weight without jackets and shoes was recorded before breakfast.

For the last week of the control period each subject kept a detailed seven-day dietary record of all food and drinks consumed.

3. Allocation to different groups and dietary instructions

Towards the end of the control month subjects were each given a

number and using this number the 51 volunteers were allocated to one of three equal-sized groups of seventeen by means of a table of random numbers.

A week before the diets were started the volunteers were addressed corporately and given a typed list of dietary instruction depending upon his group. (See Tables I, II and III).

TABLE I

Dear Mr .....

You are in Group A and are requested to follow as closely as possible starting as from 8th April, the Low Sugar Diet described below:-

This Low Sugar Diet is intended to eliminate the consumption of cane sugar (sucrose).

In order not to lose weight, compensate for the loss of sugar with protein or starchy foods such as cheese or potatoes. Non-caloric sweeteners such as saccharine or sucaryl, with which you will be provided, are allowed in tea and coffee, also diabetic jams, marmalade and tinned fruit, and non-caloric soft drinks.

AVOID

Sugars - all sugar in tea and coffee
white sugar
brown sugar
cube sugar
demerara sugar
sugar-coated breakfast cereals
Conserves - jam, marmalade, jelly, honey and golden syrup
Cakes - cakes, sweet buns and biscuits, icing and doughnuts
Dessert and Puddings - all sweet puddings and pies, ice-cream, condensed milk, jelly (except diabetic)
Sweets and Chocolate - all kinds
Drinks - soft drinks unless non-caloric fruit squash, sweetened fruit juice tinned or frozen, liqueurs
Fruits - grapes, dates, bananas, (sweetened) tinned and stewed fruit

COMPENSATE WITH

Bread - brown or white
Potatoes
Breakfast cereals
Milk
Butter
Meat
Fish
Poulty
Cheese - all kinds
Eggs
Biscuits that accompany cheese
Vegetables of all kinds
Peanut Butter
Marmite
Fruit - except grapes, dates and bananas
Yoghurt
Vegetables of all kinds
Non-caloric (diabetic) soft drinks
Nuts

Should you have any queries about this diet please do not hesitate to consult either Mrs. Manning or myself (phone 55.3921).

Yours sincerely,

J.I. Mann.

TABLE II

Dear Mr .....

You are in Group B and are requested to follow as closely as possible starting from 8th April, the Low Starch Diet described below:-

This Low Starch Diet is intended to reduce, not to eliminate entirely the daily starch consumption.

Eat only half your normal intake of starchy foods. For example, half a helping of cornflakes at breakfast and one slice of toast instead of two.

In order to maintain your weight, compensate with the non-starchy foods.

REDUCE INTAKE OF:	COMPENSATE WITH:
Potatoes	Sugar
Bread of all kinds and toast	Fruit - fresh or preserved
Buns and rolls	Jam, jelly, honey
Biscuits of all kinds	Meat
Cakes - all kinds	Fish
Cereals - all breakfast cereals	Eggs
Porridge and Rolled Oats	Cheese
Pancakes	Poultry - but not stuffing
Pastry	Milk
Rice	Chocolate
Flour - in any form	Boiled sweets
Pasta - noodles	Nuts
macaroni	Yoghurt
spaghetti	Avocados

Should you have any queries about this diet please do not hesitate to consult either Mrs. Manning or myself (phone 55.3921).

Yours sincerely,

J.I. Mann

III. II.

TABLE III

Dear Mr .....

You are in Group C and this is the Control Group which means that it is not necessary for you to change your diet.

Yours sincerely,

J.I. Mann

III. III.

Group A (low sugar) were instructed to cut out foods containing sucrose, but to maintain their weights by eating more of other foods. As this was essentially to be a practical experiment, this dietary change was requested rather than the substitution only of other carbohydrates for sucrose, which was carried out in most of the previous studies which were conducted.

Group B (reduced starch) were asked to halve their consumption of starchy food (eg. one potato instead of two) and to substitute other foods to maintain weight.

Group C (control) continued their usual diets.

The experimental diets were commenced on 8th April, 1969 and continued for $5\frac{1}{2}$ months (22 weeks) i.e. until 9th September, 1969.

The volunteers were encouraged during the dietary periods by two corporate addresses and two personal interviews with investigator and dietician during the $5\frac{1}{2}$ months. In addition, they were encouraged to communicate per telephone with us at any time should they have any queries.

4. Sampling during the test period

In addition to the three baseline fasting blood samples drawn during the control month, fasting blood specimens were taken and subjects weighed after 2, 6, 10, 14 and 18 and 22 weeks on the diet. On each occasion volunteers came to work after a twelve hour fast. Blood was taken in the insurance company's medical suite, often with the help of their medical and nursing staff. Following each sampling, each volunteer was provided with breakfast, at the expense of the company in their canteen. The experimental diet of each volunteer was always taken into account.

If a subject happened to be away at the time appointed to take his blood, arrangements were made to collect his blood within the next few days. The one subject who dropped out of the experiment was a man in Group B who resigned from the company. One of the volunteers in Group A was transferred to a different branch of the company, but special arrangements were made for

blood to be collected from him, so that he could remain in the experiment.

Three-day diet records were recorded and analysed before the 10th and 18th week blood samples during the experiment as an attempt to assess how closely the diets were being followed.

5. Post-experimental control period

At the conclusion of the $5\frac{1}{2}$ month dietary period the subjects were asked to return to their original diets as in the preceding control period. Each subject's first diet record was used to remind him what he had been accustomed to taking. After 4 weeks of this post-experimental control period (i.e. at the 26th week after commencement) a final fasting blood sample was collected and weight recorded. A final three-day diet record was kept before the collection of the final blood sample.

6. Techniques of analysis

(a) Laboratory methods

Serum cholesterol and triglyceride were measured on each blood sample and lipoprotein electrophoresis was carried out according to the method described in Part I of this study.

(b) Dietary analysis

Analysis of the 7 and 3-day dietary records kept by the volunteers was performed by the dietician using the method already described.

(c) Statistical analysis

The significance of differences was assessed by the Wilcoxon test for pair differences. This non-parametric statistical test was selected in view of the clearly described skewed distribution of serum triglyceride[22]. As this test employs the actual values at each sampling period, values for the mean only are given in all tables of results.

(C) RESULTS

1. Results of the dietary analysis

A summary of the 7-day pre-experiment dietary analysis and also the 3-day dietary analyses corresponding to the 10, 18 and 26 (post-experiment) week blood samples, is shown in Table IV and Figure 1. The total daily caloric intake and proportions of the proximate food constituents and sucrose of the control group and Groups A and B before the start of the experiment are similar to values reported for other westernised population groups[97,103,136].

TABLE IV

SUMMARY OF DIETARY ANALYSIS **Expressed as total carbohydrate, sucrose, non sucrose carbohydrate, total protein and fat as a percentage of total calories with actual caloric values derived from each in brackets.**

GROUP	Sucrose % TOTAL CALS	TOTAL CH_2O % TOTAL CALS	CH_2O EXCL. SUCROSE % TOTAL CALS	TOTAL DAILY CALORIE INTAKE	PROTEIN % TOTAL CALS	FAT % TOTAL CALS
A. Before experiment	13 (341)	43 (1129)	30 (788)	2626	17 (446)	36 (945)
10th week	2 (44)	42 (969)	35 (810)	2307	17 (392)	39 (900)
18th week	3 (56)	39 (843)	36 (787)	2161	18 (389)	41 (886)
26th week	10 (222)	43 (952)	33 (731)	2215	16 (354)	37 (819)
B. Before experiment	13 (350)	43 (1156)	30 (807)	2689	16 (430)	38 (1022)
10th week	18 (400)	46 (1021)	27 (599)	2220	15 (333)	37 (821)
18th week	15 (341)	44 (1000)	29 (659)	2273	19 (432)	37 (841)
26th week	15 (369)	45 (1106)	30 (737)	2458	16 (393)	37 (909)
C. Before experiment	15 (368)	47 (1152)	32 (784)	2450	15 (368)	35 (858)
10th week	16 (384)	50 (1200)	34 (816)	2400	16 (384)	35 (840)
18th week	16 (384)	50 (1203)	34 (818)	2405	15 (361)	35 (842)
26th week	14 (333)	47 (1119)	33 (785)	2380	16 (381)	37 (880)

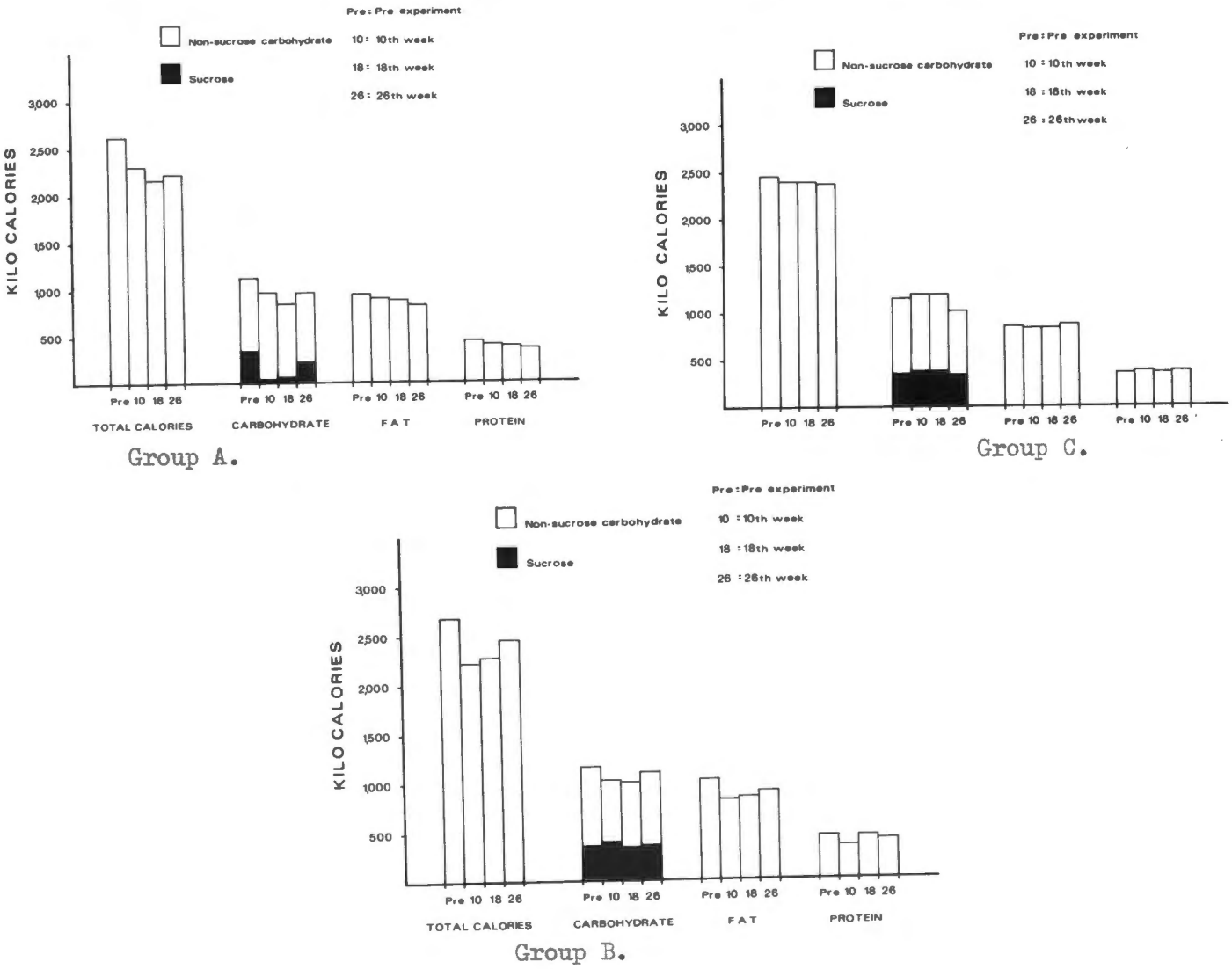


Figure 1: Summary of dietary analyses. Total daily caloric intake is indicated together with the daily calories derived from the proximate food constituents.

It can be seen that Group A succeeded in dropping their sucrose intake from 341 kCal per day (13% of total daily calories) to 44 kCal (2% of total daily calories) at 10 weeks and 56 kCal (3% of total daily calories) at 18 weeks. They did not, however, adequately compensate with non-sucrose calories and consequently their total caloric consumption fell from 2626 kCal per day to 2307 and 2161 kCal per day. When it became apparent that volunteers in this group were not compensating, they were provided with additional encouragement to do so. The majority said that despite the fact that they had given up sugar, they had no desire to eat more of other foodstuffs and had great difficulty in adequately compensating. It was not surprising therefore that at the conclusion of the experiment when the subjects after $5\frac{1}{2}$ months were asked to return to their original diet, many felt that they did not wish to return to their initial sucrose consumption. Four of the 17 subjects were pleased to be allowed sugar again. Of the remaining 13, 6 felt that they would never like to have sugar again, but agreed to try as far as possible to return to their original diet for the duration of the post-experimental control period for the month. The remaining 7 were prepared to return to their initial diet during the post-experimental control period, but felt that after this they would probably once again reduce their sucrose intake, though not as strictly as during the experiment. This is reflected in the 26 week diet check when sucrose intake had increased to 222 kCal per day (10% of the total calories) and total daily calories to 2215 kCal. There appeared to be a slight decrease in the protein, fat and non-sucrose carbohydrate of the diet with regards caloric intake derived from each of these constituents.

It had been intended for Group B to halve the non-sucrose component of their diet. As this comprised approximately 30% of their total daily calories, this would have been roughly comparable with the elimination of sucrose which comprised 13% of total daily calories. Instead of a 50% reduction of non-sucrose carbohydrate they appeared to have achieved only a 25% reduction at the 10th week. At the time the diets were sampled they did not appear to compensate

by eating more protein and fat. Mean total carbohydrate calories were reduced by 160 and 286 kCal at 10 and 18 weeks in the restricted sugar group. The corresponding reductions of carbohydrate in Group B were 135 and 156 kCal. The volunteers found the reduced starch diet much the most difficult to adhere to. There was great variation in the way the subjects attempted to carry out their dietary instructions. The numerical diet records being taken at intervals cannot show this. From their statements it seemed that most subjects in Group B did reduce their starch food at first (they had lost some weight by 2 weeks). Towards the end of the experimental period, several in Group B seemed to have given up the attempt. A typical statement was 'if you cannot eat starch, you can never have a proper meal'. This has made any changes in serum lipid levels in this group difficult to interpret.

The dietary records for Group C show excellent consistancy. All dietary components remained approximately constant between the three diet records.

Statistical analysis of the dietary data was not attempted in view of the difficulties already described in dietary assessment and consequent unreliability of the results.

2. Serum cholesterol levels during the experiment

The mean serum cholesterol levels are shown in Table V.

TABLE V

MEAN SERUM CHOLESTEROLS, mg/100ml (and change (mg) from pre-experiment mean)

GROUP	N	PRE-EXPERIMENT CONTROLS				DURING DIETS						Mean of 6 values during diets	AFTER DIETS
		I	II	III	Starting Mean	After 2 weeks	After 6 weeks	After 10 weeks	After 14 weeks	After 18 weeks	After 22 weeks		After 26 weeks
A	17	250	245	246	247	235 (-12)	224 (-23)	242 (-5)	249 (+2)	231 (-16)	242 (-5)	237 (-9)	240 (-7)
B	16	244	235	232	238	247 (+9)	238 (0)	254 (+16)	267 (+29)	249 (+11)	245 (+7)	250 (+12)	247 (+9)
C	17	248	229	227	234	232 (-2)	231 (-3)	237 (+3)	247 (+13)	234 (0)	238 (+4)	236.5 (+2.5)	241 (+7)

III. V.

The starting mean value is the mean of the three pre-experimental control samples. Group A by chance had somewhat higher serum cholesterol levels than Groups B and C but the difference between the three groups is not statistically significant.

In Group A the serum cholesterol appeared to show a slight decrease. The mean drop of the six observations during the experimental period was 9 mg/100ml - a fall of about 4%. However, only at the 6th and 18th week of the experiment was the mean cholesterol level significantly lower than the starting level (at 6 weeks $p < 0.01$ and at 18 weeks $p < 0.05$). At the 14th week there was a slight but insignificant increase in serum cholesterol above the starting level. A month after the conclusion of the diet, serum cholesterol was still 6 mg/100ml lower than the starting mean for the group.

In Group B serum cholesterol appeared show a slight increase. A mean increase of 12 mg/100ml was found for the mean of 6 values during the diet. This is a 5% increase over the starting level. The increase was, however, only statistically significant at the 10th and 14th week of the experiment (at 10th week

$p < 0.05$ and at 14th week $p < 0.01$).

During the experiment the mean serum cholesterol in the control group (Group C) remained within their pre-experimental range. The mean value at the 14th week was, however, significantly higher than the pre-experiment starting mean ($p < 0.05$). The mean of the six observations during the experiment was 2.5 mg/100ml higher than the starting value in the control group, an increase of approximately 1%.

3. Serum triglyceride levels during the experiment

The mean serum triglyceride results are shown in Table VI.

TABLE VI

MEAN SERUM TRIGLYCERIDES, mg/100ml (and change from pre-experiment mean)

GROUP	N	PRE-EXPERIMENT CONTROLS				DURING DIETS						Mean of 6 values during diets	AFTER DIETS
		I	II	III	Starting Mean	After 2 weeks	After 6 weeks	After 10 weeks	After 14 weeks	After 18 weeks	After 22 weeks		After 26 weeks
A	17	162	160	164	162	136 (-26)	126 (-36)	126 (-36)	136 (-26)	119 (-43)	115 (-47)	126 (-36)	130
B	16	145	134	155	145	136 (-10)	140 (-5)	144 (-1)	139 (-6)	136 (-10)	127 (-18)	137 (-8)	131
C	17	165	146	148	150	146 (-4)	150 (0)	146 (-4)	154 (+4)	137 (-13)	156 (+6)	148 (-2)	139

Once again, before the experiment, values tended to be somewhat higher in Group A than in the other two groups. The difference though is not statistically significant.

In Group A serum triglyceride levels fell by 26 mg/100ml after two weeks on the sugar restricted diet. The reduction was 36 mg/100ml after 6 weeks and remained reduced all the time the diet was continued. The mean reduction of serum triglyceride while on the experimental diet was 36 mg/100ml lower than the starting mean value. This is a reduction of approximately 22%. At all sampling points triglyceride levels were significantly lower than before the start of the experiment (2 - 14 weeks $p < 0.05$ and 18 - 22 weeks $p < 0.01$). The differences between sampling periods during the experimental diet were not, however, significantly different.

One month after the conclusion of the experiment, levels had increased significantly from the 22 week sampling to 130 mg/100ml ($p < 0.05$), but this level was still significantly lower than the starting value ($p < 0.05$).

The volunteers in Group B tended to have slightly lower serum triglyceride levels during the experimental diet than before the start of the experiment, averaging 8 mg/100ml or 6% lower than starting. However, only at 22 weeks was the mean value significantly lower than the starting mean ($p < 0.05$).

During the experiment there were minor fluctuations of serum triglyceride in Group C. These were not, however, statistically significant with the mean of the 6 triglyceride levels during the experiment virtually identical with the starting mean, only 2 mg/100ml or 1% lower.

Because the fall of serum triglyceride was the major change in serum lipids, the triglyceride results have been analysed further. Use has been made of the continuous control of the triglyceride measurement afforded by Group C, who did not change their diet. At each sampling the mean triglyceride concentration of Group C was taken as 100 in Table VII. The values for Group A and Group B were calculated as percentages of this moving baseline.

TABLE VII

MEAN SERUM TRIGLYCERIDES

taking Control Group C as having triglyceride value = 100 at each blood sampling

GROUP	BEFORE DIETS	ON DIETS						AFTER DIETS
	Mean before treatment	After 2 weeks	After 6 weeks	After 10 weeks	After 14 weeks	After 18 weeks	After 22 weeks	After 26 weeks
A	108	93	84	86	88	87	74	93
B	97	93	94	98	95	99	82	94
C	100	100	100	100	100	100	100	100

III. VII.

This table clearly shows that Group A who had a mean starting level of 108% of the control group, showed a significant and sustained fall of serum triglyceride which was maintained for the entire duration of the experiment and tended to increase one month after the conclusion of the experiment though the levels did not reach the initial levels. Group B appeared to show only a very slight and not significant fall in serum triglyceride during the greater part of the experiment; a significant decrease being noticed only after 22 weeks.

It furthermore seemed important to determine the importance of the magnitude of the starting serum triglyceride concentration on the observed changes during the experiment. Groups A, B and C were each divided into two sub-groups - those with triglycerides above and below starting mean triglyceride value of 155 mg/100ml for the whole group. The results are given in Tables VIII and IX.

TABLE VIII

SERUM TRIGLYCERIDE (mg/100ml) DIVIDING INTO SUBGROUPS WITH VALUES ABOVE AND BELOW STARTING MEAN (155 mg/100ml).

GROUP	SUBGROUP	N	PRE-EXPERIMENT CONTROLS				DURING DIETS						AFTER DIETS
			I	II	III	Starting Mean	After 2 weeks	After 6 weeks	After 10 weeks	After 14 weeks	After 18 weeks	After 22 weeks	After 26 weeks
A	High TGs.	7	238	229	250	239	175	168	166	187	152	155	167
	Low TGs	10	110	110	103	108	108	96	98	98	96	88	104
B	High TGs	5	204	194	199	199	175	179	206	185	184	176	180
	Low TGs	11	119	109	132	120	119	124	115	118	113	106	109
C	High TGs	9	210	186	181	192	179	186	175	187	173	200	182
	Low TGs	8	92	103	109	101	112	108	113	115	106	106	90

TABLE IX

SHOWING PERCENTAGE CHANGE IN SERUM TRIGLYCERIDES IN SUBGROUPS ABOVE AND BELOW OVERALL MEAN (155 mg/100 ml)

GROUP	SUB-GROUP	N	PRE-EXPERIMENT MEAN	DURING DIETS						AFTER DIETS
				After 2 weeks	After 6 weeks	After 10 weeks	After 14 weeks	After 18 weeks	After 22 weeks	After 26 weeks
A	High TGs	7	239	-27%	- 30%	-31%	-22%	-36%	-35%	-30%
	Low TGs	10	108	0%	- 11%	- 9%	- 9%	-11%	-19%	- 3%
B	High TGs	5	199	-12%	-10%	+ 4%	- 7%	- 8%	-11%	- 10%
	Low TGs	11	120	- 1%	+ 3%	- 4%	- 2%	- 6%	-12%	- 9%
C	High TGs	9	192	- 7%	- 3%	- 9%	- 3%	- 9%	+ 4%	- 5%
	Low TGs	8	101	+11%	+ 7%	+12%	+14%	+ 5%	+ 5%	- 11%

In Group A it is quite clear that serum triglyceride fell more both in absolute amounts and as a percentage of the starting concentration in subjects in Group A who had a mean value greater than 155 mg/100ml. All levels during the experiment in the high triglyceride subgroup were significantly lower than the starting mean ($p < 0.02$), as was the level at 26 weeks, a month after the completion of the experiment. The fall from the 14th to the 18th week was statistically significant ($p < 0.05$), but no other changes during the experimental diet were significant. In the lower triglyceride subgroup there appears to have been a small decrease but only at 22 weeks was the level significantly lower than the starting mean ($p < 0.02$).

None of the changes in both high and low triglyceride subgroups of Groups B and C were statistically significant. A fall is evident in the high triglyceride subgroup of Group B and a possible reason for significance not being reached may well be the small size of the subgroup.

4. Serum pre-beta lipoprotein during the experiment

The degrees of pre-beta lipoprotein are represented in condensed form in Tables X(a) and (b).

TABLE X(a)

The number of subjects in each grade of pre-B lipoprotein before, during and after the experimental diets.

Diet Group	N	Pre-B lipo-protein grade	BEFORE DIETS			DURING DIETS					AFTER DIETS
			I	II	III	After 2 weeks	After 6 weeks	After 10 weeks	After 18 weeks	After 22 weeks	After 26 weeks
A	17	0	3	2	3	4	2	2	4	2	2
		tr	6	3	2	6	5	7	7	9	6
		+	3	8	6	4	6	5	4	4	6
		++	4	2	3	3	4	3	2	2	3
		+++	1	2	3	0	0	0	0	0	0
B	16	0	0	1	4	2	1	0	0	0	0
		tr	4	3	7	6	5	6	6	10	12
		+	5	6	4	5	8	6	8	5	2
		++	7	6	1	3	1	4	2	1	2
		+++	0	0	0	0	1	0	0	0	0
C	17	0	1	1	0	0	0	0	1	1	0
		tr	4	4	5	7	5	5	4	4	8
		+	6	9	7	4	5	7	9	7	6
		++	4	2	4	5	6	4	2	4	3
		+++	2	1	1	1	1	1	1	1	0

Note When the pre-B lipoprotein band was graded ++ to +++ this was included in the ++ rows in this Table.

The principal change during the diet as shown in Table X(a) is that in the restricted sugar group there were fewer sera with ++ and +++ pre-beta lipoprotein and more with only a trace or + of pre-beta lipoprotein.

TABLE X(b)

MEAN (SEMI-QUANTITATIVE) GRADES OF PRE-B LIPOPROTEINS. THREE DIET GROUPS SUBDIVIDED INTO THOSE WITH TRIGLYCERIDE ABOVE OR BELOW THE STARTING MEAN.

Diet Group	N	TG sub group	BEFORE DIETS				DURING DIETS					AFTER DIETS
			I	II	III	Starting mean	After 2 weeks	After 6 weeks	After 10 weeks	After 18 weeks	After 22 weeks	After 26 weeks
A	7	high	1.9	1.8	2.4	2.0	1.6	1.6	1.4	1.1	1.0	1.4
	10	low	0.6	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.7
B	5	high	1.8	1.7	2.0	1.8	1.8	1.6	1.9	1.6	1.6	1.5
	11	low	1.0	1.0	1.0	1.0	0.9	0.9	0.8	0.8	0.7	0.8
C	9	high	2.1	1.6	1.7	1.8	1.8	1.8	1.5	1.5	1.7	1.5
	8	low	0.6	0.6	0.7	0.6	0.6	0.8	0.8	0.7	0.7	0.6

III. X(b)

As can be seen from Table X(b) those who started with higher serum triglyceride levels had greater degrees of pre-beta lipoprotein. In the high triglyceride subgroup of Group A a significant fall was noted during the experimental diet periods in the amount of pre-beta lipoprotein. In the low subgroup of Group A and in both subgroups of Group B and C the changes in pre-beta lipoprotein were not statistically significantly different.

5. Body weights during the experiment

Table XI shows the mean body weights before and during the experiment.

TABLE XI

BODY WEIGHT IN KILOGRAMS (and change (kg) from mean before experiment

Group	N	PRE-EXPERIMENT CONTROLS			DURING TREATMENT						Mean of values during diets	AFTER TREATMENT
					23 Apr	21 May	18 June	16 July	13 Aug	10 Sept		
		I	II	Mean of I and II	After 2 weeks	After 6 weeks	After 10 weeks	After 14 weeks	After 18 weeks	After 22 weeks		After 26 weeks
A	17	78.6	78.3	78.4	77.7 (-0.7)	77.4 (-1.0)	77.3 (-1.1)	77.2 (-1.2)	77.2 (-1.2)	77.2 (-1.2)	77.3 (-1.1)	78.0 (-0.4)
B	16	78.0	77.7	77.8	77.5 (-0.3)	77.8 (0)	77.9 (+0.1)	78.0 (+0.2)	78.7 (+0.9)	78.6 (+0.8)	78.1 (+0.3)	78.7 (+0.9)
C	17	74.2	73.1	73.7	73.8 (+0.1)	74.1 (+0.4)	73.9 (+0.2)	74.0 (+0.3)	73.8 (+0.1)	74.7 (+1.0)	74.0 (+0.3)	74.8 (+1.1)

It happened by chance that Group C weighed less than Groups A and B before the start of the experiment though the difference was not significant. The mean changes in weight were fairly small. The control and reduced starch groups showed slight gain in weight and both groups ended up 0.3 kg heavier than at the start. The weight in both groups was significantly higher at the 26th week sampling than before the start of the experiment (in both cases $p < 0.05$). Weight changes however during the experiment were not statistically different from the pre-experiment levels or from each other in these two groups.

The low sugar group (Group A) after 2 weeks had lost 0.7 kg in weight and then continued losing slightly until the 14th week, after which the weight remained constant. All levels were significantly lower during the experiment than before the start of the experiment ($p < 0.01$) but none of the intra-experimental weight changes were significantly lower than the preceding one. The weight one

month after the conclusion of the experimental diet was still significantly lower than the starting value ($p < 0.01$).

6. Relationship between weight reduction and serum triglyceride

Group A was the only group that lost a significant amount of weight and also showed the greatest change in serum triglyceride levels. It therefore seemed important to study the relationship between weight change and change in serum triglyceride levels. Weight change versus change in triglyceride have therefore been correlated and are shown in Table XII.

TABLE XII

CORRELATION BETWEEN WEIGHT-CHANGE AND CHANGE IN TRIGLYCERIDE DURING EXPERIMENTAL DIETS

		Number of Pairs	Correlation Coefficient (r)	Significance Limits
All changes	Group A	102	0.31	$p < 0.005$
	Group B	96	0.24	$p < 0.002$
Changes in those individuals who had high starting triglyceride *	Group A	42	0.60	$p < 0.001$
	Group B	35	0.26	NS
Changes in those individuals who lost more than 1kg of weight	Group A	48	0.29	$p < 0.05$
	Group B	42	0.28	NS **
Changes in those individuals with high triglyceride who lost more than 1kg of weight *	Group A	24	0.61	$p < 0.005$
	Group B	10	0.37	NS

* Serum triglyceride levels greater than overall mean value

** Just not significant

When considering all changes which occurred during the experiment in Groups A and B, there was a significant correlation between weight change and change in triglyceride in both groups ($r = 0.31$ in Group A and $r = 0.24$ in Group B). However, when considering this relationship in those individuals in Group A who had started the experiment with a serum triglyceride above the overall mean value of 155 mg/100ml, r was equal to 0.60. For changes in weight of greater than 1 kg, the correlation coefficient was not appreciably different from changes in the group as a whole ($r = 0.29$). Furthermore, when considering the relationship in those individuals who had high starting triglyceride values and lost more than 1 kg in weight, the correlation coefficient was not different from all individuals with high triglyceride levels, regardless of the change in weight.

It is of interest to consider the fate of those individuals with carbohydrate induced hyperlipoproteinaemia (Type IV) of Fredrickson and also those individuals with Types II and IIb (as defined earlier) in each of Groups A, B and C during the experimental diet periods. The 15 individuals with hyperlipoproteinaemia are listed in Table XIII.

TABLE XIII

Serum Lipid and Weight Changes in those subjects with Hyperlipoproteinaemia

Subject No. and Group	Values before commencement of Experimental Diets (mean of 3)			Values after 5½ months on Experimental Diet (with change from pre-experimental mean in brackets)		
	Cholesterol mg/100ml	Triglyc. mg/100ml.	Weight kg	Cholesterol mg/100ml	Triglyc. mg/100ml	Weight kg
			TYPE IV			
A3	297	296	39.8	285 (-12)	136 (-160)	83.5 (-6.3)
A12	282	337	72.6	291 (+ 9)	236 (-101)	71.7 (-0.9)
A14	224	169	72.1	219 (- 5)	110 (- 59)	68.9 (-3.2)
A56	281	384	102.5	251 (-30)	263 (-121)	98.4 (-4.1)
B21	250	192	67.6	256 (+ 6)	178 (- 14)	67.1 (-0.5)
B26	216	208	85.3	235 (+19)	138 (- 70)	83.0 (-2.3)
B27	232	232	98.4	228 (- 4)	281 (+ 49)	99.8 (+1.4)
B34	208	223	74.8	199 (- 9)	235 (+ 2)	75.3 (+0.5)
C39	236	170	78.0	279 (+43)	230 (+60)	78.5 (+0.5)
C40	229	226	80.3	215 (-14)	220 (- 6)	79.8 (-0.5)
C41	223	173	85.3	223 (0)	250 (+ 77)	87.5 (+2.2)
C42	185	161	62.6	200 (+15)	118 (- 43)	61.2 (-1.4)
C46	198	168	81.6	197 (- 1)	113 (-5.5)	80.7 (-0.9)
C47	204	179	73.0	221 (+17)	149 (- 30)	73.5 (+0.5)
C53	242	163	83.9	226 (-16)	192 (+ 29)	84.4 (+0.5)
			TYPE II (B)			
A 4	317	170	95.3	324 (+ 7)	150 (-20)	94.3 (-1.0)
A 9	330	196	85.7	337 (+ 7)	130 (-66)	81.6 (-4.1)
B23	315	208	72.1	317 (+ 2)	162 (-46)	69.4 (-2.7)
C48	351	299	80.7	343 (- 8)	400 (+101)	83.0 (+2.3)
			TYPE II			
C51	315	99	64.0	329 (+14)	78 (-21)	64.4 (+0.4)

III. XIII

In Group A the four individuals with Type IV all lowered their serum triglyceride by between 59 and 160 mg/100ml. Serum cholesterol fell slightly in three and increased slightly in one (A12). In all four there was a fall in

weight varying from 0.9 - 6.3 kg. The two subjects with Type IIb also showed a decrease of serum triglyceride associated with a fall in weight.

In Group B two of the individuals (B21 and 26) lowered their serum triglycerides. These two subjects also lost weight. Serum triglyceride increased in B27 as did body weight by 1.4 kg. In B34 there was an increase of 0.5 kg in weight associated with an increase in triglyceride of 12 mg/100ml. B23 with Type IIb lost 2.7 kg in weight and triglyceride decreased by 46 mg/100ml.

In Group C there were 7 individuals with Type IV. In three serum triglycerides were increased associated with an increase in body weight. In three serum triglyceride decreased associated with a decrease in body weight. In only one subject (C47) was a decrease in serum triglyceride associated with a 0.5 kg increase in weight. The one individual with Type IIb (C48) showed an increase in body weight associated with an increase in serum triglyceride.

DISCUSSION OF RESULTS

This field study has shown that normal well motivated men can reduce and hold their sucrose intake to less than a quarter of what they were used to. On this regime serum triglyceride averaged 22% less than before and serum cholesterol 4% lower than before the experimental diet. While the greatest fall in triglyceride occurred two weeks after the experimental diet had been commenced, it is important to note that the lower level of serum triglyceride was maintained throughout the five and a half months duration of the experiment. Serum triglyceride fell more in those individuals whose starting levels of serum triglyceride were higher. One month after the volunteers had been asked to return to their original diets serum triglyceride levels had increased, but were still significantly lower than before the experiment started.

The fall in triglyceride was associated with a small but significant weight loss, averaging 1.1 kg. The change in weight appeared to be related to the change in triglyceride ($r = 0.30$ when considering the relationship between

change in weight and change in triglyceride for all changes). When considering this relationship in those individuals who started the experiment with higher triglyceride levels the correlation was much greater ($r = 0.60$). This tends to suggest that the significant fall in serum triglyceride observed in normal men on a sucrose restricted diet in this experiment and in patients with myocardial infarction in other studies[27,114] is at least to a large extent explained by the weight loss rather than a specific metabolic effect of sucrose. This is also in agreement with the findings of other workers who in metabolic ward studies have found no significant difference in fasting serum lipid levels when sucrose was isocalorically replaced by starch with no associated change in body weight[11,57,88,111]. It is not in agreement though with the finding of MacDonald[82] who found no correlation between slight weight changes and changes in serum lipid concentration. It may be very relevant though that the subjects which he considered in this study had been fed a variety of experimental diets. It furthermore appears that in those individuals who have low serum triglyceride levels dietary manipulation has little effect in further lowering serum triglyceride.

In this experiment there were two other diet groups running concurrently with the restricted sugar group. Group C who continued their usual diet had an easy task and provided a continuous control for the weight and lipid measurements in the sugar restricted group.

Group B was intended to provide a direct comparison with Group A in that it was hoped that they would give up an amount of non-sucrose carbohydrate equivalent to the amount of sucrose given up by Group A. However, from the diet histories they have clearly not been able to achieve this and a direct comparison therefore cannot be made. Despite the fact that the triglyceride changes which occurred during the greater part of the experimental diet period in this group did not reach statistical significance, there was still a significant correlation between weight change and change in triglyceride when considering all changes ($r = 0.30$). This provides further evidence that changes in triglyceride

are chiefly dependent upon caloric balance.

It is interesting to note that serum cholesterol levels rose temporarily in all three groups at the fourteenth week sampling. This may have resulted from increased nervous tension at the end of the Company's financial year which took place at this time. Many of the volunteers reported an increased work load at this time. This phenomenon was first reported by Friedman et al[33] and later also by others[21,42,123].

The observations made on these individuals with hyperlipoproteinaemia, together with the significant correlation between change in triglyceride and change in body weight seems to suggest that caloric balance is probably the most important factor regulating serum triglyceride levels at least when dietary constituents are eaten in more or less normal proportions. Changes on different carbohydrates are probably due to caloric imbalance.

There is still no definite evidence that elevated serum triglyceride levels predispose to coronary heart disease as serum cholesterol is known to do (See Part I). This information may be expected from prospective studies which are now underway, such as the Stockholm Prospective Study[20]. In the interim all that can be said is that restriction of dietary sucrose appears to be a useful practical dietary manipulation for the lowering of elevated serum triglyceride levels, particularly in Type IV Hyperlipoproteinaemia. It seems likely though that the mechanism for this lowering of serum lipids is weight loss; individuals who give up sugar not adequately being able to compensate with other foods and it seems quite conceivable that weight loss arising from restriction of any other dietary constituent(s) would almost certainly yield similar results.

SUMMARY

Fifty one healthy office workers, aged 36 to 55, volunteered for a dietary experiment. One-third of them were asked to cut out sucrose and replace it with other foods. Another third tried to halve their dietary starch and

substitute other foods. The remainder were controls who continued their usual diets.

In the low sugar group, serum triglycerides showed a significant decrease which persisted until the diets were stopped after $5\frac{1}{2}$ months. Serum triglyceride fell more in those who had higher levels to start with. The reduction of serum triglycerides has been attributed chiefly to the weight loss which occurred in the low sugar group.

Serum lipids did not change significantly in the reduced starch group but these subjects had more difficulty following their prescribed diet and did not lose weight.

CHAPTER III - THE DIFFERENT EFFECTS OF ORAL SUCROSE AND GLUCOSE ON ALIMENTARY LIPAEMIA

(A) INTRODUCTION

While attempting to confirm a report[116] that serum triglycerides are not significantly elevated after the ingestion of a small mixed meal, it was noticed that the triglyceride levels seemed to depend on the type of carbohydrate given. Albrink et al[5] and Sullivan[120] have shown that the lipaemia which occurs after a fatty meal is diminished by the addition of glucose to the meal. Krut and Barsky[71] found that postprandial lipaemia in patients with ischaemic heart disease is decreased by intravenous infusion of glucose and insulin.

These considerations led me to examine the effects of glucose and sucrose and subsequent insulin release on alimentary lipaemia as it was felt that this might provide further information on the effects which different carbohydrates have on serum lipids.

(B) METHODS

Nine middle-aged men (aged 30-58 years) and ten younger men (20-25 years) participated in the study. The older men were studied in the metabolism ward of Groote Schuur Hospital. They were all convalescent patients who had been admitted to hospital earlier for non-metabolic illnesses and by the time of testing had normal erythrocyte sedimentation rates and liver function tests. They had all been on an ordinary hospital diet for at least two weeks before testing and their weights were stable. The ten younger men were medical students and myself, and were on diets of their own choice. They were not admitted to hospital. The mean fasting serum triglyceride level for the nine middle-aged men was 101 mg/100ml (Range: 70-135 mg/100ml); and for the ten younger men 75 mg/100ml (Range 54-99 mg/100ml).

Two formula breakfasts (Table XIV), both providing the same total calories (565 kCal) and proportions of the proximate food constituents were given to each of the volunteers.

TABLE XIV

Meal G	Meal S	Composition[130]	Caloric value in kCal (Percentage of total Cals).
Powdered egg white: 30g	Powdered egg white: 30g	Protein : 24g	96 (17)
Sunflower seed oil: 25g	Sunflower seed oil: 25g	Fat : 25g	225 (40)
Glucose : 60g	Sucrose : 60g	Carbohydrate : 60g	240 (43)

III. XIV.

Protein was given in the form of powdered egg white and fat as sunflower seed oil. The meals differed only in their carbohydrate content - one contained glucose (meal G) and the other an isocaloric amount of sucrose (meal S). The proximate food constituents were present in the proportions normally eaten. The dry constituents were mixed with 200 ml warm water in a "Waring Blender", approximately ½ hour before the formula was ingested. The glucose and sucrose test meals were given in a randomised order at 8 a.m. on two different days, usually two days apart. As a "control" experiment, five subjects in the metabolism ward were given 100 grams of glucose and sucrose without the other components of the test meal. All subjects fasted for 12 hours prior to testing.

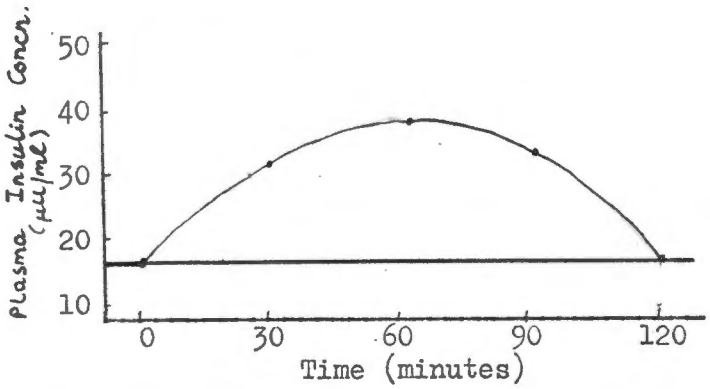
A fasting blood sample was taken on each occasion at 7.40 a.m. This was repeated at 2 and 4 hours after the ingestion of the formula in those subjects studied in the metabolism ward and at half-hourly intervals for 2½ hours in the ten younger men. The subjects were kept at rest during the experiment and did not smoke.

Blood was spun down immediately and the serum frozen at -20°C until the analyses were carried out. Serum triglyceride was measured as described in Part I. The samples taken from the ten younger subjects were also assayed for

serum insulin and blood sugar was measured as described earlier.

The degree of lipaemia at each sampling was measured as the increase in triglyceride above the fasting level. The total lipaemic response, glycaemic stimulus and insulin response to the meal were calculated in a similar way to the method described for total insulin response by Perley and Kipnis[107].

The following method was described by these authors:



$$\text{Insulin Response } (\mu U \text{ minutes}) = \text{Insulin Increment } (\mu U) \times \text{Time (min)} \times 10^{-3}$$

$$\text{INSULIN INCREMENT } (\mu U) = \frac{\frac{0+30'}{2} + \frac{30'+60'}{2} + \frac{60'+90'}{2} + \frac{90'+120'}{2}}{4}$$

This was modified as follows:

$$\text{LIPAEMIC RESPONSE (MG-hours)}$$
$$\text{GLYCAEMIC STIMULUS (MG-hours)} = 0.5\left(\frac{t^{o}+t^{30}}{2} + \frac{t^{30}+t^{60}}{2} + \frac{t^{60}+t^{90}}{2} + \frac{t^{90}+t^{120}}{2} + \frac{t^{120}+t^{150}}{2}\right)$$
$$\text{INSULIN RESPONSE (Microunit-hours)}$$

Where t = triglyceride, glucose or insulin increase above fasting concentration, at the particular sampling interval. t^{o} therefore would always equal 0.

The significance of the difference between two responses was determined by Student's t test. As a further check on the statistical significance of the results, a non-parametric test, the Wilcoxon test for pair differences was employed as it does not assume that the scores under analysis are drawn from a normally distributed population. The interrelationships between

responses were determined by calculating the correlation coefficient. These methods have been discussed in the section on statistical methods.

(C) RESULTS

(1) Middle-aged subjects

The triglyceride results are shown in Table XV (a) and (b) and Figure 2. The degree of lipaemia was significantly higher after S than after G at two hours ($p < 0.005$) and four hours ($p < 0.05$).

TABLE XV(a)

	Degree of Lipaemia following S		Degree of Lipaemia following G	
	After 2 hours	After 4 hours	After 2 hours	After 4 hours
Mean	51.4	27.9	23.8	8.6
S.E.M.	10.61	8.04	5.47	3.69

The degree of lipaemia (measured as increase in serum triglyceride above fasting level in mg/100ml) in the 9 middle-aged subjects. The degree of lipaemia was significantly higher after S than after G at 2 hours ($p < 0.005$) and 4 hours ($p < 0.05$).

III. XV(a)

Serum triglyceride levels at 2 and 4 hours after the ingestion of 100g glucose or sucrose alone do not differ significantly from the fasting level.

TABLE XV(b)

	Glucose			Sucrose		
	Fasting	After 2 hours	After 4 hours	Fasting	After 2 hours	After 4 hours
Mean	97	96	96	107	99	102
S.E.M.	9.3	7.9	11.8	9.6	9.7	11.0

Serum triglyceride levels (mg/100ml), following the ingestion of 100g glucose and sucrose alone, without the other components of the test meal in five subjects in the metabolism ward.

III. XV(b)

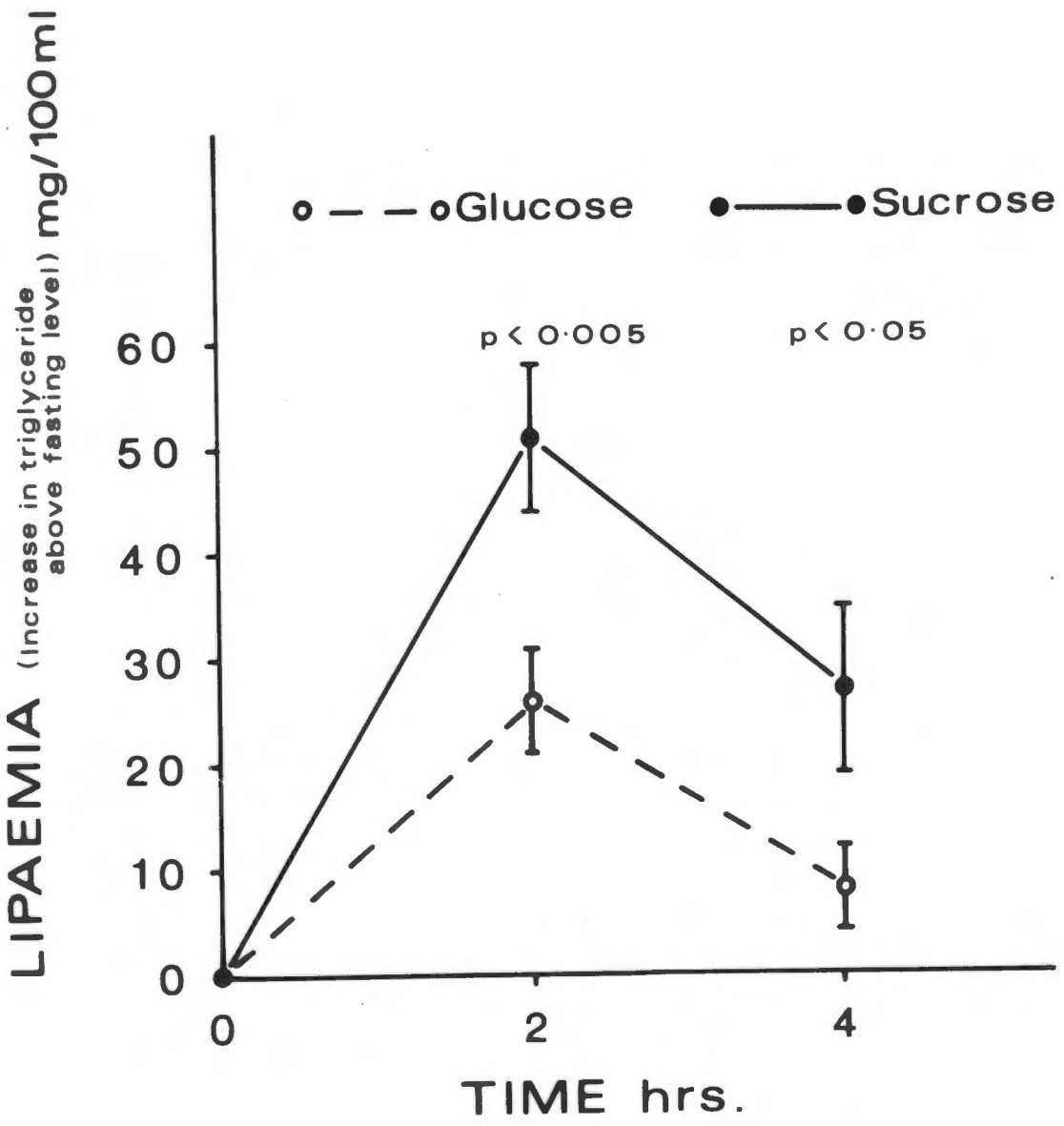


Figure 2: The degree of lipaemia following the ingestion of the formula breakfasts in the nine older subjects.
The mean values ± S.E.M. and significance levels are shown.

Glucose and insulin were not measured in these subjects.

2. Younger subjects

(i) Serum triglycerides

There was no significant difference in the degree of lipaemia for $1\frac{1}{2}$ hours after G and S had been ingested. At 2 and $2\frac{1}{2}$ hours, however, values were significantly lower after G than after S, and tended to be decreasing towards fasting values (at 2 hours $p < 0.02$ and at $2\frac{1}{2}$ hours $p < 0.005$). See Table XVI and Figure 3. The total lipaemic response to the meal (Table XVII) was significantly greater after S than after G ($p < 0.01$). Subjects 4 and 7 showed negligible lipaemic responses but the remaining eight all showed similar patterns.

(ii) Blood Sugar

Blood sugar levels were significantly higher at $\frac{1}{2}$ and 1 hour after G than after S ($\frac{1}{2}$ hour $p < 0.05$ and at 1 hour $p < 0.01$). See Table IV and Figure 2. The glycaemic response (Table XVII) was greater in all 10 cases ($p < 0.005$).

(iii) Serum Insulin

Serum insulin levels were significantly higher at 1 and $1\frac{1}{2}$ hours after G than after S (at 1 hour $p < 0.02$ and at $1\frac{1}{2}$ hours $p < 0.005$). See Table XVII and Figure 3. The total insulin response (Table XVII) was greater in all 10 cases ($p < 0.001$).

TABLE XVI

Time	Blood Sugar mg/100ml		Serum Insulin microunits/ml		Degree of Lipaemia mg/100ml	
	Glucose	Sucrose	Glucose	Sucrose	Glucose	Sucrose
0	87.3 (3.78)	89.0 (3.91)	8.3 (1.21)	8.3 (1.01)	0	0
½	124.6 (4.63)	114.6 (4.37)	55.2 (7.40)	50.7 (5.13)	-5.0 (0.63)	1.1 (0.79)
1	121.3 (5.91)	101.8 (1.69)	57.5 (10.31)	33.4 (2.59)	15.0 (5.52)	11.8 (4.18)
1½	99.9 (7.34)	95.6 (5.89)	41.5 (4.75)	26.3 (3.16)	19.7 (3.67)	22.7 (4.62)
2	80.7 (4.19)	80.5 (6.32)	20.6 (3.47)	13.3 (2.89)	8.0 (4.42)	24.3 (5.63)
2½	80.3 (3.47)	81.7 (3.29)	8.3 (0.98)	11.2 (1.13)	5.3 (5.03)	20.9 (5.90)

The mean values for blood sugar, serum insulin and degree of lipaemia at each sampling interval after G and S. The values for the S.E.M. are given in brackets. The blood sugar levels are significantly higher at ½ ($p < 0.05$) and 1 hour ($p < 0.01$) after G and S. Serum insulin levels are higher at 1 ($p < 0.02$) and 1½ hours ($p < 0.005$) after G and S. The degree of lipaemia is greater after S than G at 2 ($p < 0.02$) and 2½ hours ($p < 0.005$).

III. XVI.

TABLE XVII

Subject Number	LIPAEMIC RESPONSE		GLYCAEMIC STIMULUS		INSULIN RESPONSE	
	Glucose	Sucrose	Glucose	Sucrose	Glucose	Sucrose
1	33.00	42.25	42.50	19.25	107.00	55.00
2	58.50	69.50	23.00	15.25	64.00	40.00
3	9.50	18.00	46.50	23.50	112.50	64.00
4	No lipaemia		32.50	26.50	43.00	42.00
5	25.00	43.25	31.00	22.00	56.00	42.00
6	4.50	35.75	47.50	27.50	90.50	61.50
7	No lipaemia		75.00	44.75	60.50	25.00
8	42.25	76.75	76.00	40.50	79.25	42.50
9	21.25	28.25	35.00	27.00	85.25	59.50
10	40.50	47.25	13.50	12.00	34.00	29.75
Mean	23.45	36.10	42.25	25.83	73.20	46.13

Total lipaemic response, glycaemic stimulus and insulin response after the glucose and sucrose test meal. (Lipaemic response and glycaemic stimulus measured in mg-hours, and the insulin response in microunit-hours).

III. XVII.

(iv) Interrelationships between triglyceride, blood sugar and insulin responses

There was a close inverse relationship between the insulin response and the total lipaemic response to the meal. The correlation coefficient r was -0.66, which was significant at the 1% level. There was also an inverse correlation between the glycaemic stimulus and the lipaemic response, but the correlation coefficient r was -0.43 and not statistically significant.

The insulin response seemed to be related to the glycaemic stimulus in that there was no significant difference between ratios of insulin response to glycaemic stimulus after either glucose or sucrose. (See Table XVIII).

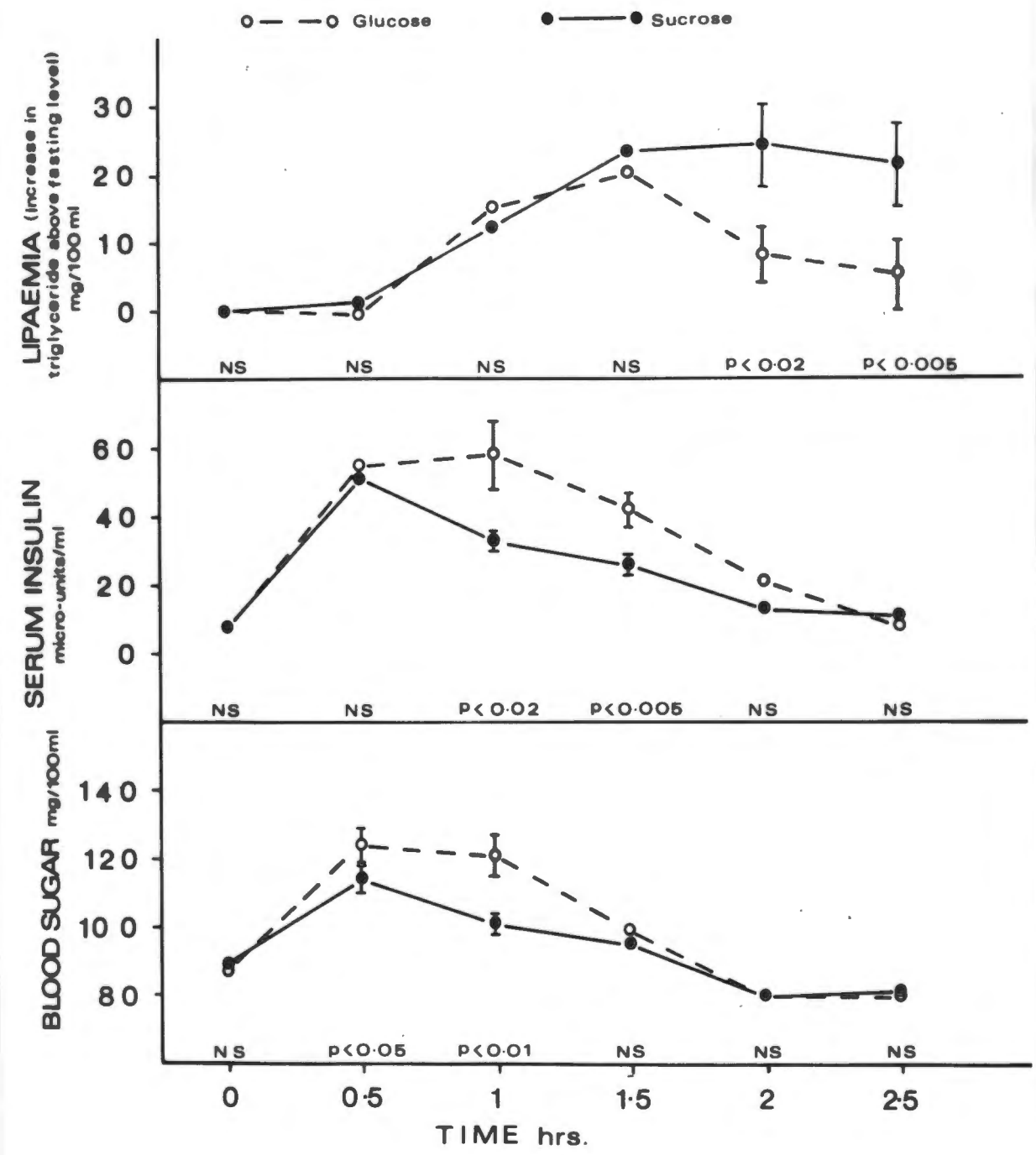


Figure 3: The degree of lipaemia together with the blood sugar and serum insulin levels found at half-hourly intervals after the formula breakfasts in the ten younger subjects. The mean values ± S.E.M. are given for those times at which statistically significant differences were found.
Significance levels are also indicated.

TABLE XVIII

The ratios between insulin response and glycaemic stimulus following the glucose and sucrose formulae.

Insulin Response / Glycaemic stimulus	Subject No.	1	2	3	4	5	6	7	8	9	10	Mean
	Glucose	2.52	2.78	2.42	1.32	1.81	1.91	0.81	1.05	2.43	2.52	1.96
	Sucrose	2.86	2.62	2.72	1.58	1.91	2.23	0.56	1.04	2.20	2.48	2.02

III. XVIII.

The correlation coefficient, r, between glycaemic stimulus and insulin response was 0.37. This is not statistically significant.

The significance limits given are derived from the value of t in the Student distribution. All the differences indicated as being significant were also found to be significant at the 1% level when using the Wilcoxon test for pair differences except the difference between blood sugar levels half an hour after the test meals, which was significant at the 5% level.

(D) DISCUSSION

This study has shown that following a formula meal similar in composition to a normal breakfast, the lipaemic response is greater when the carbohydrate component of the formula is sucrose rather than glucose. The lipaemic response after both meals was greater in the older than the younger men. This is almost certainly explained by the lower fasting serum triglyceride levels of the latter group, since a relationship between postprandial lipaemia and fasting serum triglyceride levels has been clearly demonstrated[26].

Three possible mechanisms have been considered for this greater lipaemic response after sucrose.

(1) Glucose might impair fat absorption (or sucrose might enhance it).

(2) Fructose, derived from the sucrose in meal S might stimulate hepatic[139] or intestinal[99,110] triglyceride synthesis, and so add an endogenous component to the alimentary lipaemia.

(3) Alimentary lipaemia might be cleared more rapidly in the presence of glucose.

There appears to be no physiological basis for the first possibility which in addition would seem to be ruled out by our finding that the differences between S and G were only significant in the descending portion of the triglyceride curve (Figure 3).

Endogenous triglyceride synthesis from carbohydrate does not seem to affect human serum triglycerides under the conditions of this experiment. I found no significant change in serum triglyceride after 100g glucose or sucrose without fat and neither did Swan et al[121]. Under certain conditions ingestion of glucose can decrease triglyceride levels. Havel[45] found a significant decrease after the ingestion of glucose, but then he gave 400g. Perry and Corbett[108] have demonstrated a significant (albeit a very small) fall in serum triglyceride following the intravenous injection of 50g of glucose. The possibility does, therefore, exist that hepatic lipogenesis might be relatively higher with fructose than with glucose. However the smaller degree of lipaemia after the meal containing glucose seems more likely to have resulted from enhanced chylomicron clearance.

Adipose tissue lipoprotein lipase, which plays the major role in clearing chylomicron triglycerides after a fatty meal, is potentiated by insulin[60,70]. Thus, in rats made diabetic with alloxan the enzyme activity is low and restored to normal by insulin treatment[61]. Normal and diabetic animals clear ingested or infused glycerides better when treated with insulin[43,50].

Glucose has been shown to be a more potent stimulator of insulin than any other monosaccharide both in vitro[39,94] and in man, in whom oral fructose

is much less effective than glucose[3,121]. It would therefore be expected that glucose would stimulate a greater insulin response than an isocaloric amount of sucrose, which is digested to glucose and fructose. This was suggested by the work of Swan et al[121], who showed higher peak insulin levels after 100g oral glucose than after sucrose (both taken without other food). The present investigation shows a significantly greater insulin response to glucose when either individual levels at 1 and 1½ hours, or when total insulin response, are considered. The protein and fat in the formula meals presumably contributed to the insulin secretion as well but these two constituents were kept constant in the 2 meals and they are weaker stimulators of insulin secretion than carbohydrate[94,106,109].

Infusion of insulin to hyperglyceridaemic patients results in decreased plasma glyceride levels as compared to control infusions[117]. In this particular study, protamine, a known inhibitor of lipoprotein lipase[16], prevented this insulin-induced decrease of glyceride without preventing the fall in blood sugar and plasma free fatty acids which also resulted from the insulin infusion. This suggests that insulin lowers glycerides through increased removal of glyceride by peripheral tissue, rather than by decreased endogenous synthesis as a result of reduced fat mobilization from adipose tissue.

It is therefore suggested that the more rapid clearing of the alimentary lipaemia which occurs after the glucose meal is due to the greater insulin secretion stimulated by this carbohydrate.

The wider implications of this study remain to be considered. It has previously been pointed out that the elevated serum triglyceride levels observed when a diet high in sucrose replaces a diet high in glucose or starch may be due either to accelerated influx into or decreased efflux from the serum triglyceride pool. There is ample evidence that in both man and experimental animals the fructose of sucrose is more rapidly converted to the precursors of triglyceride than glucose[46,47,48,126,139]. The evidence that impaired removal may play an

important role is less impressive and based on evidence in experimental animals[12]. This study provides some further evidence that this mechanism may also operate in man.

Insulin-stimulated lipoprotein lipase is responsible not only for the clearing of chylomicron triglyceride but also influences the removal mechanism of endogenous triglyceride[100]. It seems likely that on diets high in sucrose the pattern of insulin secretion after meals during the course of the day would resemble the pattern seen after formula S, whereas on diets high in polymers of glucose (eg. starch) insulin secretion is likely to resemble that seen after formula G. That is to say, the postprandial waves of insulin would be higher on high starch diets than on high sucrose diets. Lipoprotein lipase activity would therefore be lower on the high sugar diets and not only would chylomicron clearing be delayed but the removal of endogenous triglyceride would be impaired. This would result in an increased serum triglyceride pool and consequently higher fasting serum triglyceride levels.

Even though similar mechanisms may operate for the removal of exogenous and endogenous triglyceride and from the results of this study slower removal of exogenous triglyceride seems very likely to occur on diets high in sucrose, this does not provide conclusive evidence that the removal mechanisms for endogenous triglyceride will also be impaired on such diets.

Nestel et al[98] have compared the incorporation of radiopalmitate into plasma triglyceride fatty acid on high sucrose and high starch diets. Fractional incorporation was similar on the starch and sucrose diets (when higher fasting serum triglycerides were noted). However, when the higher triglyceride pool size with sucrose was taken into account, the total incorporation of free fatty acid was increased in all the subjects on the high sucrose diet. Because of the difficulties involved in their interpretation[100] even kinetic studies such as this cannot really confirm whether reduction in removal or increased formation of triglyceride is the major factor causing the elevated fasting serum

triglyceride on high sucrose diets or whether both mechanisms are equally involved.

SUMMARY

A formula breakfast containing protein, carbohydrate and fat was given on two occasions to nine middle-aged convalescent patients and to ten young men. The meals differed only in the type of carbohydrate given - sucrose or an isocaloric amount of glucose. Following the formula meal containing glucose, the alimentary lipaemia was cleared more quickly than after the sucrose formula. The insulin response was greater after the meal containing glucose and appeared to be related to the larger glycaemic stimulus. Triglyceride clearing showed a significant correlation with insulin response. It is therefore suggested that the more rapid clearing of alimentary lipaemia following a meal containing glucose as compared with sucrose is related to the greater insulin response elicited by glucose.

The wider implications of this study are considered.

CHAPTER IV - THE EFFECTS OF ISOCALORIC EXCHANGE OF DIETARY STARCH AND SUCROSE ON FASTING SERUM LIPIDS, PATTERNS OF INSULIN SECRETION AND ALIMENTARY LIPAEMIA

(A) INTRODUCTION

This study was carried out for the following reasons:-

1. I wished to confirm the conclusions from the field study described in Chapter II; namely that the significant reduction of serum triglyceride which occurred on the sucrose restricted diet was due to weight reduction rather than a specific metabolic effect of sucrose. This could be done by first isocalorically replacing dietary sucrose with complex carbohydrates, which is what the volunteers in Group A of the field experiment were requested to do; and then to reduce the total caloric intake by reduction of sucrose calories, which is what the volunteers in this group, in fact, did. An accurate isocaloric dietary exchange could only be carried out under the strict control of a metabolism ward.

The exchange would of course be carried out using proportions of total carbohydrate and sucrose similar to those normally consumed[97,103,136].

2. In addition to answering the above question, such a study might contribute to the morass of conflicting literature on the effects of different dietary carbohydrates on fasting serum lipids as studied in the metabolism ward. Some workers have reported little or no difference when sucrose and starch were exchanged[11,28,37,67,77,88] and others have reported elevated serum lipids on diets containing sucrose[4,6,7,8,51,57,73,79,80,83,122]. However, in only a few studies has sucrose been fed in proportions normally eaten[6,37,67,88].

3. Szanto and Yudkin[122] found elevated fasting insulin levels and abnormal insulin response to glucose in some individuals on a very high sucrose diet. Dunnigan et al[28] however, found no abnormality of insulin response to glucose when comparing responses on diets containing normal amounts of sucrose (20% of total calories). It therefore seemed most important to rather test insulin response to a more physiological stimulus (eg. an ordinary meal) on diets varying in their carbohydrate composition, but always containing a normal

amount of dietary sucrose. This appeared to be essential in order to prove or disprove the claim that carbohydrate metabolism is altered on diets containing sucrose.

4. It was shown in the study described in the previous chapter that alimentary lipaemia is more rapidly cleared after a formula meal containing glucose than an isocaloric amount of sucrose. The increased insulin secretion after glucose was suggested as the cause of this. Impaired clearance of exogenous and endogenous triglyceride was postulated as a possible mechanism for the elevated fasting serum lipids seen on high sucrose diets in man[4,6,7,8,51,57,73,79,80,83,122] in view of the fact that the same mechanism plays a role in the removal of both from the blood stream[100]. It therefore seemed important to examine the patterns of insulin secretion and triglyceride clearance after normal meals on diets which differed in the nature of their dietary carbohydrates, but in which proportions of total carbohydrate and sucrose were similar to those normally consumed[97,103,136], and also to study the relationship between triglyceride clearance and fasting serum triglyceride levels under these conditions.

5. MacDonald and Braithwaite[79] and Kuo and Bassett[73] have reported changes in the fatty acid pattern of serum lipids on high carbohydrate diets and further changes when dietary sucrose and starch were exchanged. I wished to see whether similar (or in fact any) changes took place when exchanging carbohydrates at more physiological levels.

(B) METHODS

1. Planning the diets

Three important factors were borne in mind when planning the diets. Firstly, they should contain normal proportions of the proximate food constituents (i.e. carbohydrate should comprise approximately half the total daily calories) and when sucrose was given it should be fed in amounts normally taken (i.e. less than 25% of total daily calories[97,103,136]. Secondly, carbohydrate exchange should be strictly isocaloric and thirdly the diets should be designed as simply

as possible without resorting to formula feeding. Simplicity would aid more accurate preparation of the meals in the diet kitchen.

Each subject was given three diets and each dietary period was of fourteen days duration. Diet 1 was intended to represent a fairly average western diet with regards proportions of proximate food constituents and amounts of dietary sucrose[97,103,136]. In Diet 2 sucrose was isocalorically replaced by complex carbohydrate so that the amount of total carbohydrate remained unchanged. In the third dietary period, the total caloric content of the diet was reduced by eliminating the sucrose from Diet 1 and not replacing it with other food constituents. This is to similate what Group A in the field study did, despite instructions to the contrary. During Diets 2 and 3, saccharine was used as an artificial sweetener.

Each dietary study was preceded by two weeks in the metabolism ward during which the subjects were placed on either of diets 1 and 2 and their caloric requirements determined. The caloric requirements for the 9 subjects were remarkably similar. This is not surprising in view of the similarity in body weight of all subjects excluding subject 2 (See Table XXIV).

The detailed dietary analysis of the three diets is shown in Tables IXX, XX and XXI.

TABLE IXX

DIET 1, representing an average "Western diet" with regards the proportions of the proximate food constituents and sucrose content.

		Protein (g)	Fat (g)	Carbohydrate (g)	Calories (kCal)	Sucrose (g)
BREAKFAST						
Jungle Oats	300g (cooked)	6.0	3.0	29.1	165	
Bread	60g	6.0	1.2	28.8	146	
Butter	15g		12.0		114	
Milk for day	500g	17.5	18.5	24.5	325	
Sugar for day	140g			140.0	532	140.0
Clear tea						
MID-MORNING						
Clear tea						
Bread	30g	3.0	0.6	14.4	73	
Marmite						
LUNCH						
Chicken	100g (white meat cooked)	31.6	3.4		166	
Carrots	100g (tinned)	0.9	0.2	7.1	31	
Butter	25g		20.0		190	
Bread	60g	6.0	1.2	28.8	146	
MID-AFTERNOON						
Clear tea						
Bread	30g	3.0	0.6	14.4	73	
Marmite						
SUPPER						
Bread	60g	6.0	1.2	28.8	146	
Tomato	100g (raw)	1.6		7.0	33	
Egg - hard boiled - one		6.5	5.8	0.4	81	
Butter	10g		8.0		76	
TOTAL DAILY CALORIES					2297	
Quantity of each constituent		88.1	75.7	323.3		140.0
Daily caloric intake of each Constituent		352	681	1265		532
Percentage of total daily calories		15%	30%	55%		23%

ACTUAL DAILY CALORIC INTAKE = 2513 kCal *

* Estimated by bomb calorimetry

TABLE XX

DIET 2, in which dietary sucrose was replaced by the complex carbohydrates - rice and potato. Artificial sweeteners were used.

		Protein (g)	Fat (g)	Carbohydrate (g)	Calories (kCal)	Sucrose (g)
BREAKFAST						
Jungle Oats	300g (cooked)	6.0	3.0	29.1	165	
Bread	60g	6.0	1.2	28.8	146	
Butter	15g		12.0		114	
Milk for day	500ml	17.5	18.5	24.5	325	
Clear tea						
MID-MORNING						
Clear tea						
Bread	30g	3.0	0.6	14.4	73	
Marmite						
LUNCH						
Chicken	100g (white meat cooked)	31.6	3.4		166	
Carrots	100g (tinned)	0.9	0.2	7.1	31	
Potato	77g (flakes)			70.0	266	
Butter	25g		20.0		190	
Bread	60g	6.0	1.2	28.8	146	
MID-AFTERNOON						
Clear tea						
Bread	30g	3.0	0.6	14.4	73	
Marmite						
SUPPER						
Bread	60g	6.0	1.2	28.8	146	
Tomato	100g (raw)	1.6		7.0	33	
Egg - poached -	one	6.5	5.8	0.4	81	
Rice	77g (uncooked)			70.0	266	
Butter	10g		8.0		76	
TOTAL DAILY CALORIES					2297	
Quantity of each constitent		88.1	75.7	323.3		
Daily caloric intake of each constituent		352	681	1265		
Percentage of total daily calories		15%	30%	55%		

ACTUAL DAILY CALORIC INTAKE = 2498 kCal. *

* Estimated by bomb calorimetry

TABLE XXI

DIET 3, in which the sucrose present in Diet 1 was removed and not replaced.

		Protein (g)	Fat (g)	Carbohydrate (g)	Calories (kCal)	Sucrose (g)
BREAKFAST						
Jungle Oats	300g (cooked)	6.0	3.0	29.1	165	
Bread	60g	6.0	1.2	28.8	146	
Butter	15g		12.0		114	
Milk for day	500ml	17.5	18.5	24.5	325	
Clear tea						
MID-MORNING						
Clear tea						
Bread	30g	3.0	0.6	14.4	73	
Marmite						
LUNCH						
Chicken	100g (white meat cooked)	31.6	3.4		166	
Carrots	100g (tinned)	0.9	0.2	7.1	31	
Butter	25g		20.0		190	
Bread	60g	6.0	1.2	28.8	146	
MID-AFTERNOON						
Bread	30g	3.0	0.6	14.4	73	
Clear tea						
Marmite						
SUPPER						
Bread	60g	6.0	1.2	28.8	146	
Tomato	100g (raw)	1.6		7.0	33	
Egg - hard boiled - one		6.5	5.8	0.4	81	
Butter	10g		8.0		76	
TOTAL DAILY CALORIES					1765	
Quantity of each constituent		88.1	75.7	183.3		
Daily calorie intake of each constituent		352	681	733		
Percentage of total daily calorie		19%	39%	42%		

ACTUAL DAILY CALORIC INTAKE = 1802 kCal. *

* Estimated by bomb calorimetry

Food tables were used to determine the composition of most of the foods used (See Part I). However, as the tables used are not South African and in view of the variable moisture content of food, particular care was taken with the carbohydrate foods involved in the exchange - rice, potato and sugar.

For consistency, a constant carbohydrate source was used throughout the series of experiments - "Tastic" rice, "Maggi" potato powder and "Hulett's" sugar. The moisture content of both rice and potato was checked and remained constant at approximately 9% of the total weight. The sugar was found to have no appreciable amount by weight of water. The caloric value of each of these three carbohydrates was determined in duplicate by bomb calorimetry and in each case was found to be 3.8 kCal/g dry weight.

"Attwell's" Brown Wonder Loaf" was used as the bread source during the course of the experiment since the moisture content was found not to vary for 3 days after baking. The moisture content was approximately 40% of total weight. The moisture content of other breads appeared to vary considerably and this could have caused variation in daily caloric intake. Standard foods were used to make up the remainder of the diet and were the same on all three diets. The same diet was given each day during the dietary period. This resulted in a more accurate caloric balance. The diets were prepared in the diet kitchen at Groote Schuur Hospital and each meal was checked and reweighed in the metabolism ward by the dietician, ward sister or the investigator.

A check was made on the actual daily caloric intake of each diet by homogenising all the food eaten on one day of each dietary period and then carrying out bomb calorimetry in duplicate on aliquots of the homogenate. The total daily caloric intake on diets 1, 2 and 3 using this method was found to be 2513 kCal, 2498 kCal and 1802 kCal per day, respectively. In all three cases these values were somewhat higher than the values calculated.

It was pointed out by MacDonald[87] that for strict accuracy the protein which was present in the rice and potato of diet 2 should be replaced during diet 1. Nitrogen determinations were therefore carried out by the Kjeldahl

technique and the potato flakes found to contain 6.88 mg nitrogen/g and the rice 9.79 mg nitrogen/g. This meant that the potato given daily contained 3.1 g protein and the rice 4.4 g protein. It seemed unlikely that this small amount would be significant; but nevertheless the last three subjects were given protein supplements during diet 1. Casilan was given at lunch and supper. Since Casilan was found to contain 142.93 mg/nitrogen/g (by the Kjeldahl method), 3.4 g of Casilan were added to the lunch to replace the 3.1 g of protein in the potato and 4.6 g at supper to replace the 4.4 g of protein in the rice.

Diets 1 and 2 were given to the subjects in a random order and diet 3 was always given last. The order in which the diets were given is shown in Table XXII.

TABLE XXII.

Order in which the three diets were given.

DIET	I	II	III
Subject			
1	1	2	3
2	1	2	3
3	1	2	3
4	2	1	3
5	2	1	3
6	1	2	3
7	1	2	3
8	2	1	3
9	2	1	3

III. XXII.

The experiments were all carried out between January and September 1970, in the Groote Schuur Hospital metabolism ward.

2. The Subjects and Life in the Metabolism Ward

The subjects were all men aged 30-40 years who had been admitted to the hospital earlier for non-metabolic conditions and had at the time the diets were commenced normal liver function tests and erythrocyte sedimentation rates. They had all been on ordinary ward diets before admission to the metabolism ward and then during the first 2 weeks were started on either diets 1 or 2 so that each individual's caloric requirements could be determined. Each subject remained in the metabolism ward for 8 weeks. All were ambulant and allowed ordinary ward activities during this period. All received occupational therapy during their stay in the metabolism ward and one in addition received physiotherapy. The subjects appeared contented and happy during their 2 month stay in the ward and co-operated magnificently, seldom complaining about the numerous venipunctures. They were, of course, all explained the nature of this study before the commencement. The exception to this was subject 9 who with the conclusion of diet 2, refused to continue and withdrew from the study.

3. Sampling during the course of the experiment

(a) The subjects were weighed daily on a platform scale during the course of the experiment. Weight was recorded before breakfast but after the subject had been to the toilet. They were always weighed without shoes and when dressed in their night attire.

(b) Fasting blood samples were taken on days 0, 3, 6, 9, 12, 13 and 14 of each dietary period, day 0 corresponding with day 14 of the previous diet. Serum triglyceride was measured on each of the samples, serum cholesterol on the last 3 samples of each dietary period and total lipid and serum triglyceride fatty acid patterns determined on one of the samples from day 12, 13 or 14 of each dietary period, depending upon the day on which the most serum was available.

(c) In subjects 4, 5, 7, 8 and 9, patterns of insulin secretion and triglyceride clearance after meals were also studied. On random days during the last 4 days of the diets 1 and 2, blood was taken before breakfast, lunch

and supper and then again at half-hourly intervals for 2½ hours after breakfast and lunch had been completed and at ½, 1, 1½ and 2½ hours after the completion of supper. Serum triglyceride, serum insulin and blood sugar were measured on each of these samples. These breakfast, lunch and supper experiments were carried out on different days.

In order that the responses to the 3 different meals on each of the two diets could be directly compared, the organisation of the meals on diet 1 was slightly changed on the days that these tests were carried out. The 140 g of sugar which was usually distributed during the course of the day in any way the subject desired, was on these days divided into two 70 g amounts and 70 g given with each of lunch and supper. None was given on these days at the breakfast meal which was therefore identical on the two diets. Lunch and supper respectively, contained identical amounts of total calories and total carbohydrate calories on the two diets. However, on diet 1 sucrose comprised the bulk of the carbohydrate calories at lunch and supper, whereas on diet 2 potato comprised the bulk of the carbohydrate calories at lunch and rice the bulk of the carbohydrate calories at supper. This is shown in Table XXIII.

TABLE XXIII

	DIET 1	DIET 2
BREAKFAST	Jungle Oats 300g (cooked) Bread 60g Butter 15g Milk per day 500ml Clear tea	Jungle Oats 300g (cooked) Bread 60g Butter 15g Milk per day 500ml Clear tea
MID-MORNING	Clear tea Bread 30g Marmite	Clear tea Bread 30g Marmite
LUNCH	Chicken 100g (white meat cooked) Carrots 100g (tinned) Sugar 70g Butter 25g Bread 60g	Chicken 100g (white meat cooked) Carrots 100g (tinned) Potato 77g (flakes) Butter 25g Bread 60g
MID-AFTERNOON	Clear tea Bread 30g Marmite	Clear tea Bread 30g Marmite
SUPPER	Bread 60g Tomato 100g (raw) Egg - one (hard boiled) Butter 10g Sugar 70g	Bread 60g Tomato 100g (raw) Egg - one (poached) Butter 10g Rice 77g (uncooked)

The meals on Diets 1 and 2 are the days when the breakfast, lunch and supper experiments were done. The mid-morning and mid-afternoon snacks were delayed until the last blood sample after the meal had been taken.

III. XXIII.

4. Techniques of analysis

Serum triglyceride, cholesterol and insulin and blood sugar were measured by the techniques described and expressed as mg/100ml and microunits/ml in the case of insulin. The triglyceride levels were expressed as described previously as degree of lipaemia which was the increment above fasting levels expressed in mg/100ml. The glycaemic stimulus, the insulin response and lipaemic response

to the meal were calculated as described in the previous chapter and expressed as mg/hours and microunit/hours. Both the Wilcoxon test and Student's t test were used to determine the significance of differences between body weight, serum cholesterol and serum triglyceride levels on the different diets. In all cases identical significance limits were obtained by the use of these two different tests.

The significance of differences between lipaemic response, glycaemic stimulus and insulin response to meals on diets 1 and 2 were determined by Student's t test.

(C) RESULTS

During the first two weeks in the metabolism ward caloric requirements were determined according to the diet which the subject was to be given first. Four of the five patients who commenced the experiment with diet 1 complained that they were being given too much to eat when switched to diet 2. Despite this all volunteers were always persuaded to finish their daily ration each day. On the other hand, two of the four patients who commenced the experiment with diet 2 complained that they were not getting sufficient to eat on diet 1.

The detailed results for each subject are given in Table XXIV and summarised in Table XXV(a) and Figure 4. For the purpose of statistical analysis all the subjects have been grouped together. Table XXV(b) shows in addition the mean values for body weight, serum cholesterol and triglyceride on Diets 1 and 2, separating the subjects into those in whom protein was not replaced during Diet 1 (subjects 1-6) and those in whom it was replaced (subjects 7-9).

TABLE XXIV

Body weight, serum cholesterol and serum triglyceride on Diets 1, 2 and 3 for each of the nine subjects. The value given for body weight is the mean for the last 3 days on each diet. The value for cholesterol is the mean of the 3 samples taken on the last 3 days of each diet. Triglyceride values are given for each sampling day. The mean of all these values and the mean of the last three only are also given.

DIET 1.

SUBJECT	Mean Weight kg	Mean Cholesterol mg/100ml	Triglyceride (mg/100ml)								Mean of last three
			0	3	6	9	12	13	14	Mean	
1	63.1	259	107	103	110	110	115	125	100	110	113
2	84.3	178	126	124	131	118	137	122	125	126	128
3	59.3	194	64	61	65	59	68	65	70	65	67
4	54.7	213	102	103	102	104	102	101	102	102	102
5	47.2	150	122	125	110	116	114	125	124	119	121
6	58.7	225	83	74	103	95	94	86	92	90	91
7	56.6	140	82	88	83	82	80	86	80	83	83
8	49.8	151	82	64	83	66	76	74	79	73	77
9	56.2	249	145	163	155	157	178	150	161	161	163
Mean of Subjects 1 - 8	59.2	188.8	96.0	92.8	98.4	93.8	98.3	95.4	96.5	96.0	97.8

DIET 2.

DIET 2

SUBJECT	Mean Weight kg	Mean cholesterol mg/100ml	Triglyceride (mg/100ml)								Mean of last three
			0	3	6	9	12	13	14	Mean	
1	63.5	228	100	101	131	106	127	119	137	120	127
2	84.5	182	125	128	125	126	125	124	125	126	125
3	59.1	192	70	66	70	62	65	62	68	66	65
4	54.0	213	94	83	85	85	95	101	102	92	100
5	46.6	169	108	119	107	127	116	112	122	116	116
6	58.7	219	92	92	85	101	95	95	88	92	92
7	56.5	154	80	77	85	89	90	83	76	83	83
8	49.6	142	59	73	70	80	74	80	82	74	79
9	56.1	243	157	168	164	149	160	163	145	158	156
Mean of Subjects 1-8	59.1	187.4	91.0	92.4	94.8	97.0	98.4	97.0	100.0	96.1	98.4

DIET 3.

DIET 3

SUBJECT	Mean Weight kg	Mean Cholesterol mg/100ml	Triglyceride (mg/100ml) 0	3	6	9	12	13	14	Mean	Mean of last three
1	62.1	253	137	120	122	113	96	100	94	108	96
2	83.5	193	125	127	125	120	107	103	109	115	107
3	56.2	222	68	58	61	56	65	70	67	62	67
4	53.5	234	102	88	80	83	89	82	77	83	83
5	44.7	163	124	125	120	98	90	88	84	101	88
6	57.7	235	88	83	88	80	72	76	78	79	76
7	56.6	172	76	89	80	82	85	85	74	83	82
8	48.4	162	79	72	74	72	78	77	73	74	76
9	-	-	-	-	-	-	-	-	-	-	-
Mean of Subjects 1-8	57.8	204.3	99.9	95.3	93.8	88.0	85.3	85.1	82.0	88.2	84.1

III. XXIV.

TABLE XXV(a)

	DIET 1			DIET 2			DIET 3		
	WEIGHT kg	CHOLESTEROL (mg/100ml)	TRIGLYCERIDE (mg/100ml)	WEIGHT kg	CHOLESTEROL (mg/100ml)	TRIGLYCERIDE (mg/100ml)	WEIGHT kg	CHOLESTEROL (mg/100ml)	TRIGLYCERIDE (mg/100ml)
MEAN	59.2	188.8	97.8	59.1	187.4	98.4	57.8	204.3	84.1
RANGE	47.2-84.3	140-259	67-128	46.6-84.5	142-228	65-127	44.7-83.5	162-253	67-107
S.D.	11.91	44.03	29.85	11.92	34.67	29.83	10.34	38.74	12.59
S.E.M.	3.65	14.68	9.95	3.65	11.56	9.95	4.08	10.56	4.45

Mean body weight, serum cholesterol and serum triglyceride (mean for the last 3 days on each diet) on diets 1, 2 and 3 for subjects 1 to 8.

II. XXV(a)

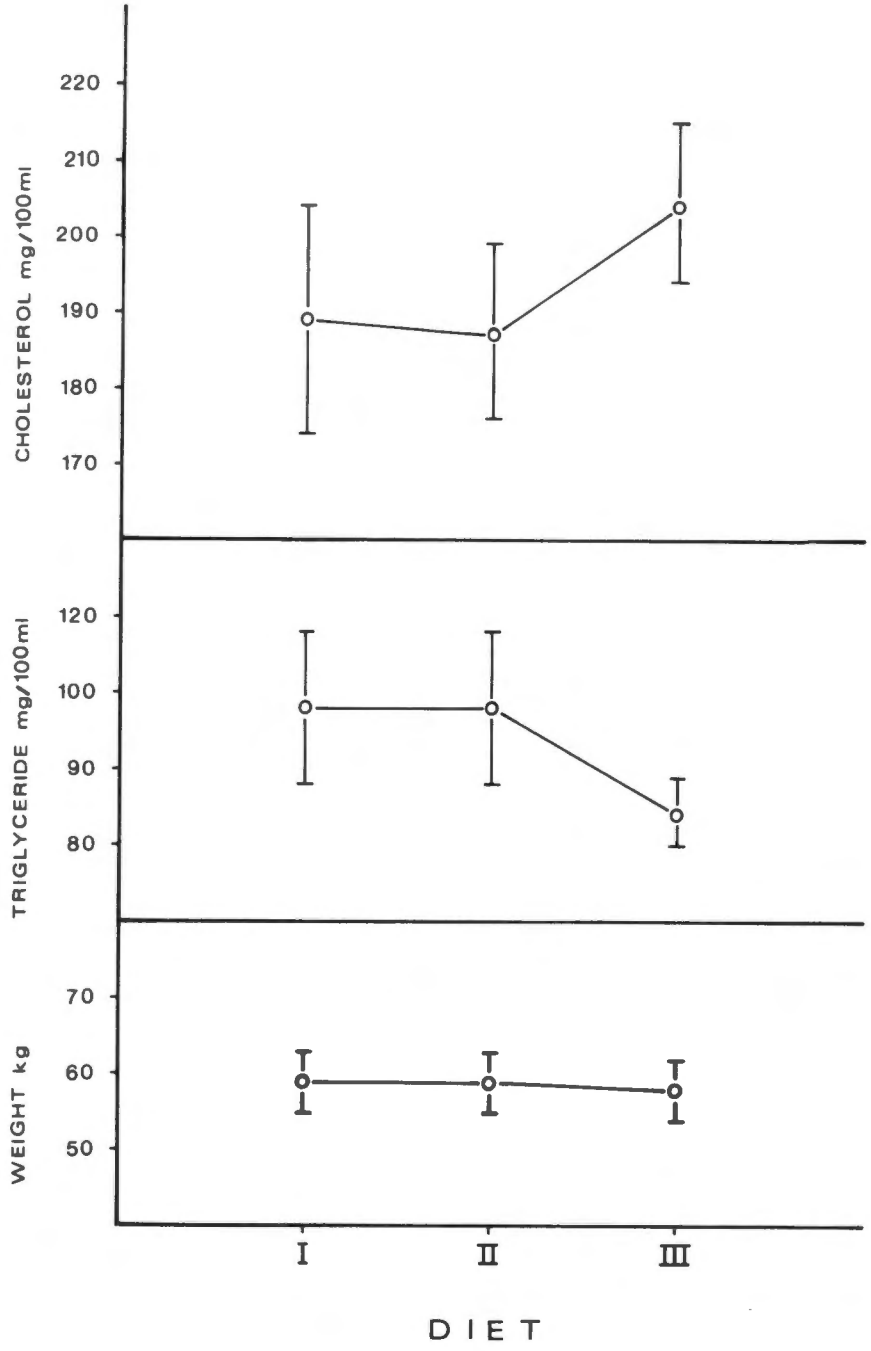


Figure 4: Mean body weights, serum cholesterol and serum triglyceride (mean for the last three days on each diet) on diets 1, 2 and 3 for subjects 1 to 8. S.E.M. also indicated.

TABLE XXV(b)

SUBJECTS	DIET 1			DIET 2		
	WEIGHT (kg)	CHOLESTEROL (mg/100ml)	TRIGLYCERIDE (mg/100ml)	WEIGHT (kg)	CHOLESTEROL (mg/100ml)	TRIGLYCERIDE (mg/100ml)
1 - 6	61.2	203.2	103.7	61.1	200.5	104.1
7 - 9	54.0	180.0	107.7	54.1	179.7	106.0

Mean values for body weight, serum cholesterol and triglyceride on diets 1 and 2 (mean for last 3 days on each diet), separating the subjects into those in whom protein was not replaced during diet 1 (subjects 1-6 and those in whom it was replaced (subjects 7-9).

III. XXV(b)

1. Body weight during the experimental diets

The mean body weight in Table XXIV given for each individual during a dietary period represents the mean of the last three daily weights on that particular diet. As is apparent from Tables XXIV and XXV mean body weight during dietary periods 1 and 2 is virtually identical, being 0.1 kg more than during diet 2 than diet 1. This difference is not statistically significant.

The mean weight during diet 3 is, however significantly lower than during diets 1 and 2 ($p < 0.05$ in both instances). The actual differences in weight are very small, 1.4 kg lower on diet 3 than on diet 1 and 1.3 kg lower on diet 3 than on diet 2.

2. Serum triglyceride levels on the different diets

There were no significant differences between fasting serum triglyceride levels during diets 1 and 2 when considering daily triglyceride values, mean of all values while on the diets or mean of the last three values while on the two diets.

However, when comparing the mean of the last three readings on diet 3 with the mean of the last three readings on diets 1 and 2, values were significantly lower on diet 3 than diet 1 ($p < 0.02$) and than on diet 2 ($p < 0.05$). Differences

were not significant when considering the mean values for the whole dietary period, even though the mean value did tend to be lower on diet 3 than on diets 1 and 2. Means of the individual daily values tended to be lower on diet 3 than on diets 1 and 2 starting from day 9, but only on day 14 were the values significantly lower on this diet than on diets 1 and 2 ($p < 0.02$).

It is of interest to point out that the fall in triglyceride which occurred during diet 3 was chiefly evident in those subjects whose fasting serum triglyceride levels during diets 1 and 2 were greater than 90 mg/100ml (subjects 1, 2, 4, 5 and 6).

3. Serum cholesterol levels on the different diets

These were only measured on each of the last 3 days of each dietary period. Mean values given for each individual are the mean of these three. Once again there is no significant difference between values on diets 1 and 2. The mean values on diet 3 are, however, significantly higher than on diets 1 and 2 ($p < 0.05$).

From the individual results shown in Table XXIV and from the mean values in Table XXV(b) replacement of the small amount of protein during diet 1 appears to make no difference to the mean body weight, serum cholesterol and triglyceride on diets 1 and 2.

4. Fatty acid pattern of total serum lipids and of serum triglyceride

It can be clearly seen from Tables XXVI(a) and (b) that there are not significant differences between fatty acid patterns of total serum lipids and serum triglycerides on diet periods 1, 2 and 3.

TABLE XXVI(a)

Total serum lipid fatty acid pattern on diets 1, 2 and 3.
Mean values are given together with S.D. and S.E.M.

FATTY ACID	DIET 1		DIET 2		DIET 3	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
C14:0	1.6	0.48	1.7	0.51	1.1	0.49
C16:0	27.6	1.63	27.9	1.88	28.2	1.64
C16:1	4.7	0.89	4.5	0.65	4.5	0.68
C18:0	8.1	0.92	7.7	1.06	7.3	1.21
C18:1	27.5	2.41	27.8	2.14	27.0	3.74
C18:2	24.4	3.45	25.1	2.38	26.5	4.26
C20:4	4.8	0.91	4.3	0.85	5.2	1.03
Others	1.3	0.62	1.0	0.53	0.7	0.64
Total Sat.	37.3	1.50	37.2	1.31	36.6	1.62
Total Unsat.	61.4	1.73	61.8	1.63	62.7	1.99

TABLE XXVI(b)

Serum triglyceride fatty acid patterns on Diets 1, 2 and 3.

FATTY ACID	DIET 1		DIET 2		DIET 3	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
C14:0	3.4	1.19	3.3	1.13	2.1	0.59
C16:0	31.0	1.60	31.7	1.81	32.1	0.95
C16:1	6.3	1.44	6.4	1.24	6.0	1.55
C18:0	5.0	0.98	5.4	1.18	5.3	0.66
C18:1	43.6	2.43	43.4	3.21	44.3	2.02
C18:2	8.6	1.45	8.8	1.51	9.1	1.02
Others	2.0	1.76	1.1	1.05	1.0	0.72
Total Sat.	39.4	1.39	40.4	2.41	39.5	1.14
Total Unsat.	58.6	1.63	58.6	3.38	59.5	1.70

III. XXVI(b)

5. Blood sugar, serum insulin and serum triglyceride levels after breakfast, lunch and supper on diets 1 and 2

The glycaemic stimulus, insulin and lipaemic responses to the breakfast, lunch and supper on diets 1 and 2 are shown in Table XXVII and Figure 5.

TABLE XXVII

Glycaemic stimulus, insulin response and lipaemic response to each of the meals on the different diets.

MEAL & SUBJECT NUMBER	INSULIN RESPONSE (microunit hours)		GLYCAEMIC STIMULUS (mg hours)		LIPAEMIC RESPONSE (mg hours)	
	SUGAR	STARCH	SUGAR	STARCH	SUGAR	STARCH
BREAKFAST						
5	69.75	62.75	17.00	15.75	9.75	6.00
4	42.25	48.50	27.50	32.75	31.50	16.00
7	53.75	54.75	22.50	28.00	7.00	8.50
8	66.25	63.25	25.50	21.00	16.50	26.00
9	79.50	79.25	14.00	18.50	25.75	20.75
MEAN	62.30	61.70	21.30	23.20	18.10	15.50
S.D.	14.51	11.55	5.69	7.01	10.41	8.33
S.E.M.	6.49	5.17	2.54	3.14	4.66	3.72
LUNCH						
5	29.50	54.50	12.00	23.75	20.50	15.00
4	41.00	56.50	39.00	59.75	47.50	33.50
7	38.50	60.00	41.50	45.00	11.50	10.25
8	59.00	73.50	31.50	41.50	38.50	34.50
9	76.75	99.00	10.00	48.25	51.00	26.00
MEAN	49.00	68.70	26.80	43.60	33.80	23.90
S.D.	18.87	18.49	14.90	13.07	17.17	10.89
S.E.M.	8.44	8.27	6.66	5.84	7.68	4.87
SUPPER						
5	36.25	16.50	17.00	5.50	0.00	0.00
4	38.75	21.50	45.50	31.50	0.00	0.00
7	41.50	55.50	30.00	47.00	7.00	10.50
8	20.00	51.50	28.50	39.00	0.00	0.00
9	43.25	94.50	24.50	33.25	28.25	7.25
MEAN	35.90	47.90	29.10	31.25	7.10	3.60
S.D.	9.31	31.33	10.46	15.61	12.23	5.00
S.E.M.	4.16	14.00	4.68	6.98	5.47	2.23

III. XXVII.

After the breakfast on the two different diets (when the stimulus was the same) the total serum insulin response was almost identical (mean value on the sugar diet 62.30 microunits-hours and mean value on the starch diet 61.70 microunit-hours). Similarly the glycaemic stimulus to the meal and the lipaemic response were similar on the two different diets. None of these differences were statistically significant.

After lunch however, on the two different diets when the stimuli differed, the glycaemic stimulus was significantly greater after the starch meal (containing potato) than after the sugar meal ($p < 0.05$), and similarly the

the total insulin response was significantly greater ($p < 0.001$). Total lipaemic response to the meal was significantly lower after the starch meal than the meal on the sugar diet ($p < 0.05$). All five subjects in whom these tests were carried out showed similar patterns.

After the supper meal on the two different diets, glycaemic stimulus and insulin response were once again higher after the starch meal than sugar meal, but this time the differences were not statistically significant, probably due to the fact that only 3 of the 5 showed this pattern. The lipaemic response to both meals was very small after the supper meal on both diets due to the fact that only 10 grams of butter were given with these meals.

The mean levels for blood sugar, serum insulin and degree of lipaemia at each sampling period are shown in Table XXVIII.

TABLE XXVIII

Serum insulin, blood sugar and degree of lipaemia at each sampling period after the meals on the two different diets. Mean values are indicated with S.E.M. in brackets.

MEAL & TIME OF SAMPLING	SERUM INSULIN (microunits/ml)		BLOOD SUGAR (mg/100ml)		DEGREE of LIPAEMIA (mg/100ml)	
	SUGAR	STARCH	SUGAR	STARCH	SUGAR	STARCH
BREAKFAST						
0	3.6 (0.68)	4.0 (0.71)	78.0 (3.45)	75.6 (4.12)	0	0
½	37.8 (7.96)	39.4 (6.85)	95.0 (4.95)	98.6 (8.14)	-2.4 (2.02)	-3.0 (1.22)
1	45.4 (5.73)	47.2 (6.40)	94.0 (5.50)	96.2 (6.13)	14.0 (2.98)	12.6 (6.43)
1½	30.0 (4.37)	29.0 (2.99)	85.6 (4.80)	82.0 (5.68)	15.1 (8.84)	13.4 (5.35)
2	21.2 (3.46)	18.0 (3.41)	79.6 (3.61)	78.8 (4.59)	12.4 (3.03)	3.6 (3.14)
2½	15.2 (3.14)	13.2 (2.85)	74.0 (3.83)	73.8 (4.09)	0.00 (2.28)	0.0 (2.06)
LUNCH						
0	6.8 (1.02)	5.6 (0.75)	80.8 (4.21)	80.6 (4.31)	0	0
½	39.2 (7.25)	47.0 (13.04)	92.4 (5.37)	100.2 (7.18)	13.0 (3.61)	12.8 (3.64)
1	39.2 (4.03)	48.4 (9.33)	110.6 (6.83)	117.6 (5.39)	19.4 (12.36)	18.2 (4.38)
1½	23.0 (2.05)	35.2 (3.44)	87.0 (3.79)	99.6 (4.83)	30.8 (12.32)	28.7 (3.81)
2	18.6 (3.16)	22.0 (3.90)	83.4 (4.83)	90.8 (5.14)	28.8 (13.69)	12.4 (2.54)
2½	16.6 (2.52)	20.0 (3.03)	78.4 (3.21)	82.6 (3.79)	23.60(16.94)	9.4 (4.83)
SUPPER						
0	8.8 (1.53)	10.0 (1.02)	85.2 (3.41)	83.4 (4.20)	0	0
½	37.0 (8.47)	38.2 (7.55)	100.8 (10.07)	110.0 (5.16)	-9.4 (4.47)	1.6 (4.29)
1	31.0 (4.61)	40.6 (8.14)	99.8 (4.73)	108.6 (4.83)	-0.4 (6.79)	-1.9 (3.21)
1½	25.2 (3.61)	37.0 (6.84)	92.9 (6.81)	98.0 (9.81)	-4.1 (7.69)	-0.2 (4.21)
2½	18.8 (3.20)	20.8 (4.59)	83.5 (3.95)	84.7 (3.80)	-8.7 (9.46)	-4.3 (5.78)

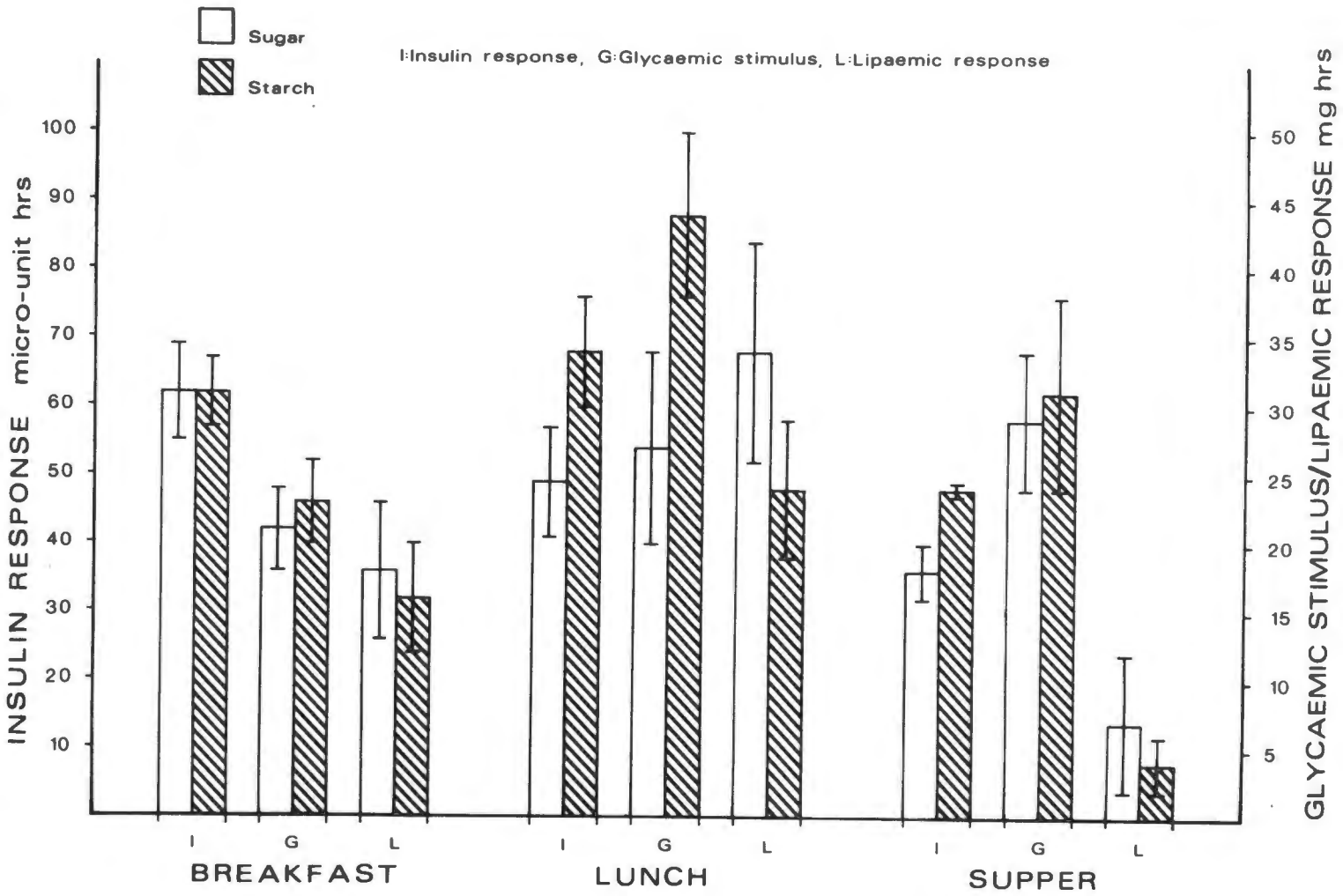


Figure 5: The glycaemic stimulus and insulin and glycaemic responses to the breakfast, lunch and supper on diets 1 and 2.

After the breakfast meal values for each of these parameters at each sampling period were once again almost identical. After lunch, however, all mean values of se um insulin and blood sugar except the sampling at 0 appeared to be higher after the starch meal than after the sugar meal. Values for the degree of lipaemia appear lower at 2 and 2½ hours after the starch lunch than after the sugar lunch. However, none of these differences reached statistical significance, probably due to the small numbers.

Likewise, after supper all levels of serum insulin and blood sugar except at 0 time were higher on the starch diet than the sugar diet but again differences were not statistically significant.

6. Interrelationships between triglyceride, blood sugar and insulin responses

The calculations for the correlations described below have been based on the values obtained for all three meals.

An inverse relationship existed between insulin response and lipaemic response. The correlation coefficient was -0.22, which is not statistically significant. There was an even smaller inverse correlation between glycaemic stimulus and lipaemic response ($r = -0.13$).

The insulin response seemed to be related to the glycaemic stimulus in that there was no significant difference between ratios of insulin response to glycaemic stimulus on both diets when different glycaemic stimuli were obtained on different meals. (See Table XXIX).

TABLE XXIX

The ratio between insulin response and glycaemic stimulus following meals on Diets 1 and 2.

INSULIN RESPONSE / GLYCAEMIC STIMULUS	DIET 1		DIET 2	
	Mean	S.E.M.	Mean	S.E.M.
	2.48	0.38	2.21	0.29

The correlation coefficient, r, between glycaemic stimulus and insulin response was 0.29 which again is not statistically significant.

DISCUSSION OF RESULTS

This study has shown that when sucrose (given in amounts normally eaten) is replaced by complex carbohydrates, there is no significant change in fasting lipid levels under the conditions of this experiment in which subjects with normal fasting lipid levels were studied. The exchange was isocaloric and there was no significant change in weight during the two 14-day dietary periods. Failure to have replaced during diet 1, the small quantity of protein present in the rice and potato of diet 2 in some subjects appears to have made no difference to fasting serum cholesterol and triglyceride levels. However, when the sucrose was eliminated from the diet and the calories provided by the sucrose not replaced, certain changes were noted. The mean serum triglyceride levels had started to fall by the ninth day after the commencement of this reduced calorie diet. On the fourteenth day, and when considering the mean values for the last three days of each dietary period, the values were statistically significantly lower on diet 3 than on the other two diets. On the other hand, serum cholesterol levels, when considering the mean values for the last three days on each diet, were significantly increased on the reduced calorie diet when compared with diets 1 and 2 during which they did not differ significantly. These changes coincided with the significant weight reduction which occurred during the third dietary period.

The serum triglyceride results tend to confirm the conclusions of the field experiment described in Chapter I, namely that the fall in serum triglyceride was due chiefly to the weight loss which occurred in the group who were requested to restrict their sugar intake and not due to a specific metabolic effect of sugar. The fact that the subjects in this study felt that they were being given too much to eat when switched from diet 1 to diet 2 may well explain the weight loss in this sugar restricted group despite the fact that they were

specifically asked not to lose weight. Starch foods are more bulky than sucrose and it is probably difficult for an individual to voluntarily replace the sucrose in his diet with starchy foods. The fall in serum triglyceride in diet period 3, after levels had remained unchanged in periods 1 and 2, also lends support to the theory of those who suggest that changes in caloric balance[11, 111] can explain the observed differences in fasting serum lipids when complex carbohydrate replaces sugar[4,6,7,51,79,80,122]. It has been claimed that sucrose rather than complex carbohydrate causes elevation of serum lipids when the fat component of the diet is saturated[8,84]. However, from the results of this study it appears that even this phenomenon is only apparent when sucrose is fed in abnormally high proportions, as was the case in these[8,84] studies. In the present study the bulk of fat was saturated (provided chiefly as butter) but sucrose was given in more physiological amounts. The finding of Dunnigan et al[28] (published shortly before the completion of this study), are also confirmed; namely that when dietary sucrose at normal levels in normal people was replaced isocalorically by complex carbohydrate, there was no change in the fasting serum lipid levels. His conclusions are based on a study very similar to this one, except that he did not include a third dietary period, during which calories were reduced.

The finding that serum cholesterol was elevated during the third dietary period was rather an unexpected one. Several workers have demonstrated elevated serum cholesterol levels during acute starvation[15,29], with the highest levels found at 72 and 96 hours after the commencement of the starvation diet. Other workers[56] have found no significant increase of serum cholesterol over the same time interval when feeding a 500 kCal diet to overweight subjects. On more physiological weight reducing diets, most investigators have found that weight loss was associated with a fall in serum cholesterol levels[34,38,55,129] though others have observed no changes[19,95,96]. It should however be pointed out that in three of the studies in which the weight reduction was associated with

a fall in cholesterol, the diets fed had a fairly low total fat content[38,55,129] and a high proportion of polyunsaturated fat[38,55]. In one study serum cholesterol fell despite a fairly high cholesterol intake on a diet which was low in total calories (900 kCal/day) and moderately low in total fat content (40 g/day)[34]. In two studies serum cholesterol did not fall despite weight reduction[95,96]. In these cases normal amounts of fat were fed (80 g/day). In only one study was there no fall in cholesterol associated with a weight reducing diet moderately low in fat (40 g/day)[19]. In the present study total calories were reduced in diet 3, but the amount of fat given (which was nearly all saturated) remained unchanged and the proportion of total daily calories provided by fat actually increased. It may be that this is an important factor, but further study is clearly required to determine the cause of the elevated cholesterol levels during this reduced caloric dietary period.

In this study there were no differences in the fatty acid patterns of serum triglycerides and total serum lipids on the different diets suggesting that differences reported by other workers[73,79] occur only when starch and sucrose are fed and exchanged at abnormally high proportions of total calories.

Measurement of the glycaemic stimulus, insulin response and lipaemic response to the meals on the different diets provided interesting results. Unfortunately these tests were only carried out on 5 of the 9 subjects. They had to be carried out on selected people whose veins were not too difficult to puncture since when these tests were carried out, a large number of venipunctures had to be performed.

Glycaemic stimulus, insulin response and lipaemic response to the breakfasts were virtually identical on the two different diets. The actual meal was identical on the two diets thus showing that these responses to a physiological stimulus (such as breakfast) did not differ significantly when a diet containing a normal amount of sucrose is compared with one containing complex carbohydrates. This is again in keeping with the findings of Dunnigan et al[28] that insulin response to glucose does not differ significantly under circumstances

such as these and tends to minimise the practical significance of Szanto and Yudkin's finding that on diets containing abnormally high proportions of sucrose, certain individuals showed abnormalities of insulin response[122].

A different pattern was observed after lunch and supper when the effects of potato and rice were compared with sucrose. From the study described in the previous chapter and the results of other workers[3,39,94,121], it was expected that higher levels of serum insulin might be found after the complex carbohydrate, since these consist of polymers of glucose and would, therefore, be expected to be more potent stimulators of insulin secretion than an isocaloric amount of sucrose, which consists of glucose and fructose, a very poor stimulator of insulin secretion.

After lunch when potato was compared with sucrose, this was in fact, found to be the case when significantly higher insulin levels were found on the starch diet. As was found in the previous study, the lipaemic response was found to be significantly smaller after the starch meals when the insulin response was greater. This smaller lipaemic response after starch meal appeared to be due to more rapid clearing of ingested fat, since triglyceride levels did not appear to be different for the first 1½ hours, but appeared to be lower at 2 and 2½ hours after the starch meal.

As in the previous experiment, the differences in insulin responses appeared to be due to differences in glycaemic stimuli in that there was no significant difference between the ratios of insulin response to glycaemic stimulus on the two different diets when different stimuli were obtained.

However, when considering the supper meal, when rice and sucrose were compared, the mean values for glycaemic stimuli and insulin responses were once again higher after the starch meal than after the sucrose, but the differences were not statistically significant, probably due to the fact that only three of the five subjects showed this pattern. Lipaemic response was negligible on both diets after supper, since only 10 grams of butter (the principal

source of fat) was given with each meal.

In summary then it appears that when starch replaces sucrose at physiological levels in people with normal fasting lipid levels, there is no significant difference in fasting serum lipid levels or in glycaemic stimulus, insulin response and lipaemic response to a physiological stimulus (in this case a normal breakfast). However, after other meals on diets differing in their carbohydrate content, differences are apparent. Insulin secretion depends upon the potency of the carbohydrate component of the meal to stimulate this hormone - carbohydrates containing polymers of glucose being more potent stimulators of insulin secretion than sucrose which contains glucose and fructose. The triglyceride clearance is more rapid when there is a greater insulin secretion. It appears therefore from this study that while physiological biochemical changes may occur when starch replaces sucrose fed in normal amounts, these changes do not result in changes in the fasting serum lipids.

Delayed triglyceride clearance has been postulated as a mechanism for elevated serum triglyceride levels observed on high sucrose diets in man. If this mechanism does in fact operate it does not apparently do so under the conditions of this experiment, i.e. when sucrose is fed at normal levels.

There remains a need for long-term comparative studies of starch and sucrose feeding to establish whether different patterns of insulin secretion, glucose tolerance and triglyceride clearance will become apparent which are not evident in relatively short-term experiments. Such a study would be extremely difficult to carry out as it has already been shown in the field experiment described in Chapter I that restriction of dietary sucrose with isocaloric replacement by other foods is practically impossible for a free living individual. An experiment of this nature would therefore need to be done under strict supervision in a metabolic ward such as the present study and would necessitate very long periods of hospitalization.

Finally there remains a need for a study such as the present one in

which starch is substituted for sucrose (within the normal range of sucrose intakes) in people with various types of hyperlipoproteinaemia and the effects of this manipulation on the fasting serum lipids and patterns of insulin secretion, glucose tolerance and triglyceride clearance studied. There is considerable evidence for the presence of abnormalities of carbohydrate metabolism in certain varieties of hyperlipoproteinaemia[31,35,75,113] and such a study would provide both useful practical information and further insight into mechanisms.

SUMMARY

The effects of isocaloric exchange of dietary starch and sucrose on fasting serum lipids, patterns of insulin secretion and alimentary lipaemia have been studied in 9 healthy male volunteers. After meals on the two diets different patterns of insulin secretion were observed; and appeared to depend upon the potency of the carbohydrate component of the meal to stimulate this hormone. Triglyceride clearance in turn depended upon the insulin secretion. However, despite these changes, no significant differences were observed in fasting serum lipid levels on the two diets. Furthermore, insulin response to a physiological stimulus (a normal breakfast) was identical on the two diets. Restriction of dietary sucrose without replacement by other dietary constituents resulted in a fall in fasting serum triglyceride level and elevation of fasting serum cholesterol.

CHAPTER V - GENERAL CONCLUSIONS

The findings of each of three dietary experiments described in this part of the study have been summarised at the end of Chapters II, III and IV. I wish therefore in this final chapter merely to draw a few general conclusions and discuss their possible clinical significance.

It has been shown in a field experiment that voluntary sucrose restriction in healthy male volunteers resulted in a significant fall in serum triglyceride which was maintained for five and a half months. There was little change in serum cholesterol levels. These changes were attributed chiefly to the weight loss which occurred during the period of sucrose restriction. In a shorter dietary experiment in which starch isocalorically replaced the sucrose present in a westernised diet, there was no change in fasting serum lipid levels. After meals on the two diets different patterns of insulin secretion were observed and appeared to depend upon the potency of the carbohydrate component of the meal to stimulate this hormone. Triglyceride clearance in turn depended upon the insulin secretion. Insulin response to the same physiological stimulus (in this case a normal breakfast) was identical on the two diets.

Any clinical significance of these experiments seems to lie chiefly in the dietary prevention of atherosclerosis. The association between cholesterol and coronary heart disease has been clearly established[102]. A large eight-year controlled clinical trial of a polyunsaturated fat diet, low in cholesterol was conducted in Los Angeles[25]. The experimental diet produced a sustained fall in serum cholesterol as compared with the control group and fatal atherosclerotic events (sudden death due to coronary thrombosis and cerebral infarction) were significantly less in the experimental group. The investigators responsible for conducting this trial have admitted that their trial, as with most of its predecessors, has fallen short of providing a definite answer. Despite this, however, there seems to be fairly general agreement that a diet low

in fat, which should be chiefly polyunsaturated and low in cholesterol plays some role in the dietary prevention of atherosclerosis[89,92].

Certain workers[23,134,136] have suggested a closer association between intake of sugar and mortality from coronary heart disease than the better described association between intake of dietary fat and mortality from this disease[17,54]. Epidemiological evidence of this nature is far from conclusive in view of the association between consumption of sucrose and saturated fat[54] and sugar consumption and cigarette smoking[13]. Furthermore, other epidemiological studies have failed to confirm this association[52,104].

The hyperlipidaemic effect of sucrose has been postulated as a possible atherogenic mechanism of this carbohydrate[4]. The results of this study confirm the findings of other workers that a diet containing sucrose in physiological amounts is not hyperlipidaemic in normolipaemic individuals when compared with an isocaloric amount of starch[28,37,67]. Sugar restriction does appear to be a useful and practical dietary manipulation for the reduction of serum triglyceride in individuals with elevated fasting serum triglyceride (Type IV Hyperlipoproteinaemia of Fredrickson). The mechanism of this reduction in triglyceride observed in this study seemed to be weight reduction rather than a specific metabolic effect of sucrose. Furthermore, the significance of an elevated fasting serum triglyceride alone is not completely clear and must await the findings of Prospective Studies which are now underway[20,36].

An abnormal insulin response to glucose observed in certain individuals on a very high sucrose diet has been suggested as further evidence that a high sucrose diet may be atherogenic[122]. Once again the present study has indicated no difference in insulin response to a physiological stimulus when a diet containing physiological amounts of sucrose were compared with diets containing isocaloric amounts of starch.

An abnormal insulin response to glucose may well occur on diets containing abnormally large amounts of sucrose. However, during the course of

these studies, seven day diet histories were obtained from approximately 100 subjects. The mean daily sucrose intake was 89 grams and the highest 147 grams. Two experienced workers in this field[22,101] have found similar results and agree that the abnormally large amounts of sucrose used in the above-described experiments must be rare indeed in the free living population.

In view of these observations, I would agree entirely with the view of McGandy et al[89] that from knowledge available at the present time there seems no justification for recommending that the population at large change the nature of its dietary carbohydrate for the purpose of the dietary prevention of atherosclerosis.

It may well be that sucrose even in physiological quantities may have a specific effect in individuals with Type IV Hyperlipoproteinaemia[58] but comparative studies of sucrose in normal amounts and other carbohydrates are still to be carried out in such subjects.

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SOME FACTORS INFLUENCING SERUM TRIGLYCERIDE IN MAN

A brief summary indicating the contribution to knowledge made by this thesis.

(A) Epidemiological studies have been conducted in order to clarify further the factors influencing serum triglyceride levels. From these studies it appears that the single most important factor influencing serum triglyceride levels may be the frequency of physical activity. This in turn presumably reflects a caloric balance which is not excessive. The well-described variation with age appears to be modified by the degree of physical activity. Evidence is also presented suggesting that the relation between smoking and serum triglyceride levels which has been reported by some workers may be explained on the basis of the relationship between smoking and physical exercise.

The individuals with elevated serum lipids in each of the population groups were classified according to the system described by Fredrickson and co-workers[1]. The most common abnormality was Type IV Hyperlipoproteinaemia (the "carbohydrate induced" variety). No cases of this condition were found in physically fit adult males and it is therefore suggested that this apparently common condition may consist of two sub-groups - the more common variety being due to caloric excess and a far smaller group to true primary familial Type IV Hyperlipoproteinaemia.

No evidence was found in this study to suggest that race or diet per se influence serum triglyceride.

The pronounced skewness in the frequency distribution of serum triglyceride has previously been demonstrated by Carlson and Linstedt[2]. These workers did not, however, study in detail subjects under the age of 20 years. In the present study a fairly large group of 16-18 year old healthy males was studied and a significantly skewed distribution was found even in this group.

(B) Three dietary experiments were carried out in an attempt to clarify further the precise role which different dietary carbohydrates play in influencing serum triglyceride levels.

(1) It was shown in a field experiment that voluntary sucrose restriction in healthy male volunteers resulted in a significant

fall in serum triglyceride which was maintained for 5½ months. There was little change in serum cholesterol levels. These changes were attributed chiefly to the weight loss which occurred during the period of sucrose restriction.

(2) A formula breakfast containing protein, carbohydrate and fat was given on two occasions to nine middle-aged convalescent patients and to ten young men. The meals differed only in the type of carbohydrate given - sucrose or an isocalorie amount of glucose. Following the formula meal containing glucose, the alimentary lipaemia was cleared more quickly than after the sucrose formula. The insulin response was greater after the meal containing glucose and appeared to be related to the larger glycaemic stimulus. Triglyceride clearing showed a significant correlation with insulin response. It was therefore suggested that the more rapid clearing of alimentary lipaemia following a meal containing glucose as compared with sucrose was related to the greater insulin response elicited by glucose.

(3) In a shorter dietary experiment the effects of isocaloric exchange of dietary starch and sucrose (in physiological proportions) on fasting serum lipids, patterns of insulin secretion and alimentary lipaemia were studied in 9 healthy male volunteers. After meals on the two diets different patterns of insulin secretion were observed; and appeared to depend upon the potency of the carbohydrate component of the meal to stimulate this hormone. Triglyceride clearance in turn depended upon the insulin secretion. However, despite these changes, no significant differences were observed in fasting serum lipid levels on the two diets. Furthermore, insulin response to a physiological stimulus (a normal breakfast) was identical on the two diets. Restriction of dietary sucrose without replacement by other dietary constituents resulted in a fall in fasting serum triglyceride level and elevation of fasting serum cholesterol.

The clinical significance of these experiments is discussed on page 205 of the thesis.

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EFFECTS ON SERUM-LIPIDS IN NORMAL MEN OF REDUCING DIETARY SUCROSE OR STARCH FOR FIVE MONTHS

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Summary 51 healthy office workers, aged 36–55, volunteered for a dietary experiment. A third of them were asked to cut out sucrose and replace it with other foods. Another third tried to halve their dietary starch and substitute other foods. The remainder were controls who continued their usual diets. In the low-sugar group, serum-triglycerides showed a significant decrease which persisted until the diets were stopped after $5^1/_2$ months; triglycerides fell more in those who had higher levels to start with. The reduction of serum-triglycerides can be attributed, at least partly, to weight-loss. Serum-lipids did not change significantly in the reduced-starch group, but these subjects had more difficulty following their prescribed diet and did not lose weight.

INTRODUCTION

DIETARY sucrose has been named as a possible factor predisposing to ischæmic heart-disease.[1] Yet the longest time that the effects of restricting dietary sugar have been measured in man is 10 weeks.[2] In that experiment 11 men who had returned to work after myocardial infarction showed reductions of serum-triglycerides which were significant at the beginning of sugar restriction but had become not significant by the 10th week. Their serum-cholesterols fell slightly and the patients lost an average 3 lb. in weight at the beginning of sugar restriction.

All the other experiments in man in which dietary sucrose was restricted have been shorter, lasting up to a month. Serum-lipids were found higher on high-sugar, low-starch diets than on comparable low-sugar, high-starch diets in some of these trials,[3–7] but not in others.[8–12] The lack of firm data pointing to a predictable and sustained effect of substituting one type of carbohydrate for another[13,14] led us to make the following field experiment.

METHODS

51 volunteers were recruited from the head office of a large insurance company. They were male office workers, many of managerial status, 35–53 years old and in apparent good health.

Before the diets were started, 3 fasting blood-samples were taken at weekly intervals for baseline serum-lipid measurements. Next the volunteers were divided randomly into three groups of 17. Each man was given a list of the dietary instructions for his group. These were explained at a meeting before starting the diets and at interviews throughout the experiment.

Group A (low sugar) were instructed to cut out foods containing sucrose but to maintain their weight by eating more of other foods.

Group B (reduced starch) were asked to halve their consumption of starchy foods (e.g., one potato instead of two) and to substitute other foods to maintain weight.

Group C (control) continued their usual diets.

The experimental diets were taken from April to September, 1969.

Fasting blood-samples were taken 2 weeks after starting the diets, and thereafter 4-weekly for $5^1/_2$ months (22 weeks) after which the diets were stopped. Final control blood-samples were drawn 4 weeks later. Bloods were taken in the company's medical suite, where the subjects were weighed on a platform scale without jacket or shoes. They then went to have breakfast in the cafeteria before starting their day's work. All the volunteers persisted cheerfully throughout the experiment except 1 in group B who resigned from the company.

All subjects kept 7-day records of their usual diets during the preliminary control period. 3-day diet records were kept twice during the experimental period, after 10 and 18 weeks, and again a month after the men had returned to their usual diets. The tables of Hardinge et al.[15] were used to calculate dietary sucrose and starch. Serum-cholesterol was measured by the method of Abell et al.[16] and triglycerides by a modification of the van Handel and Zilversmit procedure,[17] using triolein (British Drug Houses) as standard. Lipoprotein paper-electrophoresis was carried out on every serum-sample, using the method of Lees and Hatch.[18]

RESULTS

The initial mean serum-triglycerides in the 51 subjects ranged from 51 to 325 mg. per 100 ml. Their frequency distribution was significantly skewed, with a tail to the right. The course of serum-triglycerides and changes in body-weight is summarised in table I. In group A, serum-triglycerides started rather higher

instance, the overall figure given by Professor Tripathy for urban diabetes over the age of 10 years was 9·2%. Admittedly his criteria for diagnosis are laxer than ours.

It may be perfectly true that there has been a tenfold increase in diabetes among poor rural Indians who have become urbanised South African Indians, but urbanisation is apparently the important factor rather than the transfer to South Africa per se. There may thus be a great difference between the prevalence of diabetes in the whole of India compared to Indians in South Africa, but much less difference between urban-dwelling Indians in India and urban-dwelling Indians in South Africa.

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SERUM-LIPIDS IN SUGAR-CANE CUTTERS

SIR,—We would like to contribute the following observations to the interesting discussions on sugar intake in your journal.

Because it has been claimed that large intakes of sugar can raise serum cholesterol and/or triglycerides [1,2] we have lately taken the opportunity to measure serum-lipids in a group of Pondo sugar-cane cutters working on the Natal coast. They are migrant workers who have been reported to consume large quantities of sugar during the cane-cutting season.[3,4]

Each year, before the start of the cane-cutting season, large groups of African labourers are recruited from Pondoland. In their homeland there is little employment for these people, and the majority are occupied chiefly in herding small numbers of cattle and cultivating small plots of maize. Their diet contains a very high proportion of complex carbohydrate. Sucrose, protein, and fat intakes are low.

During their stay in the Natal sugar belt their diet is very different. We examined men working for the Tongaat Sugar Company. We estimate that their rations, supplied by the company provide, daily, approximately 5000 C. and adequate amounts of all nutrients. Of particular interest is the amount of sucrose consumed. 130 g. is supplied to each man as part of the daily ration. In addition, the men generally chew enough cane to provide about ½–¾ lb. (227–341 g.) of sucrose daily.[3]

With the help of Dr. W. Mukheiber (factory medical officer) and Mr. J. Potgieter (fields manager) we examined 43 of these Pondo cane-cutters (aged 25 to 50 years) the day after their arrival in Tongaat. 28 of the same individuals were examined again after 3 months of sugar-cane cutting. On each occasion body-weight was recorded and venous blood was taken after an overnight fast. The samples were taken by plane to Cape Town, where the serum was separated the same afternoon. Cholesterol [5] and triglycerides [6] were measured and lipoprotein paper electrophoresis carried out.

The mean serum-lipid concentrations and body-weight in the 28 subjects who were examined both on arrival from Pondoland and after 3 months' work cutting sugar are shown below.

Date	Body-weight (kg.)	Serum-cholesterol (mg./100 ml.)	Serum-triglyceride (mg./100 ml.)
April, 1970	59·0	145	92
July, 1970	61·1	142	94

Despite a statistically significant gain in weight, both cholesterol and triglyceride values remained unchanged. There were no remarkable features on lipoprotein electrophoresis.

There are two possible reasons why they exhibited no increase in serum-lipids: firstly, most of their sugar intake was unrefined, direct from chewing cane [4]; secondly, we believe the more important reason was the great daily energy expenditure. Cutting sugar-cane is very heavy physical work and it has been estimated that a very good cutter can cut and move by hand up to 7 tons of sugar-cane a day.[3]

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ETHICS AND ECONOMICS IN BLOOD-SUPPLY

SIR,—Professor Titmuss, in his discussion of the role of giving in blood-supplies,[1] is likely to mislead many of your readers (a) by suggesting a distinction between "economic" and "social" man and (b) by asserting that payment for blood causes higher social costs, acute and chronic shortages of blood, and a high risk of infection of recipients through viral hepatitis and other diseases, compared with donation. Although I do not "urge" and have not "urged" the introduction of payment to blood suppliers in Britain, as Titmuss asserts,[2] the case for payment (if there is or ever will be one) does not stand or fall on these claims.

(*a*) Man is a social animal with economic problems that are a subset of "social problems". They are not mutually incompatible categories. As a science, economics is the study of the *means* of achieving ends and only the ends can ever justify any specific means. In this sense nobody will dispute that "ethics" comes before "economics". So far as I know nobody (except Titmuss) has ever suggested otherwise.

(*b*) The problem of post-transfusion hepatitis was barely discussed in my original study with M. H. Cooper of the role of "price",[3] and I should like briefly to indicate some of the economic possibilities here. It is clearly not "price" *per se* that causes the disease but the social condition of those who are frequently drawn by "price" to supply blood. A variety of possibilities suggest themselves once this fallacy has been identified:

A. Assume there is no clinical test by which hepatitis carriers can be identified before donation. One could (1) collect all blood, including infected blood, and distribute as usual on the grounds that it is better to incur a risk (of less than unity) that recipients contract hepatitis than to suffer even greater shortage. This seems a wrong policy since better choices are available. It is a valid criticism of Americans that they have largely adopted this policy; (2) offer a sufficiently high price that an excess supply is produced from amongst which those individuals with obviously undesired characteristics may be rejected—this is costly; (3) offer a "price" *in kind* of a sort to appeal to the appropriate social class (e.g., theatre tickets, free parking, Wine Society membership)—this is costly; (4) discriminate at the point of collection by offering "prices" only to certain prechosen categories of population (e.g., university students, teachers, residents of well-to-do districts)—this too will be costly.

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TABLE I—MEAN SERUM-TRIGLYCERIDES (mg. PER 100 ml.)

Group	Before (mean of 3)	On experimental diets				After 1 mo.
		2 wk.	6 wk.	10–18 wk. (mean of 3)	22 wk.	
A: low sugar ..	135	113 (−1·7)	105 (−2·3)	106 (−2·7)	96 (−2·8)	108 (−2·0)
B: reduced starch	121	113 (−0·7)	117 (0)	116 (+0·6)	106 (+1·7)	109 (+1·9)
C: control ..	125	122 (+0·2)	125 (+0·9)	121 (+0·5)	130 (+2·2)	116 (+2·4)

Changes in body-weight (lb.) are shown in parentheses.

than in the other groups. On the low-sugar diet triglycerides fell by 22 mg. per 100 ml. after 2 weeks and by 30 mg. per 100 ml. after 6 weeks. There was an accompanying loss of weight, likewise maximal in the first 2 weeks. Weight was steady from 14 to 22 weeks, and triglycerides stayed at the new lower level. Reduction of serum-triglycerides (mean of six) throughout the low-sugar diet averaged 22% and was statistically significant by the sign test. In group B there was only an average 6% reduction of triglycerides. This group did not lose weight except in the first 2 weeks.

The changes of serum-triglycerides on the low-sugar diet were much greater in the subjects whose baseline values were above average (table II); this subgroup in general showed moderate pre-β-lipoprotein bands. The intensity of these bands decreased with the serum triglycerides.

The men who lost more weight on the low-sugar diet (group A) had larger reductions of serum-triglycerides. For example, after 6 weeks the mean triglyceride change was −61 mg. per 100 ml. in subjects who lost more than 5 lb. It was −25 mg. in men who lost less than 5 lb. and −12 mg. in those who did not lose weight.

Serum-cholesterol changed little during the experiment. The starting concentration and mean of six measurements during the diets were 247 and 237 mg. per 100 ml. in the low-sugar group, 238 and 250 mg. in the reduced-starch group, and 234 and 237 mg. in the controls.

According to the diet records at 10 and 18 weeks, group A reduced their average sucrose from 85 to 12 g. per day; but they did not eat more of other foods, so their total calorie intake fell. Group B lowered starch consumption by about 25% (175 calories per day), but their average intake of other foods was not increased sufficiently to compensate for this at the times the diets were recorded. There was great variation between individuals and between the two dietary records in group B. The volunteers found the reduced starch diet difficult to follow. Dietary records were very consistent in group C.

TABLE II—HIGH AND LOW SERUM-TRIGLYCERIDE SUBGROUPS OF LOW-SUGAR GROUP (A)

Starting-serum-triglyceride subgroup	No. of volunteers	Serum-triglycerides		
		Mean before diet	Mean of 6 values on diet	Change (%)
>135 mg. per 100 ml.	7	199	139	−30
<135 mg. per 100 ml.	10	90	81	−10

When the time came to return to their usual diets, many men in group A claimed they had lost the taste for sweet food. The dietary records showed that a month after the experiment they were eating only 55 g. of sucrose a day.

DISCUSSION

This field trial has shown that well-motivated men can reduce and hold their sucrose intake to less than a quarter of what they are used to. On this regimen serum-triglycerides averaged 22% less, and serum-cholesterol 4% less, than before. Triglycerides fell more in those who started with mild-to-moderate hypertriglyceridæmia, in agreement with the metabolic-ward experience of Kaufmann et al.[19]

In the low-sugar group serum-triglycerides fell synchronously with loss of weight, which appeared to result from reduced calorie intake. Several workers have concluded from metabolic-ward experiments that serum-triglycerides do not fall when starch is substituted for sucrose if care is taken to make the change isocaloric, with no loss of weight.[10,11,14] Although the group average weight stopped falling in the last 2 months, when presumably the subjects were no longer in negative calorie balance, their serum-triglycerides remained at the new low level.

The relevance of this study to the pathogenesis of ischæmic heart-disease cannot be decided without further information on the epidemiology of serum-triglycerides, such as that being gathered in the Stockholm prospective study.[20]

We are grateful to the Old Mutual Life Assurance Company, Pinelands, Cape Town, for facilities and to Dr. A. de Beer and the medical staff. We specially thank the volunteers for their conscientious participation. Financial support was received from the South African Sugar Association. The work was performed in the Clinical Nutrition Unit of the University of Cape Town and the South African Medical Research Council.

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THYROID HORMONAL EFFECTS ON CARDIAC FUNCTION IN THE CONSCIOUS DOG

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In the intact conscious morphine-sedated dog the functional state of the myocardium could be correlated directly with the thyroid status of the animal. The effect of experimentally induced hyperthyroidism was to increase myocardial contractility by actions both on the B-adrenergic receptors as well as on the myocardium directly, as demonstrated by selective blockade with propranolol. In the hypothyroid state a profound decrease in myocardial contractility could be shown, but responsiveness to B-adrenergic stimulation was maintained until gross myxoedema developed.

Responsiveness to exogenously administered catecholamines was greatly increased at low dose levels, but failure to maintain normal dose-response curves occurred at higher dose levels. The effect of thyroxine was well established in one week, that of triiodothyronine could be shown to occur within twenty minutes.

These data suggest that thyroid hormone may act as a physiological determinant of the contractile state of the myocardium and may provide an explanation for the functional derangements of myocardial function in both thyrotoxicosis and hypothyroidism.

THE EFFECTS OF TWO DIFFERENT CARBOHYDRATES (GLUCOSE AND SUCROSE) ON THE CLEARING OF ALIMENTARY LIPAEMIA

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A formula meal containing protein, carbohydrate and fat was given on two occasions to each of ten volunteers. The meal varied only in the type of carbohydrate given – glucose being ingested on one occasion and an isocaloric amount of sucrose on the other.

Blood was taken before ingestion of the formula and at half-hourly intervals for two and a half hours subsequently. Blood sugar, serum insulin and serum triglyceride were measured on each sample. Following the formula meal containing glucose, the alimentary lipaemia tended to be cleared more quickly than after the sucrose formula. The insulin response (as measured by the area under the insulin response curve) was greater after glucose had been ingested and appeared to be related to the glycaemic stimulus.

The insulin responses showed a significant inverse correlation with T.G. clearance. The inverse correlation between glucose response and T.G. clearance are not statistically significant.

RADIO-IMMUNO-ASSAY OF INSULIN

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The principles underlying the radio-immuno-assay technique for the determination of serum insulin concentrations, established by Hales and Randle, are described, and the routine application of the method to large numbers of specimens is discussed. The technique has been in use in our laboratory for nearly six years, and approximately 30,000 individual determinations have been carried out.

Our normal fasting values as well as serial values established during oral glucose tolerance tests compare well with those reported by other workers in the field. However, these data are not amenable to conventional statistical treatment due to the wide range of individual variations encountered even in normal fasting values. Presenting the values on a logarithmic scale does not resolve this problem, nor does the conversion of the insulin : glucose ratio to the "insulinogenic index", although the latter procedure may reveal additional information.