THE COMMON FATTY ACIDS OF HUMAN DEPOT FAT.

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TO RHODA

With love and affection

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INTRODUCTION

The current renaissance in biological research has evoked widespread interest in the field of lipid metabolism. While extensive studies during the past decade have greatly expanded our knowledge of the subject, these researches have been directed primarily to the serum lipids. By far the greatest accumulation of lipid in the mammalian organism is found in the depot fat. This tissue had, until fairly recently, been generally regarded as a semi-static food store, expanding and shrinking in response to a relative excess or deficit of dietary calories. This simple concept is no longer tenable. The pioneering work of a few workers in the field during the past few decades has more recently interested many others and the accumulated evidence has established the fat depot among the more active organs in the mammalian organism.

Despite what is now an extensive literature on the metabolic activity of this tissue there is a relative hiatus in our knowledge of an aspect so fundamental as the fatty acid composition of this tissue in man. Adipose tissue contains about 30% water and of the dry weight approximately 90% is lipid. This lipid is almost exclusively triglyceride. It is the triglyceride fatty acid composition that is the subject of this study.

Studies in man on the fatty acid composition of adipose tissue have been reported from time to time, but few reports have been either accurate or comprehensive. The earliest report, using classical methods of fatty acid analysis, to give data comparable with that obtained by modern techniques, was made in 1943. Since this study was commenced there have been two more communications, and this appears to be the total extent of detailed studies in this aspect of adipose tissue to date.

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The difficulty has obviously been in obtaining samples for analysis. The classical method of fatty acid analysis required a generous supply of tissue, not readily available, which had to be obtained from post mortem material. This always raises the possibility of an alteration in composition by autolytic processes. The advent of fatty acid analysis by gas-liquid chromatography has greatly facilitated the study of this tissue. Less than 1 mg. of lipid, which can be readily obtained by a simple procedure in living man, is more than enough for an accurate fatty acid analysis. This methodological advance has stimulated this study on the fatty acid composition of human depot fat.

BACKGROUND TO THIS STUDY

Extensive research in the lipid field has established that there are certain trends with age and sex in normal man of both a qualitative and quantitative nature. The role of the diet has received particular attention and this variable has been shown to be a most important factor in determining lipid values. Ischaemic heart disease has also been shown to be associated with deviations from normal lipid metabolism.

THE EFFECT OF AGE

Changes in fat metabolism that occur with age have been studied in a number of respects, and these may be profound. Changes with age in the composition of tissue lipids affect even the so-called essential lipids, or element constant of Terroine[1], both absolutely and in relation to each other[2]. The significance of these changes, while regarded as being of a physiological nature in childhood, is not certain in adult life[3].

Blood Lipids

The serum lipid components have been widely studied in relation to age. Serum cholesterol concentration in particular has received widespread attention in this regard. The level in new-born infants is low[4,5]. This is found even where the serum cholesterol concentration has been compared in White and Bantu infants whose mothers showed marked differences[6]. There is then a very rapid rise[5] which continues more slowly so that by the age of 3 years the levels in both sexes approximates to that of young adults[7].

During adult life the trends with age are influenced by several factors, including sex. In adult men the serum cholesterol concentration has been shown to rise with age until the middle of the fourth decade[7,14,15,16]. After this age, studies have shown conflicting findings. Some investigators have reported a further

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increase until around 60 years of age[15,12] whereas others have not been able to confirm this[10,7,17]. It has been shown in some communities that there is no trend with age in the adult male[18,19,20]. These differences in age trends have been attributed to differences in the dietary fat intake[13]. In Johannesburg[21] it was found that Bantu men, with a very low fat intake, do not show a rise in serum cholesterol concentration with age, but that age-matched White men, who derive a large proportion of their calories from fat, do show a rise. Urban Bantu men, living in the same city, on a "Europeanized diet", also show an increase in their serum cholesterol concentration with age. In Cape Town, studies on White, Cape Coloured and Bantu males have shown marked differences in serum cholesterol concentration between the racial groups in both young men (20 - 29 years)[22] and older men (40 - 58 years)[23]. The concentration in the Bantu is low, in the Cape Coloured it is intermediate and is high in the White males. Further, in each racial group the mean serum cholesterol concentration has been shown to be related to income; the higher income group having a higher mean level in both young men and older men. These differences in serum cholesterol concentration in the Cape Town racial groups have been correlated with dietary fat intake, particularly animal fat[23]. In general there is a trend for serum cholesterol to rise with age in the White and Cape Coloured, but not in Bantu men on comparing the age groups 20 - 29 years and 40 - 49 years in each race[13].

It does therefore appear that in adult men a rise in serum cholesterol concentration with age cannot be regarded simply as a function of age. Other factors, notably the dietary fat intake and particularly animal fat, have been shown to play an important role. The question of race has been reviewed[13,21,24] and possible genetic factors do not seem to be of primary importance in determining serum cholesterol concentrations. Again the dietary factor correlates.

/Females.....

Females have been found to show variations in serum cholesterol concentration independent, at least in part, of those factors affecting the concentration in men. It has been shown in a single community that girls between 3 and 12 years of age have a significantly higher serum cholesterol concentration than do boys of the same age range. From 12 to 27 years the levels in the sexes are about the same and from 28 to 42 years the men exceed the women by a significant amount. Women between 53 and 57 again overtake the men and, after this age, level off to the concentration found in men of the same age[7]. A trend for serum cholesterol concentration to increase with age in adult women has been established[7,12]. This trend, however, cannot be regarded as universal in view of the above findings in men. No similar studies have yet been reported on women in the racial groups in Cape Town. It would however not be unexpected if, other than sex factors possibly influencing serum cholesterol levels at a particular age, similar general trends to those in men of the same race were found.

Other serum lipid factors have also been studied in relation to age. The concentration of the serum phospholipids has been shown to follow very closely that of cholesterol in both men and women[7]. In one study[17] where no change in total cholesterol or phospholipid was found with increasing age a significant increase in both of these components was shown in the alpha-lipoprotein fraction over the entire age range from 26 to 72 years. An increase in serum glycerides with age has also been reported[17].

The significance of these latter findings cannot yet be assessed. Whether they are true functions of age or, as has been shown in regard to serum cholesterol, are dependent on other factors, remains to be determined.

Total Body Fat

The greatest quantitative change in tissue lipids reported to occur with age is the increase in the size of the adipose organ. This is fully discussed in Chapter 2. While the available evidence indicates that this change is a function of age, it has

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yet to be established as a universal phenomenon. Again it is possible that other factors may affect this trend.

Depot Fat Fatty Acid Composition

The possible effect of age on human depot fat fatty acid composition has been studied. Both reports[25,26] state that there was no change with age. From what is reviewed above it is plain that some of the reported trends with age are not universal phenomena and would appear not to be a function of age but related to other factors, notably the diet. Trends with age nevertheless do occur in some groups although not in others. From the two published reports, to date, on the relationship between age and depot fat fatty acid composition it is not stated whether the subjects sampled showed a change with age in any of the other lipid components. It is therefore not yet known whether a change that occurs with age in, for example, serum lipids or total body fat is paralleled by a change in depot fat composition.

The population of Cape Town provides particularly interesting material because of the established differences between the racial groups in serum cholesterol concentration. The trends with age are different in the racial groups. It is therefore of interest to determine whether these different trends with age are mirrored by differences in the depot fat fatty acid composition.

THE EFFECT OF SEX

Several aspects of fat metabolism have been compared in males and females. Certain differences between the sexes have been clearly established while others are less well founded. These differences are usually attributed to the effect of the respective gonadal hormones but a simple, direct relationship is by no means always clear, let alone an understanding of their mechanisms of action.

Total Body Fat

The considerable differences between the sexes in the proportion of body weight

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represented by fat is well established in man and in several animal species. The differences in the distribution of body fat between males and females has also been clearly shown (See Chapter 2).

Blood Lipids

Differences between men and women have also been demonstrated in some of the blood lipid components. Russ, Eder & Barr[27] studied the protein-lipid relationships in normal individuals and compared a group of young women (18 - 35 years) with young men of the same age range. They found that the plasma of young women contained more alpha- and less beta-lipoprotein than in the young men. In groups of men and women, aged 45 - 65 years, they found no differences. Similar studies on men and women by the ultra-centrifugation technique gava analagous results[28,29]. It was further shown that the beta-lipoprotein cholesterol concentration, the total plasma cholesterol and cholesterol phospholipid ratio in normal young women reach their lowest point at the time of ovulation[9], when the secretion of oestrogens has been shown to be maximal[30]. Similar cyclic changes were not found in men. At the menopause a sharp rise in serum cholesterol concentration[7] and a reversal of the beta-/alpha-lipoprotein ratio has been found[10].

Gonadal hormonal lack has also been studied in these respects. Oophorectomised women showed a higher total serum cholesterol concentration than normal women of the same age[31] while eunuchs had a lower serum total cholesterol and a lower beta-lipoprotein with higher alpha than normal men, regardless of age[32]. It thus appears that oestrogen reduces both the total lipoproteins and the beta-/alpha-lipoprotein ratio, whereas androgens have the opposite effect. The administration of oestrogen preparations to post-menopausal women[33] and to subjects with myocardial infarction[11,27] appear to have had the expected effect while androgens tended to produce an opposite but not quite so dramatic effect[27,34].

/The relationship.....

The relationship between the male sex hormones and plasma lipids has since been shown to be much more complex than at first appeared to be the case. The removal of protein from the diet of men has been reported to produce a marked fall in serum cholesterol and in the beta-lipoproteins without any change in the alpha-lipoproteins[35,36,37]. With the addition of methyl testosterone (50 mg./day) there was surprisingly a further marked fall in both alpha- and beta-lipoproteins. These effects have been attributed to the decrease in dietary protein[38]. Although these dietary manipulations did not alter the fat or calories in the diet, there was a change in carbohydrate intake. In view of the obvious complexity of these relationships the observed effect cannot be so readily ascribed to the effect of protein alone. There is in fact evidence that the protein anabolic effect of testosterone is dependent on the presence of insulin in an as yet undefined manner[39] and thus implying a possible role for carbohydrate in these relationships.

The dietary fat content, both qualitative and quantitative, has been shown to affect the serum cholesterol concentration and particularly the beta-lipoprotein fraction. The marked differences in total serum cholesterol concentration that have been found to exist between White, Cape Coloured and Bantu men resident in Cape Town are due primarily to the differences in the beta-lipoprotein fraction. These differences have been correlated with the amount of animal fat consumed by the above racial groups[23]. Further, the fluctuations in total serum cholesterol concentration that have been produced by altering the type of dietary fat has again been shown to be due to parallel changes in the beta-lipoprotein fraction[40].

It would thus appear that dietary manipulations may produce changes in the blood lipids similar to those thought to be characteristic of oestrogenic activity. An interesting communication in this regard has recently been made[41]. It was reported that on altering the diet of long-term White male prisoners from a high fat

/to a low fat.....

to a low fat Bantu type of diet gave rise to a marked increase in the urinary oestradiol/ oestriol ratio. Conversely, giving Bantu prisoners a high fat diet produced a striking lowering of this ratio, thereby implying that diet could change or influence hormonal secretion.

The only clear point to emerge from the available evidence on the effect of sex hormones on blood lipids in man is the complexity of the relationship. It seems that oestrogen is responsible for certain qualitative and quantitative differences between males and females. Similar differences however can be produced by suitable dietary manipulations and dietary manipulations have been shown to alter the response to administered testosterone. The alteration in hormonal secretion following on dietary manipulations needs to be confirmed. Animal experiments designed to elucidate the relationship between blood lipids and sex hormones have not contributed further to our understanding because of species differences and differences in observations on the same species(38).

Our present state of knowledge on this subject may be summed up by stating that oestrogens and androgens have a distinct effect on some of the circulating lipids. Their effects are to some extent antagonistic but not directly so. Nutritional factors profoundly influence the effects of the sex hormones on the circulating lipids and may themselves produce changes simulating the effects of hormones. There appears to be a relationship between nutritional and endocrine factors in the regulation of the circulating lipids but the nature of this relationship is not known; nor is the mechanism of action of the sex hormones in regard to the plasma lipids known.

The Fat Content of Specific Tissues

Animals studies have shown that there is a sex difference in the liver lipid content of some animal species. This was first noted by Greisheimer(42) in 1930. She found that male rats had a lower liver fat and higher liver glycogen than did the females on a variety of dietary regimens common to both sexes. Deuel and his

/co-workers.....

co-workers reported a series of studies on these sex differences. They were unable to confirm Greisheimer's findings in unfasted rats but noted the same sex differences with fasting for periods up to 4 days[43]. Guinea pigs showed the same trends with sex in regard to liver fat, but not glycogen, which was higher in the females after fasting for 48 hours[43]. Subsequent studies on liver glycogen (which Deuel[3] believes to behave in a manner opposite to that of fat) showed that these differences were related to ovarian hormonal secretion. Ovariectomized rats did not show differences from males in liver glycogen content[44], nor were sex differences evident in immature rats (26 - 29 days old) or old rats (17 - 24 months old)[45].

Feeding rats choline-free diets resulted in a greater content of glycogen and lower fat in the male as compared with the female rat in the unfasted condition[46]. Similar sex differences between male and female rats have been reported by Best et al.[47]

These sex differences in the liver fat content have been shown to be due entirely to the neutral lipid fraction. In comparing males and females, the latter with a higher total lipid content, the males were in fact found to have more cholesterol[48] and phospholipid[48,49] than the females. The neutral fat fraction is therefore clearly considerably greater in the female rat. This liver fat in the fasting state is almost certainly derived from the fat depots (Chapter 2).

A sex difference in the liver fat of hens has also been demonstrated[50]. Laying hens have been shown to have a higher liver fat content than both non-laying hens and male birds. This difference again was due to an increase in the neutral fat fraction.

It is therefore established that in the rat, guinea pig and hen, liver lipid is either present in greater amount in the female, or readily stimulated by fasting to show an increase; and that this increase is due to an increase in the neutral lipid component which is presumably derived from the depot fat. These differences appear to be due to the effect of oestrogens.

/Lipid Turnover.....

Lipid Turnover

The elaboration of the differences in liver glycogen and lipid content between the sexes, primarily by Deuel and co-workers, appears to have arisen out of a chance observation. In investigating the "ketolytic" action of certain carbohydrates it was noted that women produced more urinary ketone bodies on fasting than did men. Deuel and Gulick[51] studied this and found that on fasting, the acetone bodies excreted by women was 3 to 4 times as great as that in men. Men were able to tolerate a 7-day fast in reasonable physical comfort whereas women could not manage for this length of time and required to be given sugar at a much earlier stage. The acetonuria produced by women increased on the average, from the first day to the fourth day of a fast, from 0.02 - 6.56 gm./day; whereas in men the corresponding time period produced an increase from 0.02 - 2.66 gm. The CO_2 combining power of the blood over the same period showed a fall in women from 53.6 to 26.9 volumes %; whereas in men the change was slight - 52.1 to 46.6 volumes %. These differences between the sexes could not be accounted for by obesity. Expressing the ketone body production in gm./square metre of body surface gave figures of 10 - 13 in the females and 3.5 - 4.0 in the males.

Sex differences were similarly demonstrated when male and female rats and guinea pigs were given ketogenic acids, like acetoacetic and butyric, as their sodium salts in their diets. Females again showed a greater ketonuria[52]. Similar sex differences in ketonuria were demonstrated in alloxan diabetic rats[53]. There was thus evidence that, on fasting, a greater accumulation of neutral fat appeared in the liver of female rats and that this was associated with a higher degree of ketonuria indicating a greater degree of fat breakdown in the female than the male. It was further shown that after the production of fatty livers in both male and female rats fed a choline-free diet, the rate of lipid clearance after refeeding was greater in the female than in the male[46]. This increased rate of fat clearance occurred together with an increased ketonuria in the female.

/These findings.....

These findings by the Deuel group led them to conclude that the fat stores in the female were more labile than in the male, indicating that at least a quantitative variation must exist in the intermediary metabolism of these lipids as related to sex[3].

Essential Fatty Acids (E.F.A.)

This subject has recently been comprehensively reviewed[54] and there seems little doubt that the so-called essential fatty acids, linoleic, linolenic and arachidonic, are obligatory nutrients for normal growth, development and maintenance of health in the rat. Their role in man is as yet uncertain, but they may be necessary nutrients for children. The problem is as yet unresolved. It appears that essential fatty acids cannot be synthesized by the mammalian organism and must be provided in the diet.

The role of these fatty acids in rat metabolism was discovered only after the rigid exclusion of all fat from the diet by the feeding of ether-extracted foods. Such a diet is clearly artificial and does not occur in nature; nor is it certain what other nutrients may have been destroyed by such a procedure. There is, however, some evidence that a relative E.F.A. deficiency may be brought about by an increased demand for linoleic acid under certain dietary conditions such as a high fat diet, particularly of saturated fat, and the cholesterol content.

A sex difference in the requirements for E.F.A. has been claimed in rats. It would appear that females require less linoleic acid than males. This is based on the assessment that on a fat-free diet females are able to survive for longer than males; that growth in females is relatively less retarded than in males; that reproductive functions are relatively less affected in females; that females appear to be relatively more resistent to lethal doses of X-irradiation and that females appear to require less linoleic acid for the cure or prevention of some of these deficiency manifestations.

Depot Fat Fatty Acid Composition

It would appear from the above that there is a difference between the sexes in both qualitative and quantitative aspects of lipid metabolism. There is good evidence

/that sex.....

that sex hormonal factors determine these differences but that dietary factors may, interestingly, provoke similar changes. The local population provides material with which it may be possible to assess the interplay of these factors with regard to depot fat composition. If there should be sex differences in the depot fat fatty acids it would be of interest to establish whether these differences occur in each racial group, or whether the different dietary habits accentuate or diminish possible sex differences.

Linoleic acid is found in relative abundance in the depot fat of man. If the intake of dietary linoleic acid was comparable in men and women of the same race then, from the above evidence of a lesser linoleic acid requirement in females, one would expect that there would be a progressive accumulation of this fatty acid in females. This should be reflected in a higher proportion of linoleic acid in female depot fat. This hypothesis appears not to have been examined to date.

THE EFFECT OF DIET

The manifold role of the diet in determining lipid patterns in general and in respect to trends with age and sex have already been indicated. The importance of dietary factors in determining the depot fat fatty acid composition is established (Chapter 2). It has however recently been shown that the depot fatty acid composition in man is particularly resistant to changes. These changes are brought about only very gradually after a radical change in the nature of the dietary fat[25]. It would therefore be safe to assume that the composition of human depot fat does not respond in any detectable manner to minor fluctuations in diet quality, but represents the overall effect of that diet in so far as dietary factors are able to influence its composition.

In addition to the time lag in producing changes in depot fat composition, it would appear that a rather atypical diet must be fed to man for such an effect to be demonstrable. Such an approach in testing the effect of diet on depot fat composition is therefore both impractical and unrealistic.

/The local.....

The local racial groups are again of particular interest in this regard. The dietary habits of each group are fairly characteristic, providing a dietary pattern that is largely maintained for life. The effect of this diet would therefore be represented in the depot fatty acids. Further the dietary habits of the local population represent between them the qualitative extremes of human nutriture for a large proportion of mankind. It would therefore be possible here to obtain data on the effect of natural diets, within these extremes, on the depot fat fatty acid composition that would be representative of a large proportion of mankind, as well as indicate the manner in which these different diets influence the depot fat composition.

Another aspect of depot fat composition that may be of particular interest in the local racial groups stems from serum lipid analysis on White and Bantu subjects[55]. It was found that despite marked quantitative differences in the cholesterol and triglyceride components of the plasma, the fatty acid composition of these fractions was remarkably similar in the racial groups. This balance in serum fatty acid composition could conceivably be maintained by selectively drawing on the depot fatty acids. If this is so it would be another point of interest in determining whether differences exist between the racial groups in depot fat composition.

ISCHAEMIC HEART DISEASE

The increasing incidence of ischaemic heart disease in the Western world is widely held to be associated with, if not due to, an altered fat metabolism. The marked differences seen in the prevalence of this disease among the local racial groups has been shown to bear a striking relationship to serum cholesterol concentration among members of these groups[23]. These differences in serum cholesterol concentration have further been shown to reflect differences in the dietary fat intake, both qualitatively and quantitatively[23]. The effect of dietary fat on serum cholesterol concentration has been attributed to the composition of the dietary fat[40].

/A fatty acid.....

A fatty acid believed by some to have special protective qualities in this regard is linoleic acid, a common constituent of oils that have been shown to lower serum cholesterol concentration. This property is,however, not unique to oils containing an abundance of linoleic acid. Certain fish oils with a linoleic acid content no greater than that found in beef fat, which raises serum cholesterol concentration, are as effective[40]. The oils with a high linoleic acid content are however more commonly part of the average diet than are fish oils. Thus although oils with a high linoleic acid content may not be unique in possessing the property of lowering serum cholesterol concentration, such oils are probably of great importance because they would in general provide the primary dietary factor that lowers serum cholesterol in man.

Several recent studies on blood lipid fatty acids[56,57,58] have revealed no striking differences in the proportion of linoleic acid between subjects with ischaemic heart disease and controls. These findings, as in the findings among the local racial groups, again focus attention on the depot fat as a potential source of balancing the circulating fatty acids. The possibility that this fatty acid may be low in the depot fat of subjects with ischaemic heart disease to account for the apparently normal levels in the blood lipids merits consideration.

There may, in addition, be other differences in the depot fat composition that could indicate an altered fat metabolism associated with this disease.

/PURPOSE OF THE STUDY.....

PURPOSE OF THE STUDY

The fatty acid composition of depot fat is determined in large measure by the nature of the diet and particularly its fat content (see Chapter 2). Cape Town with its three racial groups, White, Cape Coloured and Bantu, subsisting on diets distinctly different, both in the relative proportion and type of dietary fat, provides material of particular interest. The dietary habits of these three racial groups represent between them extremes of human nutriture. The subjects available here can thus provide data on the depot fat fatty acids, both qualitatively and in their relative proportion, that would be representative of a large part of mankind.

The effect of age, sex, diet and ischaemic heart disease on adult lipid metabolism, and particularly serum lipids, has been widely investigated and certain trends have been established in man. The opening of a new avenue of investigation on an aspect of lipid metabolism heretofore rather neglected makes it desirable to assess the influence of these factors on the fatty acid composition of human depot fat.

The purpose of this study is to :

1) Determine the fatty acid composition in the depot fat of subjects from each of the local racial groups. The combined data will therefore provide details of the type and proportion of fatty acids to be found in a cross section of man subsisting on diets representing extremes of human nutriture.

During the course of this study it became apparent that 6 fatty acids consistently constituted more than 90% of the total fatty acids. The determination of the proportion of each of these fatty acids was found to be highly reproducible. In comparing the relationship between the depot fat composition and various other factors between groups only these 6 fatty acids will be considered.

/ 2) Compare.....

2) Compare the depot fat composition between subjects in the third and fifth decades of life.

3) Compare the depot fat composition between males and females.

4) Compare the depot fat composition between the local racial groups in both males and females.

5) Determine the depot fat composition in subjects with proven ischaemic heart disease.

The serum cholesterol concentration and the degree of body fatness of the subjects studied will be estimated. These factors will be helpful in determining whether the subjects sampled show the general trends established with regard to age, race and sex. The dietary details of the subjects sampled will also be collected. This too will be helpful in assessing whether the subjects sampled from the various racial groups eat the diets typical of their groups.

The reasons for selecting subjects between the ages of 20 - 29 years and 40 - 49 years is: (i) Because of the established differences between them with regard to trends in serum cholesterol concentration. (ii) The relationship between age and depot fat composition is to be assessed in females as well. Post-menopausal women were therefore avoided because this would introduce a potent variable. (iii) The greatest difference in serum cholesterol concentration between subjects with and without ischaemic heart disease is shown during the fifth decade. The disorder in fat metabolism associated with this disease is therefore possibly most marked at this age level. If there is a difference in depot fat composition between subjects with and without this disease it may be expected to express itself most clearly at this age.

/ABBREVIATIONS USED.....

ABBREVIATIONS USED

Throughout this study 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 unsaturated bonds in the molecule also indicated by a number. Thus myristic acid is C14 : 0; palmitic, C16 : 0; palmitoleic, C16 : 1; stearic, C18 : 0; oleic, C18 : 1; linoleic, C18 : 2, etc. It has become common practice to identify fatty acids in this manner in studies using gas-liquid chromatography as the method of fatty acid analysis.

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CHAPTER 2.

ANATOMY AND PHYSIOLOGY OF DEPOT FAT - A REVIEW.

Depot fat forms a considerable proportion of the various basic mammalian tissues. The view that this tissue is simply a storehouse for excess dietary calories was widely held, and until fairly recently there appeared to be no good reason for changing this concept. The fact that there were obvious fluctuations in the size of this tissue with overeating and starvation did not seem to provoke consideration of possible dynamic aspects related to deposition and mobilization of fat. Research into the metabolic activity of adipose tissue during the last two decades has completely changed this concept. There is now conclusive evidence establishing the depot fat among the more active body tissues.

The passive role of depot fat in body physiology was a view repeatedly challenged for more than a century, but the arguments put forward did not win general acceptance. It is therefore correct to acknowledge the work of those who, on the basis of their studies with limited analytical apparatus, had the insight and imagination to anticipate the vast functional potential of this apparently static tissue.

ANATOMY

DEFINITION

Depot fat may be defined as an accumulation of closely packed cells which have a special fat-forming function. These accumulations form continuous masses which are distributed in the connective tissue in various parts of the body.

/ TYPES OF ADIPOSE.....

TYPES OF ADIPOSE TISSUE

There are 2 types of adipose tissue - "white" fat and "brown" fat. These names distinguish the tissues on their difference in colour. White fat is the common depot fat. Brown fat is found in all mammals who hibernate and also in some non-hibernators. In those animals who have brown fat it is distributed in the region of the neck, interscapularly, in the axillae and groins, around the kidneys, the great vessels and about the spine and sternum. Histologically it has a gland-like appearance. It is lobulated and built up of fairly large polygonal cells with a relatively abundant cytoplasm. Functionally it is believed to be the hibernating gland - extracts of it having reduced the metabolic activity of the recipient animals. The subject of brown adipose tissue has recently been reviewed(1). It is clear that while brown fat and white fat have many features in common, they are distinguished by still more. This is in relation to the various metabolic activities of the two tissues, brown fat in general being the more active. It has been thought that brown fat occurs in man, but this is very doubtful. The evidence is based on the similarity in histoligical appearance of embryonic fat or fat depleted human adipose tissue and brown fat. This appearance however in human adipose tissue is temporary, taking on the typical fat cell structure with fat impletion(2). The concensus of opinion is that brown fat does not occur in man. It will not be further discussed here.

CELL ORIGIN OF ADIPOSE TISSUE

For more than a century there has been speculation, controversy and debate on the cell origin of adipose tissue. In 1902 Batty Shaw(3) reviewed the literature. The two broad concepts then commonly held were:

(1) Adipose tissue is a specific tissue or organ.

(2) Adipose tissue is simply fat laid down in connective tissue.

A relative minority of writers tried to wed these two concepts and still others put forward additional views.

/The early studies.....

The early studies on this tissue were entirely histological. The arguments advanced in support of the first concept were based on the observations that large polygonal cells with finely granular cytoplasm were found at sites in embryos or young animals which later develop into fat stores. These cells were shown to go through a stage of fat impletion soon after birth when droplets of fat appeared in these cells giving them a "mulberry-like" appearance. The histological appearance of these cell collections suggested a gland-like structure and the tissue was termed the fat forming organ.

The supporters of the second concept found that they could not confirm that fat was laid down in cells rich in cytoplasm and that the blood supply to the tissue was too poor to maintain an active metabolism. Further, on depletion of fat from the depots, the fat cell was said to revert to an ordinary fibroblast. A view shared by no less a man than Virchow [4].

It is of interest that the histological appearance of the developing adipose tissue cell as described by those holding the first view is identical to that described by Wells[2] almost a century later. The views of these early workers appear to have been found unacceptable by the general body of histologists for all this time. In this regard there is a case in man reported by Strandberg[6], illustrative of the individuality of fat tissue. A full thickness skin and subcutaneous tissue graft was taken from the abdominal wall of a girl aged 12 years to cover a burn on the dorsum of the hand. When the child grew older and "acquired the rotundity of a matron", the grafted area laid down fat in the order of that at the original site "giving rise to a grotesque boxing glove effect". This was thought to indicate the specific property of a specialised tissue, not dependent upon the original innervation or blood supply.

In discussing this point with a plastic surgeon[7] he stated that it is indeed a practice to remove all the subcutaneous fat in performing a skin graft. The reason given, however, was that it better ensures the viability of the grafted tissue. He

/was not able.....

was not able to confirm Strandberg's observations.

Wells[2] points out what should be rather obvious, namely that fat is not laid down in all connective tissue. He cites as examples the eyelids, ears and nose, and membranes like the meninges. He notes that these sites remain fat-free, regardless of the degree of general adiposity. This he feels is strong evidence against the concept of simple fat deposition in connective tissue. He does not neglect to mention special fat sites, such as the sucking pad, to which one may also add the fat pads behind the eyes, which do not always behave as does the rest of the adipose organ. In an admirable review of the subject from the structural, embryological and pathological viewpoints, Wells argues strongly in favour of the tissue behaving as an organ. He points out that the cells from which adipose tissue is derived are developed from the main mesenchymal tissue and can be distinguished from other connective tissue cells. These cells, in their embryological state, have a gland-like structure and assume their typical signet-ring shape after passing through a "mulberry-like" appearance when fat droplets appear in the cells during fat impletion. When depleted of fat they revert to the glandular structure. This latter appearance has apparently often posed diagnostic difficulties on which he has frequently been consulted.

Relationship to the reticulo-endothelial system (R.E.S.)

There is evidence that adipose tissue is related to the reticulo-endothelial system. This concept is in harmony with the gland-like histological structure of the unfatted fat organ as well as with the function it can fulfil along with its usual fat-storing function.

Wasserman[8] demonstrated the affinity of fat tissue to the reticular perivascular mesenchymal cells; a concept that was anticipated by Klein[3] in 1873, who considered that the fat lobules of the mesentery and omentum were transformed perilymphangeal lobules. Bell[9] in 1909 also commented on the resemblance of pre-adipose tissue to a lymph gland.

/The other evidence.....

The other evidence indicating a relationship between adipose tissue and the reticulo-endothelial system is based on certain functional similarities.

Portis[10] showed that the cells of the omentum are able to form antibodies, thus demonstrating a functional relationship. The amount of antibody formed was different in dogs, guinea pigs and rabbits and this was correlated with the occurrence in the adipose organ of aggregates of specific reticulo-endothelial cells. Histologically these cells are considered to closely resemble primitive adipose cells before fat storage sets in[2].

According to Wasserman[8] the blood-forming function may be re-established in depleted adipose tissue under appropriate conditions. This is in analogy with the behaviour of bone marrow which may adopt the function of fat storage in addition to its blood-forming function.

Vital dyes are stored by adipose tissue, just as in the reticulo-endothelium. Bremer[11] using a special technique to demonstrate the protoplasmic layer of fat cells examined the adipose tissue of rats who had been given massive doses of trypan blue. He was then able to demonstrate the presence of dye granules in the protoplasmic layers of the fat cell which were expanded by hypotonic solutions. McCullough[12] showed in the omental fat of rats that trypan blue is included in the fat cells, especially after their depletion of fat. This he regarded as evidence of the macrophagial origin of adipose tissue. Chang[13] showed that the immature adipose tissue cell had the power of phagocytosis. McCullough[12] in 1944 summarised the conclusions from previous work as indicating that adipose arises from:
(1) Primitive mesenchyme; (2) Possibly primitive reticulo-endothelial elements and more particularly from macrophages of loose connective tissue.

Feigenbaum, Byers and Friedman[14] have shown that blocking the reticulo-endothelial system with india ink interfered with the ability of dogs, rats and

/rabbits.....

rabbits to clear plasma after ingestion of cholesterol dissolved in olive oil, which these animals were previously able to do. They attribute this lack to a failure of the liver Kupffer cells to take up the lipid. The high level of total lipid and chylomicra in these animals suggest, however, that the adipose tissue might have also been inactivated by this manoeuvre. This is in view of the above reports and also that adipose tissue plays an important role in clearing lipaemia. (See later).

The present state of our knowledge of adipose tissue based on its morphology indicates that it is a special tissue with a distinct and separate origin and function from connective tissue and with considerable evidence that it may be closely related to the reticulo-endothelial system.

BLOOD SUPPLY OF ADIPOSE TISSUE

The blood supply of adipose tissue formed part of the observations made by earlier workers[3]. There was then considerable controversy as to the abundance of its vasculature. Gersh and Still[15] in an excellent paper presented evidence that should finally settle the problem. They state that "....in embryos, differentiation of sites occupied in adult life by fat consists of a rapid circumscribed proliferation of mesenchymal cells honeycombed with sinusoid-like blood channels and capillaries. The capillary net appears to become coarser and less prominent as fat cells continue to differentiate until, in well-developed fat sites, capillaries seem to be inconspicuous, at least in sections fixed in conventional ways".

In accordance with this seemingly suppressed vascularity and the relatively small cytoplasmic volume of fat tissue, it had been assumed that the metabolic activity of the tissue was low.

These workers used two methods for demonstrating capillaries in adipose tissue.

(1) Rapid fixation of fat and contained blood by freezing and drying the tissue in intact animals. This demonstrated the number and dimensions of the capillaries which then contained blood.

/(2) Intra-.....

(2) Intra-arterial injection of the whole animal with india ink. This was used as a means of determining the total number of capillaries present in the tissue.

With these techniques they were able to demonstrate loose meshes formed by the capillaries running around the fat cells, few cells escaping direct contact with at least one capillary. This vascular structure differs from that in other organs. Comparing lean and fattened animals they showed that the capillary density was greater in lean adipose tissue. Calculating the ratio of capillary surface area to volume of fat (S/V) they found that in well developed fat sites (for total capillary bed) S/V = 51.9, and in poorly developed sites S/V = 222.2. This is at a level found in muscle tissue with a relative poor blood supply.

As they correctly point out, to equate S/V in this manner is misleading since it does not reflect blood supply in relation to protoplasm, which is, after all, the active part of the cell. Recalculating these ratios in relation to protoplasmic volume, S/V in fat-rich tissue was found to be 2159.0 for total capillary surface and 977.6 for the open capillary surface.

They were thus able to show that the blood supply in adipose tissue, in relation to active metabolic tissue, is in fact much richer than is found in most muscles. This is then good evidence that on the basis of blood supply the fat cell has abundant potential for a high degree of metabolic activity.

INNERVATION OF ADIPOSE TISSUE

It had long been taken for granted that adipose tissue did not have a nerve supply. Evidence, both direct and indirect, has since accumulated establishing that there is a nerve supply to adipose tissue and that this exerts a controlling influence on its metabolism.

Direct Evidence

Nordmann[16] in 1925 demonstrated that nerves enter adipose tissue in company with

/with blood.....

blood vessels and he suggested that nervous factors control depot fat metabolism through their effects on the blood vessels. Boeke[17] was able to confirm Nordmann's findings and in addition demonstrated that nerve fibres independent of the blood vessels enter the fat areas and that single nerve fibres appeared to be wound about each fat cell. Boeke claimed that these nerve fibres were derived from the sympathetic nervous system. This latter paper is widely quoted and appears to be the only report to date demonstrating that the fat cell in white adipose tissue is directly supplied with nerve fibres. No ganglia have been described in adipose tissue nor have any sensory end-organs been noted[18].

A direct nerve supply to the fat cell in brown adipose tissue has also been reported[19]; this, too, is thought to be derived from the autonomic nervous system.

Indirect Evidence

The indirect evidence demonstrating that the depot fat is innervated is based on alterations in the metabolic activity of this tissue following on nerve section or stimulation, as well as on the action of both the effector agents of the sympathetic nervous system and the drugs which block the action of these effector agents. This evidence will be reviewed later.

SUMMARY

Evidence is presented to show that the adipose tissue cell is a special type of cell, that it is of mesenchymal origin and probably related to the reticulo-endothelial system. The cellular arrangement in the fat depleted stage suggests that it is a gland. It has a generous blood supply, compatible with a high degree of metabolic activity, and a nerve supply that exerts a controlling influence on this metabolic activity.

/DISTRIBUTION AND SIZE.....

DISTRIBUTION AND SIZE OF THE ADIPOSE ORGAN

Adipose tissue is found in varying amounts in different body sites. The chief deposit of this tissue is subcutaneous. Other sites where body fat is found in substantial amounts is the abdominal mesentery, the omentum and the perirenal and pericardial regions. Relatively small amounts are deposited at other sites. The adipose organ is thus distributed very widely throughout the body and accounts for the vast bulk of the total body fat content. Total body fat analyses therefore closely reflect the adipose tissue as such and these analyses in general are regarded as representative of adipose tissue[20].

A number of different techniques, both direct and indirect, have been used to estimate the size of the adipose organ.

Direct Methods

Direct determinations of total body fat have rarely been done in man[20,21]. This laborious procedure involves a total body dissection and an ultimate fat extraction. While these methods have been invaluable in providing basic data on general body composition in man, they are of little practical value in determining trends in relation to other variables such as age and sex.

Indirect Methods

These methods cannot be as accurate as the direct methods of analysis, but many have proved to be reliable and reproducible and have provided much data on the size and distribution of the adipose organ in man. These indirect methods involve the use of a number of techniques. These are :

(1) Anthropometry, including the measurement of skinfolds by calipers and X-rays;

(2) Body density measurements;

(3) Total body water determinations; and

(4) Fat soluble indicators.

/The whole subject.....

The whole subject was reviewed by Keys and Brozek[20] in 1953 and there has been little added to date. All of these indirect methods have shortcomings, some of which are more serious than others. The technique that appears to be the most accurate is estimation of body density and the conversion of this, using a formula evolved by Rathburn and Pace[22], to the percentage of body weight accounted for by fat.

Body density measurements, total body water determinations and the use of fat soluble indicators are all laboratory procedures and by no means practical for general use. These techniques have at this stage, however, provided much basic data on body fat and there is a surprisingly good correlation between these methods of determination and anthropometry. Of the body measurements that are easily made, the estimation of skinfold thickness by X-ray techniques, or with calipers, has been shown to be a very good indicator of the relative degree of body fatness-leanness. Measurements of skinfold thickness at certain sites, above the nipple, the posterior aspect of the arm and below the scapula, have been shown to give a high degree of correlation with specific gravity studies[23]. This simple procedure therefore provides a ready means of rapidly and accurately assessing the relative degree of body fatness between subjects.

These methods between them have provided data on the size and distribution of the adipose organ in man. These have been shown to vary with both age and sex.

THE EFFECT OF AGE

Fluctuations in the size of the adipose organ with age during adult life have been demonstrated in both men and women. The body fat in adult men has been estimated by the use of total body water determinations[24], specific gravity studies and skinfold measurements[25,26]. These data indicated that there was a progressive increase in the proportion of body fat with age in both men and women during adult life until about the middle of the sixth decade. These findings have not always been consistently reported, others having found evidence indicating a reverse trend[27,28]. Keys and

/Brozek[20].....

Brozek[20] point out that these latter reports were based on subjects who were not representative of the general population, their body weight showing a decline with age; this is apparently not characteristic of the American population, based on standard weight tables. These discrepancies serve however to emphasize the complexity of the problem. While Keys and Brozek are confident that there is a trend for body fat to increase with age, this should strictly be applied to the American population only. The findings of an increase in body fat with age appear to be dependent, at least in part, on an increase in total body weight with age. Whether this latter trend is general in man is not known, since studies have not been done widely enough. Kekwick[29] considers that such a trend must be commonplace, at least in the Western world, but he gives no evidence for this statement. The trends after the age of 60 years are not at all clear since the studies on older age groups are inadequate.

THE EFFECT OF SEX

The proportion of body weight represented by fat has been shown to be higher in the female than in the male. The evidence that this is a true biological variant is more conclusive than the data with respect to variations with age because the sex difference has also been shown in animals. Deuel et al.[30] have shown this to apply to rats maintained on several different fat diets, each diet being kept constant for the sexes. Hurst[31] has shown similar trends in rats. These sex differences have also been shown in guinea pigs[32] and cattle[33].

Differences between men and women, both in general and at the same age level have been studied[34,35,36,26] and there appears to be little doubt that women have a relatively greater content of body fat than do men. Kekwick[29] has recently summarized the available evidence in man on the relationship between body weight and the percentage of body weight accounted for by fat. He has, for the sake of convenience,

/taken a .....

taken a standard 70 kg. man and 60 kg. woman and compared the sexes at various age levels (Table I).

	25 years	40 years	55 years
MALES	14%	22%	25%
FEMALES	26%	32%	38%

TABLE I. Approximate percentage of body weight accounted for by fat in "standard" men and women at increasing age levels. (Taken from Kekwick[29]) It can be seen that women in general have relatively more fat than men and that the proportion of fat increases progressively with age in both men and women during adult life.

Distribution of Fat

The anatomical distribution of depot fat also varies between the sexes. The greater amount of body fat in the female is accounted for largely by an increase in the subcutaneous deposition[34]. Further, the distribution of this subcutaneous layer also differs between the sexes; women show a relatively greater deposit in the extremities and men on the trunk[36].

These sex differences appear to stress the role of hormonal factors in determining the amount and distribution of body fat, over and above factors like diet and calorie intake that would affect total body fat. It is, however, of interest that sex differences in man appear early in life (7½ years)[37], and that ovariectomy has been found in female rats to produce an immediate rise in total body lipids[38]. It is similarly a common impression that oophorectomized women and post-menopausal women become fat; this however has apparently not been objectively assessed. These anomalies serve to indicate that sex differences might be determined by factors other than oestrogen, although it is by no means clear what these other factors might be.

/COMPOSITION OF.....

COMPOSITION OF ADIPOSE TISSUE

Calculations from densitometric measurement by Keys and Brozek[20] have indicated that "obesity" tissue is made up of 62% pure fat, 31% water and 7% cell solids. Their calculations based on body water determinations led them to conclude that "obesity tissue" was made up of 62% pure fat, 24% cells and 14% extracellular fluid. These findings with regard to fat and water content are in very close agreement with direct chemical determinations[39]. The dry matter of adipose tissue consists of about 5% protein and about 90% lipid. The lipid is almost exclusively triglyceride (more than 99%) with traces of cholesterol and phospholipid[39]. Björntorp[40] found that the mean triglyceride content of subcutaneous adipose tissue from 6 normal subjects represent 60.6% of the dry weight with a standard deviation of 19.1%. This is at a lower level than that cited above. He confirmed that the cholesterol and phospholipid components represented only trace amounts (0.4 and 0.4%) respectively. Hirsch et al[41] have also confirmed that the lipid of adipose tissue is almost exclusively triglyceride. The cholesterol fraction representing 0.3% of the total lipid and phospholipid was present only as a trace. Hirsch et al further fractionated the non phospholipid, non sterol moiety of depot fat on silicic acid and found that this was indeed pure triglyceride and did not contain any di- or mono-glycerides.

/METABOLISM OF ADIPOSE TISSUE.....

METABOLISM OF ADIPOSE TISSUE

ACTIVITY OF THE TISSUE

Oxygen Consumption

In vitro studies on adipose tissue have indicated a high degree of metabolic activity. This activity of the tissue however is highly dependent on the nutritional state of the animal prior to tissue sampling. Mirski[42] found that fat depleted adipose tissue obtained from animals fasted for a few days had an oxygen consumption of 0.12 c.m.m./hr./mg. of fresh tissue and a respiratory quotient (R.Q.) of 0.64. When the fasted animals were first refed for 1 day with a high carbohydrate diet the oxygen consumption rose to 0.18 c.m.m./hr./mg. of tissue and the R.Q. reached values in excess of 1.0. Further, the incubation of tissue in serum gave an oxygen uptake twice that found on incubation in buffer solutions. The evaluation of oxygen uptake in relation to tissue weight is very variable, depending upon the relative amount of fat contained therein. When these findings were calculated on the basis of nitrogen content, or on fat-free dry weight, the oxygen uptake of adipose tissue gave values equal to about half that found in the liver[43].

This evidence has clearly established that adipose tissue is a metabolically active tissue and that this activity is of an order found in the more active organs of the body. The enormous metabolic potential of the adipose organ as a whole can be readily appreciated when the size of this tissue, representing 14 - 38% of the total body weight in adults, is compared with, for example, the size of the liver.

Lipid Turnover

Studies[44,45,46,47] using deuterium and radioactive carbon as labels have indicated that in rats and mice half of the adipose organ is mobilized and redeposited in the incredibly short time of 5 - 20 days, depending upon nutritional conditions. Again the enormous size of this tissue must be considered to appreciate the activity of the whole adipose organ.

/Comparing the.....

Comparing the exchange rates of fat in the liver with that in the depots[45,49] it was found that the rate in the liver was 2 - 6 times that in adipose tissue. Shapiro[39] argues that if a large part of the adipose tissue fat were derived from the liver, it would be expected that the ratio of exchange rates in these two tissues would increase in favour of the liver when the isolated tissues are tested. Since this was not so, he concludes that the bulk of newly formed fat deposited in adipose tissue is synthesized in the tissue itself; the total amount of fat formed in adipose tissue exceeds that in the liver in view of the discrepancy in the size of the organs.

Recently more support has been given for the high rate of fat turnover. Fredrickson and Gordon[48] in a review on fat transport point out that plasma free fatty acids++ (F.F.A.) labelled with radio-active carbon is cleared from the blood stream in man at an extremely rapid rate. Half of the injected material is removed in 1 - 3 minutes, representing a clearance of about a quarter of the plasma pool per minute. The plasma F.F.A., probably originating almost entirely from the depots in fasting man, further serve to indicate the phenomenal activity of this tissue.

Kekwick[29] has calculated the theoretical amount of fat being mobilized and redeposited in an average 40 years old 70 kg. man on the basis of the data on turnover rates to be between 0.8 and 1.5 kg./24 hrs. This phenomenal figure is all the more remarkable when one considers that even on a high fat diet the total daily fat intake would rarely exceed 150 gm./day. Fat intake then cannot account for more than 10 - 20% of the total amount of fat deposited and mobilized. It is therefore clear that the animal organism is able to convert nonlipid material into fatty acids at very significant rates.

This seemingly phenomenal rate of turnover has led some to doubt whether the

/tracer studies.....

++ Free fatty acids (F.F.A.), non esterified fatty acids (N.E.F.A.) and unesterified fatty acids (U.F.A.) are terms used synonymously. They all refer to a fatty acid-albumin complex. The term F.F.A. has been adopted for general use. The term "free" has not that literal meaning, but refers to fatty acid free of glycerol.

tracer studies could represent the whole adipose organ. Chalmers et al[49] have suggested that the most recently deposited fat may be the most readily mobilized, a suggestion also put forward by Hirsch et al[41]. If this were so then it could explain what appears to be a level of activity too great to be readily accepted by these groups of workers. The view of Chalmers et al. however is only a suggestion; they were not investigating the turnover rates of fat from adipose tissue. Hirsch et al. base their supposition on very indirect evidence. They found that on feeding corn oil containing linoleic acid, to human volunteers, at 40% of the daily caloric intake, produced only a very gradual alteration over many months in the depot fat linoleic acid content. They suggested that the depot fat may be compartmented, in the metabolic sense, a small proportion showing an active turnover rate and the vast bulk being a rather static store. If this were so then the activity of this small proportion must indeed be phenomenal when considering the data of Mirski[42] on oxygen uptake of the whole tissue. There is probably a simpler explanation for the findings of Hirsch et al. than is suggested by them. Their calculations largely assume that the linoleic acid ingested is simply deposited, largely ignoring the fact that it is again remobilized as F.F.A. at what appears to be a phenomenal rate[48]. The mobilization of a particular fatty acid does not guarantee that the fatty acid will not be degraded, and if so, will again be reassembled to that of the original arrangement and redeposited as such. This is particularly so with regard to linoleic acid, which as far as is known[50], the organ is unable to synthesize. The converse argument to that of Hirsch et al. may be put forward. The fact that a particular fatty acid which the organism is unable to synthesize is not readily accumulated in the adipose tissue even when fed at a high intake serves to confirm that the turnover rate of this whole tissue is indeed great; this fatty acid being no sooner deposited and diluted in the whole depot than it is again mobilized and oxidised but not necessarily resynthesized to the original fatty acid. Evidence has in fact recently been

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published to indicate that linoleic acid is degraded, at least in part, and is rebuilt with available acetate, labelled with C^{14} in human adipose tissue[117].

In summary the bulk of the available evidence indicates that adipose tissue has a high degree of metabolic activity. There have been suggestions that this activity is not confined to the whole tissue but only part of it. The latter are only suppositions based on indirect evidence and which may be differently interpreted. Further speculation would not be profitable without additional evidence. One must at this stage favour the evidence presented by the tracer studies, but nothing that the true position may be more complex.

FATTY ACID SYNTHESIS

It is well known that the ingestion of carbohydrate, if calories are in excess of requirements, will result in the expansion of the depot fat. Evidence has accumulated to show that this fat may be synthesized from carbohydrate by the adipose tissue itself.

It has been shown by Wertheimer[51] that where excess carbohydrate is fed to rats after they have been undernourished or starved, or when forced to ingest excess quantities of carbohydrates, increased fat deposition has been preceded by the deposition of glycogen in the adipose tissue. It was thus concluded that glycogen deposition precedes fat deposition. This is also seen in embryonic tissue where the glycogen content of the fat cell is high prior to fat impletion and falls with the accumulation of fat[52]. It has further been shown that this glycogen is formed in the adipose tissue cell. The incubation of adipose tissue in a medium containing radioactive glucose gave rise to the appearance of labelled glycogen in the cell[53]. The correlation between glycogen and fat in the adipose cell and the respective times of their appearance suggest that glycogen may be the precursor of fat synthesis. Alternately glycogen could be a by-product of fat synthesis from glucose[39].

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It is of interest that adipose tissue glycogen does not behave like liver glycogen since it is not degraded to glucose in vitro[42]. Thus glycogen once trapped in the fat cell would not provide carbohydrate as fuel.

Conclusive evidence for the ability of adipose tissue to synthesize fat was provided by Shapiro and Werkheimer[54] who incubated this tissue in serum enriched with deuterium oxide and were able to recover deuterated fatty acids from the tissue. These findings were subsequently confirmed by the use of C^{14} labelled precursors of fatty acids - acetate, glucose and pyruvate[53,55,56] which were incorporated into adipose tissue fatty acids. It was in addition shown by degradation of the fatty acids synthesized from acetate and glucose that these precursors participated in the complete synthesis of the fatty acid molecule and not merely by the addition of 2 carbon units to existing fatty acids[57]. It has recently been shown that human adipose tissue is able to incorporate C^{14} acetate in vitro[117].

The comparison of the rate at which radioactive glucose is converted to fatty acids as against CO_2 in both liver and adipose tissue indicated that a much greater percentage of this fuel was made available as energy via the adipose tissue than via the liver[56]. It was further shown that on injection of 1-C^{14}-acetate or uniformly labelled glucose, into mice, during a time of refeeding after a period of starvation, that the level of radioactivity in fatty acids was higher, three minutes after injection, in the mesenteric fat, lungs and intestines than it was in the liver. The blood, taken at the same time, was practically devoid of newly formed fatty acids making it unlikely that transport from organ to organ had occurred. Favarger and Gerlach[58] concluded from this that the role of the liver in depot fat synthesis was negligible. This point is emphasized by the earlier experiments of Masoro, Chaikoff and Dauben[59] who showed that the extrahepatic tissue does play the dominant role in fatty acid synthesis. On the injection of C^{14} labelled glucose into hepatectomised rats the fatty acid synthesis from glucose was the same as in normal rats.

/SUMMARY.....

SUMMARY

The adipose tissue has thus clearly been established as the main site of fatty acid synthesis in animals. The fact that fatty acids can be synthesized in the adipose tissue from acetate indicates that any foodstuff which may normally be broken down to acetate provides a source of fatty acid for the adipose organ. The greater rate of conversion of glucose to fatty acid via the depots than to CO_2 via the liver indicates that the adipose organ is directly concerned with a major part of the energy metabolism in the animal organism.

MOBILIZATION AND DEPOSITION OF DEPOT FAT

In the light of what is reviewed above, it is clear that adipose tissue is directly concerned with a major part of the energy metabolism in the animal organism. In 1936, Schoenheimer and Rittenberg(60) showed with their now classical deuterium experiments, that mobilization and deposition of depot fat was a continuous dynamic process. The mechanisms underlying this movement of fat into and out of the fat depots are, however, still incompletely understood. Bloor(61) pointed out that enhanced fat mobilization during inanition proceeds generally in spite of an increased fat content in the blood. It is therefore unlikely that there is a simple homeostatic mechanism by which a low blood fat concentration would stimulate an increased mobilization from the depots. Indeed, if the adipose organ plays an important role in energy metabolism it can be expected that it would be under the influence of a variety of controlling factors, responding to the fluctuating needs of the organism.

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A number of approaches have been used in the study of these factors, producing a considerable body of evidence to show that both humoral and nervous mechanisms are involved in the regulation of adipose tissue metabolism.

HUMORAL REGULATION

Pituitary Gland

A number of pituitary factors or hormones from both the anterior and posterior hypophysis have been implicated in the mobilization of fat from the depots. Best and Campbell[62] in 1936, showed that following on the injection of an anterior pituitary gland extract into normal rats there was a decrease in the depot and an increase in the liver fat. Studies using deuterated fatty acids[63], and labelling newly synthesized fat with deuterium from the body water[64] have confirmed that the increased liver fat in these circumstances is due to an increased mobilization from the depots. The release of depot fat normally provoked by fasting appears also to be dependent upon a pituitary factor, this response having been inhibited in animals by hypophysectomy[65].

Weil and Stetten[66] in 1947 found that the urine of fasted rabbits when precipitated by alcohol and extracted in a manner similar to that used for the extraction of the active pituitary principle gave rise, on injection into mice, to an accumulation of fat in the liver. They named this substance "adipokinin". No direct proof that this factor was derived from the pituitary was provided but similar studies with the urine from fasted man give indirect support for this contention[49]. This latter group of workers[49] elaborated the conditions under which this substance was produced in man, showing it to be dependent on fasting, a low calorie intake,or carbohydrate deprivation. They found that it was also present in the urine of subjects with increased fat catabolism, e.g. diabetic ketosis and carcinomatosis. This substance also had <u>in vitro</u> activity releasing F.F.A. from adipose tissue. No such substance could be provoked to appear in the urine of patients with deficient pituitary activity and thereby implying that a normally functioning pituitary is necessary for the elaboration of this substance.

/The activity.....

The activity of the pituitary fat mobilizing hormone is influenced by the secretions from the adrenal gland since the pituitary hormone was inactive in adrenalectomised animals[65]. This naturally raised the possibility that the fat mobilizing hormone was either adrenocorticotrophic hormone (A.C.T.H.), or an adrenal factor, but neither A.C.T.H. or cortisone alone were active. If, however, the pituitary extract was given together with cortisone fat mobilization was restored in adrenalectomised animals. It did therefore appear that the pituitary fat mobilizing hormone was neither A.C.T.H. nor cortisone, but that either of these made the animal more responsive to the pituitary factor.

The role of A.C.T.H. as a fat mobilizer has recently been reviewed[67]. The evidence indicates that it does have a role in lipid mobilization but that it is unlikely to be the fat mobilizing hormone for reasons similar to those given above.

Lipid Mobilizing Hormone

Cortisone (5 mg./kg.) when injected into dogs, rats and horses, gives rise to a factor which can be separated from the plasma by dialysis. This dialysate, on injection into fasted mice, rats, rabbits, guinea pigs and man, gives rise to a marked elevation of plasma lipids which persists for up to one week following a single dose. This substance has been named the "lipid mobilizer" or L.M. factor[68]. This factor was not elaborated by hypophysectomized animals receiving cortisone. The pituitary was therefore obligatory for its release. This factor was considered not to be growth hormone as it was found to be heat stable, dialysable and had no growth-promoting qualities. It did, however, appear to be indistinguishable in its action from a posterior pituitary factor obtained from hogs[69] and was considered to be either elaborated by or stored in the posterior pituitary. Work in man[70] further indicated that this L.M. factor not only released depot fat but gave rise to a striking increase in plasma cholesterol, lipid phosphorus and cholesterol excretion. This response was in

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addition dependent upon appropriate nutritional conditions, no response occurring on a high fat diet or prefeeding with glucose. The dietary conditions that are necessary for the elaboration of this factor appear to be identical to those giving rise to the appearance of a lipid mobilizing substance in the urine[49,66].

The nature of the pituitary hormone or hormones responsible for promoting lipid mobilization is not clear. A number of pituitary hormones have been shown to have this property. These include A.C.T.H., growth hormone, thyrotrophic hormone and prolactin. The problem in establishing the active principle is great. As has been indicated above with regard to A.C.T.H. it is most likely that pituitary hormonal preparations are by no means pure. Trace amounts of this potent active principle could therefore account for the varying degrees of activity attributed to the various known trophic hormones.

Adrenal glands

Cortisone

The adrenal gland plays a most important role in the regulation of fat mobilization. Cortisone has already been shown to be concerned with the elaboration of pituitary factors promoting depot fat mobilization, acting in a facilitatory capacity. There is also much evidence to prove that the adrenal medullary hormones are even more directly concerned with fat mobilization, and that the action of the latter is facilitated by the former.

Wool and Goldstein[71] treated rats with ethionine. This substance, by interfering with the lipotropic action of methionine, gives rise to an increased fat content in the liver even under normal conditions. By adrenalectomizing the animals, fatty livers were prevented. Injections of cortisone did not however raise the liver fat content to the levels found in the unoperated controls. When adrenaline was added to cortisone in the operated cases the fatty infiltration seen in the controls was reproduced. Adrenaline alone was unable to do this.

/The role.....

The role of adrenaline alone was also investigated. Demedullated rats or rats treated with ergotamine, blocking the action of adrenaline, did not develop fatty livers on being given ethionine. By giving adrenaline alone the fatty infiltration was reproduced in the demedullated animals. Cortisone has therefore again been shown to be inactive by itself, but facilitated the mobilization proceds produced by adrenaline. These findings have recently been confirmed in dogs[72], showing that not only is there a release of F.F.A. from the depots, but that serum cholesterol and phospholipids also rise after 24 hours.

Medullary Hormones

Adrenaline and nor adrenaline have both been shown to play a major role in the mobilization of depot fat. The relationship of adrenaline to cortisone in fat mobilization has already been discussed. Studies with adrenaline in both animals, man and *in vitro* have revealed marked effects on lipid mobilization that may be produced by this hormone alone.

Most of this response has recently been assessed in relation to the increase of free fatty acids (F.F.A.) in the plasma. The source of this F.F.A. in the fasting state is almost certainly from the depot fat[48]. It is therefore fair to assume that the effect of adrenaline on F.F.A. levels in the blood reflects its action on depot fat mobilization.

Among the first to show that plasma F.F.A. in man is increased following the injection of adrenaline were Gordon and Cherkes[73] and Dole[74], both independently. It has also been shown in dogs[72,75] and *in vitro*[76,77,78,79]. Nor adrenaline *in vitro* has a similar role in its effect on adipose tissue[76].

The mechanism by which this hormone acts has also been studied *in vitro*. It has been shown that adrenaline produces a release of F.F.A. primarily by accelerating the breakdown in adipose tissue triglyceride to free fatty acids[79] and with this increased triglyceride breakdown there was a concomitant release of glycerol[80].

/The F.F.A.....

The F.F.A. release by intravenous nor adrenaline is greater and more sustained than that produced by adrenaline in the plasma of man[81]. This difference in response is probably accounted for by the fact that adrenaline will give rise to an increase in blood glucose and the elevation in blood glucose will tend to promote deposition of fat in adipose tissue and thus counteract to some degree the effect of adrenaline.

PANCREATIC HORMONES

Insulin. This hormone, essential in carbohydrate metabolism, also influences the metabolism of adipose tissue.

It had long been recognised that insulin gave rise to an increased fat deposition and this was thought to be due merely to appetite stimulation. Wertheimer[51] in 1945 showed that this was not so, but that insulin promoted synthesis of glycogen in adipose tissue. It will be recalled that glycogen deposition was shown to precede fat deposition in adipose tissue. Wertheimer further showed that the glycogen thus induced to appear in adipose tissue disappeared simultaneously with the cessation of action of insulin on the blood sugar. Stetten and Boxer[82] found that alloxan diabetic rats utilize glucose for fatty acid synthesis at only 5% of the normal rate. After giving such animals insulin their ability to deposit glycogen was fully restored[85]. Further, the increase in liver fat shown to follow administration of anterior pituitary extract was partially inhibited by insulin[84]; this also indicating the enhanced fat deposition, counteracting the fat mobilizing activity of the pituitary factor.

It has been shown in both man[74] and dogs[75] that the increase in plasma F.F.A. with fasting is decreased by giving either glucose or insulin and that glucose alone was ineffective in decreasing the plasma level of F.F.A. in diabetics unless they were simultaneously given insulin[83].

In vitro studies support these findings. The rate of evolution of F.F.A. into the incubating medium, while increased by first fasting the donor animal, or adding

/adrenaline.....

adrenaline to the medium, was decreased by the addition of glucose and insulin to the medium or previously feeding the donor animal carbohydrate[78]. Similarly the uptake of glucose by adipose tissue from the incubating medium is enhanced by the addition of insulin[86].

The role of insulin in the fatty acid balance of adipose tissue would therefore appear to be that of accentuating or promoting the uptake or utilization of glucose. Insulin thus has a well-defined role in the control of adipose tissue metabolism. By acting through its effects on carbohydrate metabolism, it suppresses the release of fatty acids from adipose tissue.

Glucagon has recently also been implicated in the control of fat metabolism. Since adrenaline and glucagon are believed to share a common mechanism in stimulating glycolysis in the liver, Steinberg et al.[87] thought it of interest to determine whether glucagon would mimic the effect of adrenaline on adipose tissue. They found that glucagon given intravenously produced a profound fall in plasma F.F.A. levels during the initial hyperglycaemic phase, but that on return of the blood sugar to normal values there was a marked (200 - 800%) and prolonged rise in F.F.A. levels. It is by no means as yet clear whether this action of glucagon occurs normally, but there is little doubt that it can affect depot fat metabolism. Its mechanism of action would appear to be through its effect on blood sugar levels, but how it promotes F.F.A. release by adipose tissue is not certain.

Thyroid gland. This gland has also been implicated in fat metabolism.

Mackay and Sherril[88] produced direct experimental evidence demonstrating the effect of the thyroid gland on the adipose organ. Three sets of adult rats were fed the same high-calorie, high-fat diet for 310 days, one group acting as control, another were thyroidectomized, and the third were given thyroid extract. At the end of this period the total body weight of the control and thyroidectomized sets of rats was the

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same but the body fat content differed radically. In the control group this represented 31.1% of the body weight, whereas in the thyroidectomized group it was 6.4%. The thyroid-fed group had a fat content representing 14.2% of their body weight, but showed a net decrease in total body weight. There are then clearly gross effects on total body fat content in relation to thyroid activity.

Indirect evidence in man to support the animal work was presented by Plummer[89]. In reviewing the nutritional state of 200 patients with myxoedema it was found that over a third of them were under standard weight for height and the remaining 61.5% were either normal or above normal weight. The body weight in these cases is partly made up by myxoedematous tissue which is shed during the period of negative nitrogen balance when given treatment with thyroid. In the overweight group, the weight then shed was greater than the amount they were estimated to be overweight. Plummer concluded that the myxoedematous subjects were in fact grossly underweight when allowance is made for the myxoedematous tissue.

This is thought to be in keeping with the concept that, both in animals and man, lowering of the metabolic activity of the organism as a whole is associated with the shrinkage of the adipose organ[29]. It is as well to point out however that the converse does not hold, i.e. hyperthyroidism is not associated with an increase in the size of the adipose organ.

Direct evidence that the thyroid has a role in fat metabolism has also recently been published. The low fasting respiratory quotient (R.Q.) and the increased oxygen uptake as well as the predisposition to ketosis in hyperthyroid subjects suggests that mobilization and oxidation of fat are accelerated in these cases. It has been shown that the plasma F.F.A. concentration is significantly increased when the basal metabolic rate (B.M.R.) is elevated in subjects with both spontaneous and induced hyperthyroidism. Euthyroid subjects given L-triiodothyronine show within 6 hours an increased oxygen

/consumption.....

consumption and a rise in plasma F.F.A. levels[90]. Rats made hyperthyroid by giving them L-thyroxine (1 mg./kg./day for 20 - 40 days) showed a significant rise in the nitrogen content of adipose tissue. This was thought to indicate an increase in the active cytoplasm of adipose tissue[91] and thus account for the increased metabolism of this tissue. The values were however referable to wet weight of tissue. The nitrogen content could therefore merely reflect a change in fat content and do not necessarily reflect an absolute increase in the adipose tissue cytoplasm. In vitro work on this tissue showed that on addition of insulin to the incubating medium there was an increase in both glucose uptake and CO_2 production, reflecting an increased turnover, both synthesis and oxidation, by adipose tissue from hyperthyroid rats.

The activity of the thyroid gland influences the metabolism of adipose tissue. Indirect evidence in animals and man indicates that thyroid hypofunction is associated with a decreased adipose tissue metabolism and hyperthyroid activity with an increase. Hyperthyroidism appears to produce an increase in fatty acid synthesis and oxidation by adipose tissue but the mechanism by which this is brought about is not known.

SUMMARY

Pituitary, adrenal, pancreatic and thyroid hormones all have a regulatory function in the metabolism of adipose tissue. The nature of the pituitary hormone/s is not established but they are clearly potent in promoting fatty acid release from adipose tissue. The adrenal cortical secretions appear not to have a direct effect but a permissive action. The role of adrenaline seems to be clearly established as promoting an increase in the rate of conversion of fat (triglyceride) to fatty acid in the adipose tissue cell. Insulin acts by promoting the uptake of glucose with its resultant conversion to fatty acid, thus promoting synthesis of fat. Glucagon has a complex role, initially affecting the level of glucose, with a resultant effect on the depot fatty acid dynamics and later stimulating a release of F.F.A. Thyroid activity appears to correlate directly with adipose tissue turnover, but the mechanism is not known.

/NERVOUS REGULATION.....

NERVOUS REGULATION

The evidence that adipose tissue is directly innervated has been reviewed above. Indirect evidence in support of the view that adipose tissue is under nervous control is plentiful.

Wertheimer[92] showed in 1926 that sectioning of the spinal cord at levels between the first and seventh thoracic vertebrae prevented fatty infiltration of the liver in phlorizinised fasted dogs. Sectioning of the nerves to the liver, or spinal cord sections below the seventh thoracic vertebra, did not prevent fatty infiltration in these animals. He concluded that high cord section prevented fat mobilization from the depots. More direct evidence on the nervous regulation of depot fat was provided later by a number of investigators using comparable techniques. Beznak and Hasch[93] performed unilateral section of those nerves supplying the perirenal fat tissues in cats, rabbits and rats. They found that on a normally balanced, but inadequate diet, so that the animals were losing weight, there was 100 - 300% less fat lost from the sectioned side. In addition they found that the feeding of fat stained with Sudan III resulted normally in equal staining of both perirenal fat bodies. On feeding the stained fat and sacrificing the animal 18 hours later, the denervated side contained about one-quarter of the stained material found on the intact side. They were further able to show that unilateral ganglionectomy at the cervical, lumbar and sacral regions produced similar effects on the pericardial, abdominal and subcutaneous fat depots, these sites corresponding to the respective sympathetic innervation. They concluded that the fat depots were under the control of the sympathetic nervous system. At about the same time Hausberger and Gujot[94] and Kure et al.[95] confirmed these findings in both "brown" (interscapular fat bodies) and white fat.

Kure et al. made several further observations. They found that stimulation of nerve fibres to a fat body by bruising, or the injection of canthardin, resulted in a

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greater fat content in the ipsilateral fat body. They also found that separation of the posterior spinal root, or extirpation of its ganglion, produced the opposite effect to sympathetic denervation. Further, when an animal was brought to a state of under-nutrition, then to one of over-nutrition, the absence of normal innervation resulted in a reduction at that site of both the decrease and the increase in fat seen in the respective nutritional states. They concluded that the autonomic nervous system as a whole maintained a "tone" in the movement of fat into and out of the fat depots. Clement[96] in 1947 using the same experimental technique, confirmed the earlier findings with respect to both "brown" fat (interscapular fat body) and "white" fat (perirenal fat). He too concluded that the sympathetic nervous system influenced fat mobilization.

Several autonomic nervous system blocking agents (Dibenamine, Dibenzyline and ergotamine) have recently been shown to decrease the fatty infiltration of the liver in rats treated with carbon tetrachloride[97], ethanol or ethionine[98]. The administration of hexamethonium (5 mg./kg.), has been shown to reduce the serum F.F.A. level in dogs[99], Similarly epidural procaine instillation, or high spinal section, reduced the serum F.F.A. concentration in dogs[99]; thus confirming of the work of Wertheimer[92] 33 years earlier.

The potent role of adrenaline and nor adrenaline, the effector agents of the sympathetic nervous system, in controlling depot fat metabolism has been reviewed above. Nor adrenaline rather than adrenaline is today considered to be the effector agent of the sympathetic nervous system. The greater overall potency of the former in releasing F.F.A. from adipose tissue further attests to the importance of the sympathetic nervous system in regulating depot fat metabolism.

Psychologically stressful situations have also been shown to produce a rise in serum F.F.A. levels in man[100]. The mechanism of action being interpreted as acting through the sympathetic nervous system and giving rise to the release of adrenaline.

/ SUMMARY.....

SUMMARY

There is clearly much good evidence to indicate that the nervous system, presumably by acting through the sympathetic effector agents, has profound effects on the metabolism of depot fat. It is still to be confirmed whether the parasympathetic nervous system which has been reported as playing a role is indeed active.

UPTAKE AND RELEASE OF FAT BY ADIPOSE TISSUE

The synthesis of fat from carbohydrate and acetate by the adipose tissue has already been discussed. There is also evidence that the adipose tissue is able to take up fat directly and that degradation to 2 carbon units prior to uptake is not a necessary prerequisite. Indeed such an inference may be drawn from the fact that the adipose tissue in some animals, including man, takes on the composition of the dietary fat (see later). If this were not so, then the fatty acid composition of depot fat would be determined by the synthetic processes of which the tissue is capable and the resultant composition would bear no necessary resemblance to that of the dietary fat.

FAT UPTAKE

The incubation of fat depleted adipose tissue, produced by prolonged fasting of the donor animal, in blood or serum, produced a decrease in the fat content of the medium. At the same time there was an increase in the fat content of the tissue. This was demonstrable by both chemical analysis[101] and histologically[39]. The proof that the disappearance of the fat from the medium was the result of the uptake by the tissue was later demonstrated by the use of C^{14}-labelled fatty acids[102]. This uptake appears to be confined to neutral glycerides, free fatty acids and artificial sorbitan esters (Tweens). Phospholipids and cholesterol esters do not yield fatty acid to adipose tissue[101].

Mechanism of Uptake

The mechanism by which fat is taken up by adipose tissue is not fully determined. In vitro studies have indicated that the uptake of free fatty acids (F.F.A.) is achieved more readily than that of triglyceride since the addition of sodium fluoride to the medium prevented the uptake of the latter but not the former. This led to the

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assumption that the ester linkage is attacked during the process of uptake of triglyceride by the adipose tissue cell[39]. It would therefore appear that there may be separate mechanisms involved in the uptake of fatty acid as against triglyceride in the initial stages.

In the uptake of fatty acid by adipose tissue the bulk (60 - 90%) of the assimilated fatty acid in the tissue obtained from refed rats is present in an esterified form. In adipose tissue obtained from starved rats only 20 - 50% is esterified. Further, the total uptake of fatty acid by adipose tissue from refed rats is greater than in adipose tissue obtained from starved rats. It was therefore assumed that the esterification reaction plays a role in fatty acid uptake.

An adenosine-triphosphate (A.T.P.) and coenzyme A (CoA) dependent fatty acid activating system similar to that found in liver[105] and intestinal mucosa[106] has recently been described in adipose tissue[39]. This enzyme system catalyses the reaction:

$$\text{Fatty Acid} + \text{CoA} + \text{ATP} \xrightarrow{Mg.^{++}} \text{Fatty Acid - CoA} + AMP^{+} + \text{P-P}^{++}$$

The fatty acid - CoA complex may now transfer its fatty acid to a glycerol compound to form the ester linkage thus:

Fatty acid - CoA + "glycerol" $\longrightarrow$ glycerol - fatty acid + CoA.

Free glycerol does not appear to serve this purpose since radioglycerol was not esterified in this manner[39]. It has since been suggested[107] that this "active" glycerol may be alpha-glycerophosphate in analogy with the liver esterifying systems[105].

Recently more evidence has been brought forward to support the above concept. It has been shown that the esterification process is dependent on the availability of glucose for the synthesis of glycerolphosphate[86], the latter being necessary in the esterification reaction. This finding, of the importance of available glucose, would

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x AMP = adenosine monophosphate

xx P-P = pyrophosphate

support the earlier work which showed that the proportion of fatty acid esterified in adipose tissue is much greater in the tissue obtained from refed animals than that obtained from starved animals. This mechanism then could explain the means by which fatty acid is taken up by the adipose tissue.

The assimilation of triglyceride is not yet understood. The presence of a lipaemia clearing factor lipase in adipose tissue has been demonstrated[103,104]. The liberation of free fatty acids from triglyceride in the incubating medium by adipose tissue lipase has been shown to be inhibited by alterations in pH or on the addition of sodium pyrophosphate or at high concentrations of sodium chloride. These inhibitory processes call to mind the inhibition of triglyceride assimilation by sodium fluoride. It would therefore appear that this lipase activity could account for the splitting of the ester bond and thus make fatty acid available for assimilation by the adipose tissue cell. It was shown however that the uptake of fatty acid was more pronounced when incubated with tissue from rats refed for 1 - 2 days following a period of starvation than with that obtained from fasted rats[101,102]. The uptake of esterified fatty acid, on the other hand, is optimal in starved tissue[39]. The liberation of lipase by adipose tissue is however greatest in adipose tissue obtained from refed rats[103,104]. This is under conditions when the assimilation of fatty acid, but not fatty acid esters, is greatest.

The splitting of fatty acid esters by the adipose tissue lipase and the subsequent uptake by adipose tissue of the liberated fatty acid, according to the above mechanism, would not seem to be the method by which fatty acid esters are assimilated.

FAT RELEASE

Fat, in being released from the depots, moves from a lipid phase where it is soluble into an aqueous phase where it is insoluble. This physico-chemical problem was well appreciated in attempting to study, in vitro, the mobilization of adipose tissue.

/It was found.....

It was found that the only medium which would allow release of fat from adipose tissue was one containing serum albumin[39]. Further, the fat released was in the form of fatty acid which was attached to albumin. It did also appear that a small amount of triglyceride found in the beta-lipoprotein complex was liberated from the adipose tissue in this form. The possibility that fat may be liberated from adipose tissue as triglyceride is, however, thought very unlikely[107]. The general view of workers in this field is that the adipose tissue releases its lipid exclusively in the form of fatty acid which in the serum is bound to albumin as a fatty acid-albumin (F.F.A.) complex.

The *in vivo* demonstration of this is well established[73,74]. Marked arterio-venous differences in the F.F.A. concentration in both animals and man have been repeatedly shown. In veins draining almost exclusively adipose tissue, e.g. the saphenous vein, this difference is most marked.

Mechanism of Release

The liberation of free fatty acid from the ester in the adipose tissue cell involves the splitting of the glycerol bond. This lipolysis has recently been demonstrated in adipose tissue *in vitro*[86]. It is further thought[107] that this lipolysis is brought about by a lipase present in adipose tissue[108] or by a lipoprotein lipase [103,104]. The possibility of the latter factor being responsible is, on general grounds, not very likely. The fasting state, which is associated with a release of fat from adipose tissue, is not the nutritional condition optimal for release of lipoprotein lipase or lipaemia clearing factor[103,104]. If this lipolysis is brought about by a lipase then it would appear not to be the lipase that is usually demonstrable in adipose tissue. Thus while it is not certain what the nature of this lipase might be, there is little doubt that the form in which fat is released from adipose tissue, both *in vivo* and *in vitro* is as fatty acid.

/SOURCES OF DEPOT FAT.....

SOURCES OF DEPOT FAT

Fats isolated from the animal body contain a complex mixture of lipids. In adipose tissue, true fats or triglycerides, as has already been indicated, form about 99% of the lipid present. Other tissues and organs contain varying, but relatively abundant, amounts of phospholipids, cerebrosides, cholesterol and other unsaponifiable lipids. Only the depot fat will be considered here.

DEPOSITION OF DIETARY FAT

That the nature of the dietary fat is reflected in the depots has been known for some time from the indirect evidence based on animal feeding. Deuel[110] has reviewed the early literature and cites the following work. Lebedev in 1883 exhausted the fat from the depots in dogs by starvation. On feeding linseed oil he found that their body fat had a melting point below zero; feeding mutton fat elevated the melting point to a higher level than that of the normal dog depot fat. Henriques and Hansen in 1901 fed barley to some hogs and corn to others. The latter deposited a fat with a lower melting point and higher iodine value than the former, conforming to the relative degree of unsaturation in the barley and corn fats. This "curiosity", of animal body fat being influenced by the diet, took on economic significance when hogs in the United States were fed surplus peanuts with the resultant production of "soft pork" and oily lard which was unmarketable.

Fatty acids not normally present in the body have also been shown to be deposited in the depots when fed. Winternitz[111] in 1898 fed iodized fats and demonstrated their presence in the depots, and Munk[112] in 1884 isolated erucic acid from the fat of dogs fed rape-seed oil. More recently Barbour[113] demonstrated the deposition and subsequent utilization of dietary "iso-oleic" acid, derived from partial hydrogenation of oleic acid, in the body fat of rats. The more recent evidence based on feeding labelled fats and *in vitro* studies reviewed above directly confirm the early evidence that dietary fat is deposited in the fat depots.

/INFLUENCE OF DIETARY.....

INFLUENCE OF DIETARY FAT ON DEPOT FAT COMPOSITION

There is some controversy about the reasons that account for the divergence in the depot fat composition found in different animal species. Hilditch and Lovern[114] proposed that the differences in body fat composition between species was not adventitious but the result of the altered metabolism related to each species. They considered that every animal species lays down its own typical fat. Shorland[115] is of the opinion that the ingested fat is of greater importance in determining the fatty acid composition rather than the ability of the animal to lay down its own typical fat.

The composition of the depot fat however is dependent on several variables. These depend upon the nature of the dietary fat, the relative abundance of this fat, and the synthetic ability of the organism. Shorland[115] points out that where animals, such as certain fishes, have little ability to synthesize fat from non-fat sources, the depot fat will closely resemble in composition the ingested fat. Certain amphibians with some synthetic ability will deposit a fat which need not resemble that ingested. The composition in this instance will depend upon the degree to which this organism is able to alter the deposited fat and the type of fatty acid it can synthesize from carbohydrates and proteins. In animals like certain ruminants where the dietary constituents are degraded prior to absorption, the depot fat composition will depend on the synthetic ability of these animals and bears no necessary resemblance to dietary fat.

Man and many other mammals enjoy a role similar to that of some amphibians in their ability to determine the fatty acid composition of depot fat. The changes they are able to bring about in fatty acid composition are, however, rather limited. (This will be discussed in a subsequent chapter). On a mixed diet the composition of human depot fat will be determined largely on the balance between the fat derived from dietary sources, the degree to which he can synthesize fat from non fats and the alterations his metabolic processes can produce in the deposited fat. The resemblance of human depot fat to dietary fat is thus potentially very variable.

/CARBOHYDRATE.....

CARBOHYDRATE AS A SOURCE OF DEPOT FAT

The concept that the animal organism is able to convert carbohydrate to fat is by no means new. Deuel[110] cites Lawes and Gilbert, and Meiss and Strohmer who in 1866 and 1883 respectively, presented the first experimental proof for the conversion of carbohydrate to fat. These workers used a balance study technique and the correctness of their conclusions attests to the accuracy of their early methods. They equated the intake of carbon with the output in the form of urine, faeces and CO_2. They allowed for stored carbon, as determined from the nitrogen retained, and the available carbohydrate store was based on carcass analysis. The ingested carbon exceeded that for which they could account either in the excreta (including CO_2) or recoverable as carbohydrate from the carcass. Their conclusion that ingested carbohydrate was converted to fat is certainly confirmed.

Other indirect methods that have been used to demonstrate the conversion of carbohydrate to fat are based on the respiratory quotient[110] (R.Q.) ++ when animals were fed high carbohydrate diets.

The arguments used based on R.Q. determinations are determined by the principle that when there is fat formation from carbohydrate an oxygen-rich substance is being converted into one poor in oxygen. Oxygen is liberated in the conversion, and less in consequence is taken from the outside for general oxidative purposes. The R.Q. may therefore rise above unity. In practice however even if the R.Q. is not greater than unity it does not necessarily mean that there is no conversion of carbohydrate to fat

/taking place.....

++ $\text{R.Q.} = \dfrac{\text{Volume } CO_2 \text{ produced}}{\text{Volume } O_2 \text{ consumed}}$

R.Q. is a concept that stems from the work of Lavoisier in the 18th century. He demonstrated that the production of animal heat was a result of oxidation of carbon in the body, similar to that of a burning candle. Since the relative amount of oxygen and carbon contained in the molecules of the 3 basic foodstuffs differ, the relative volumes of oxygen consumed and of CO_2 produced during the metabolism of each type of food also varies. It has been calculated that the R.Q. of glucose is 1.0. For the combustion of fat, rich in carbon and hydrogen but relatively poor in oxygen, the R.Q. is 0.71. In the same way the R.Q. of protein has been determined to be 0.8 and alcohol 0.67. (Best and Taylor, Physiological basis of Medical Practice; Williams and Wilkins Company, Baltimore 1945).

taking place. Fats and proteins both have an R.Q. below unity. If either of these are being catabolised the total R.Q. for the organism must fall and may go below 1.0.

The direct demonstration based on in vitro work and tracer studies confirming the conversion of carbohydrate to fat has been reviewed above. The evidence that this conversion takes place in the adipose tissue itself has also been presented.

Dietary carbohydrate has clearly been established as a source of depot fat.

PROTEIN AS A SOURCE OF DEPOT FAT

Balance studies similar to those used in demonstrating the conversion of carbohydrate to fat have been used in establishing the conversion of protein to fat. Studies using the concept of respiratory quotient have confirmed the balance studies[110].

More direct evidence for the conversion of protein to fat has been difficult to obtain. The conversion of protein and amino acids to carbohydrates has however often been demonstrated[110]. If one accepts that carbohydrate is converted to fat, the conversion of protein to fat via carbohydrate is readily explained. Deuel[110] has again reviewed the early literature and quotes work where it was shown that dogs, depleted of liver and muscle glycogen by a 10-day fast, layed down glycogen on being fed codfish, which contained only inappreciable amounts of carbohydrate. Since fat did not contribute to the glycogen store it was concluded that protein had been converted to carbohydrate.

The conversion of amino acids to carbohydrate is well known and established in the literature[110]. Amino acids give rise to glycogen. This property however is not general to all amino acids. Leucine, lysine, methionine and tryptophane have been shown to be non-glycogen formers. The end products of hydroxylysine and phenylalanine are apparently also not known. The majority of the amino acids however have been shown to form carbohydrate.

Evidence has recently been published to show that adipose tissue is itself able to form fat directly from isoleucine[116]. Uniformly labelled C^{14}-isoleucine incubated

/with adipose.....

with adipose tissue and liver slices showed that the recovery of C^{14} from fatty acids in adipose tissue was 50 - 100 times greater than that in the liver. The rate of oxidation of isoleucine to CO_2 was also greater in the adipose tissue than in the liver. The finding of more labelled fatty acid in adipose tissue than liver therefore cannot be accounted for merely by accumulation of these fatty acids. It would appear that not only is adipose tissue adept at handling fat and carbohydrate at a rate probably greater than that of the liver but it seems able to convert at least one amino acid to fat. This conversion appears to take place via the intermediates acetate, propionate, methylmelonate - all of which were recovered from adipose tissue in active form.

SUMMARY

The ultimate proof that fat, carbohydrate and protein contribute to the depot fatty acids is contained in the evidence, reviewed above, that the common catabolite of most foodstuffs, acetate, is built up into fatty acid by adipose tissue including human adipose tissue. The source of depot fat is thus potentially almost all foodstuff. There is abundant evidence that the adipose tissue cell takes an active part in the taking up of fat and in the conversion of non-fat material to lipid.

/FACTORS AFFECTING.....

FACTORS AFFECTING THE COMPOSITION OF DEPOT FAT IN MAN

From what is reviewed above it is clear that the fatty acid composition of depot fat will depend upon the balance between a number of variable factors.

It has been shown in man that dietary fatty acids appear in the depot fat. The proportion of these acids will however depend on their relative contribution to the total fat content of the adipose organ. The depot fat is further able to bring about changes in fatty acid composition. These changes include an alteration in chain length and the introduction of a single double bond between carbons 9 and 10 along the fatty acid chain. Unnatural fatty acid e.g. trans isomers, common dietary constituents today, are also reflected in the body fat.

The degree to which depot fat will resemble dietary fat is markedly influenced by the contribution to the fat pool from carbohydrate and protein as well as the net result of the alteration the adipose organ is able to introduce. If these latter factors are considerable then the depot fat composition need have no necessary resemblance to dietary fat. In fact it appears that in man the latter factors are able to maintain a very constant fatty acid composition[41,117]. The net effect of dietary fat composition therefore appears to be of little apparent importance in determining the fatty acid composition of human depot fat, the depot being able to maintain a fairly constant composition.

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CHAPTER 3

MATERIALS AND METHODS

CLINICAL MATERIAL AND CLINICAL METHODS

SUBJECTS

The subjects for this study were drawn from patients attending Groote Schuur hospital. They included apparently healthy males and females in the third and fifth decades of life and were sampled in approximately equal numbers from each of the local racial groups - White, Cape Coloured and Bantu. White males with proven ischaemic heart disease in the fifth decade of life were also sampled.

Problems of Sampling From a Hospital Population

It was aimed in this study to establish values for depot fat composition as found in the local population groups. The subjects however were sampled from a hospital population and it must be made clear at the outset that this hospital population is a selected group. A means test applied at Groote Schuur hospital automatically excludes from attendance the upper income group in the local population. This affects primarily the White section of the community. The White hospital population is therefore not proportionally representative of the resident White population. Further, the White patients that do attend are more representative of the lower income group of White residents than of the general White population. The means test is less likely to affect the proportional representivity of the Cape Coloured and Bantu subjects. Other factors may however be operative in introducing selection in the hospital population in these latter groups. It is therefore not possible to select subjects from this hospital population that will be any more representative of the general population than is the hospital population.

/It was attempted.....

It was attempted to obtain from the hospital population an unselected sample of apparently healthy subjects in each of the racial groups studied. The method of selection and criteria for selection will be elaborated later.

Admission to hospital exposes the subject to environmental factors that are not part of his normal experience. Certain of these might have a bearing on some aspects of this study. An attempt was therefore made to minimise these factors so far as possible. It is not claimed that they were eliminated. The environmental factor of greatest moment is probably of a psychological nature. While there is evidence that the nervous system influences mobilization of depot fat (Chapter 2), there is no evidence that this mobilization is likely to influence its fatty acid composition. From what is known of depot fat, it is the constancy of its composition that is the outstanding feature. Stressful situations are known to alter serum cholesterol concentration. It must be accepted that admission to hospital could have produced such a change. Whether the change thus produced would be different from that provoked by venepuncture outside hospital is still an open question.

The effect of the diet on lipid metabolism is of course profound (Chapter I). The difference in the hospital diet from that normally taken by the subject is an important consideration. The Bantu patients in particular will be eating a diet that is markedly different from their usual. There is thus clearly a possibility that the subjects of this study, on being fed the hospital diet which may differ markedly from that usually eaten by them, could show changes in their serum cholesterol levels. This possibility is most likely in Bantu patients as a group and in individuals among the White and Cape Coloured subjects. Blood was therefore collected from each subject within 24 hours of admission. This meant that they usually did not have more than 2 meals in hospital before blood was sampled. The change in serum cholesterol concentrations that may have been produced by a change in their diet has been minimised as far as possible. That some change may have taken place is, of course, possible.

/Depot fat.....

Depot fat was usually collected on the day of admission or occasionally on the next day. The effect in man of a radical change in the amount and type of fat in the diet on the depot fat fatty acid composition has, since these studies were commenced, been shown to produce a very gradual alteration over many months. It can therefore be concluded that the hospital diet could have had no detectable effect on the fatty acid composition of the depot fat in any of the subjects sampled.

The group of subjects in apparently normal health were drawn from surgical wards, with the exception of a few sampled directly from the out-patient department. Because of the possibility that exposure to surgical procedures, however minor, may affect lipid metabolism, all specimens were sampled prior to the performance of any surgical procedure.

Although the subjects of this study were sampled from a selected group it is of interest that they show many similarities pertinent to certain aspects of lipid metabolism that have been established for the local racial groups. This data will be presented in Chapter 4.

SELECTION OF SUBJECTS

It was aimed to sample a minimum of 10 apparently healthy subjects in each racial group - White, Cape Coloured and Bantu - at the age levels 20 - 29 years, and 40 - 49 years, both males and females. In addition, 12 subjects with proven ischaemic heart disease were also studied.

Method of Selection

The number of patients admitted to this hospital with relatively minor conditions and in apparent good health is not unduly large. There was thus no randomization in the statistical sense of the potentially suitable subjects. There was however no form of deliberate selection from the subjects potentially suitable. The wards to whichpatients potentially suitable may have been admitted were regularly visited on admission days. All of these subjects were then assessed as to suitability

/on the basis.....

on the basis of certain criteria. If they met these criteria, and had no personal objection to the performance of the necessary procedures, they were sampled. Sampling from the various race, age and sex sub-groupings was carried out simultaneously and depended upon the availability of suitable subjects.

Criteria For Selection

Apparently healthy subjects. These subjects were drawn from patients admitted to surgical or gynaecological wards, admitted for the treatment of relatively minor conditions such as varicose veins, hernia, haemorrhoids, uterine curettage, etc. Seven subjects with similar relatively minor complaints were drawn directly from the out-patient department.

The criteria for normal health are never easy to define and sampling from a hospital population would appear to be the last place to expect to find healthy subjects. For the purpose of this study subjects were considered to be in probable normal health if they had no condition in which a metabolic disturbance might be present. In the light of present knowledge it is unlikely that a patient with a relatively simple surgical condition, who is otherwise in good health, has a disturbed metabolism. The possibility that there may be an underlying illness in any apparently minor condition cannot be categorically ruled out. Particular care was therefore taken by means of personal interrogation, scrutiny of the clinical records and discussion with the doctors in charge of the cases to ensure, so far as possible, that no underlying illness was present. Where the full diagnosis was in doubt, the subject was either not sampled or, if sampled, the case was followed up to confirm the simple nature of the final diagnosis.

It was also confirmed that all the females sampled were pre-menopausal. It is not unlikely that a number of the older females were very near the menopause.

/In addition.....

In addition to the points of specific interest relevant to each condition, particular attention was paid to general conditions that may have an effect on fat metabolism. These points were:-

1) Any notable change in dietary habits during the preceding 6 months.
2) Any change in body weight, either gain or loss, of more than a few pounds during the preceding 6 months.
3) Endocrine disorders - where a patient had been referred for investigation or specific treatment of such a condition. Diabetes mellitus was specifically excluded in so far as a history of having the condition and the detection of glycosuria will reveal it. It is possible that a latent endocrine disorder may have been undetected.
4) Ischaemic heart disease and peripheral vascular disease were specifically excluded by personal interrogation and in addition all subjects in the older age group had an electrocardiograph taken to ensure, as far as possible, freedom from this disease.

Any subject with unfavourable features with regard to the above points was not sampled.

Subjects with ischaemic heart disease. These subjects were drawn from about 700 patients who, for a variety of reasons, attend the anticoagulant clinic at Groote Schuur Hospital. The hospital records of males in the 40 - 49 year age group were scrutinized and those with unequivocal evidence, both clinical and electrocardiographic, of ischaemic heart disease, were contacted. The first 12 who agreed to submit to the necessary procedures made up the sample. Ten of these subjects had unequivocal electrocardiographic evidence of having had a myocardial infarction. In two cases there was electrocardiographic evidence of myocardial ischaemia only. All of these 12 subjects have been treated as a homogeneous group on the basis of

/evidence of.....

evidence of ischaemic heart disease. Further, the analytical data on the subjects with only ischaemic electrocardiographic changes falls within the range of values found in those with definite myocardial infarction.

Ischaemic heart disease is used in the sense that the effects of the disease are due to narrowing or occlusion of a coronary artery due to atherosclerosis and/or thrombosis. Conditions such as cardiac valvular disease, polycythaemia, Buerger's disease etc., which may produce similar effects were excluded. Again care was taken to exclude from this group subjects who had recently changed their dietary habits or altered their body weight or in whom there was evidence in the clinical notes of an endocrine or other pertinent disorder.

All of these subjects were taking Dindevan (phenindione) at a dosage that maintained their prothrombin index at an optimal level of between 50 and 60% of normal, but with a possible range of 40 - 70%

The number of subjects in each race and sex and at each age level studied, both those apparently healthy and those with ischaemic heart disease, is shown in Table I. It can be seen that it was not possible to keep strictly to the age groups 20 - 29 years and 40 - 49 years in the Bantu subjects. For the sake of convenience these groups will subsequently be referred to as the 20 - 29 and 40 - 49 year age groups.

/ TABLE I.....

RACE	SEX	n	MEAN	(S.D.)	RANGE	CLINICAL STATE
WHITE	M	12	25.2	(2.3)	22 - 49	APPARENTLY HEALTHY
	M	10	44.4	(3.3)	40 - 49	
	F	10	24.8	(2.6)	23 - 29	
	F	14	45.4	(2.6)	40 - 49	
CAPE COLOURED	M	13	24.2	(2.0)	20 - 28	APPARENTLY HEALTHY
	M	11	45.0	(2.5)	41 - 48	
	F	10	24.4	(3.0)	20 - 29	
	F	12	43.7	(2.9)	40 - 49	
BANTU	M	11	28.1	(3.7)	21 - 31	APPARENTLY HEALTHY
	M	10	44.6	(4.4)	40 - 50	
	F	14	25.6	(3.4)	21 - 30	
	F	10	45.3	(4.5)	40 - 50	
WHITE	M	12	45.3	(1.7)	43 - 49	ISCHAEMIC HEART DISEASE

TABLE I. Showing the mean age in years, one standard deviation, (S.D.), the age range in years, the number (n), and clinical state of the subjects sampled; grouped according to race and sex. Giving a total of 137 apparently healthy subjects and 12 with ischaemic heart disease.

INTERPRETATION OF ELECTROCARDIOGRAMS

All electrocardiograms in both the group with ischaemic heart disease, and those specially done in the older age groups of healthy subjects, were interpreted by the staff of the Cardiac Clinic at Groote Schuur hospital. The criteria for the electrocardiographic diagnosis of myocardial infarction and myocardial ischaemia at this Clinic have been reported by Schrire[1].

/ DIETARY ANALYSIS.....

DIETARY ANALYSIS

A dietary history was obtained from the subjects studied. The details of each subject's diet was obtained by recall. The specific food items, and average quantities of each, eaten on each day during a typical week, were taken as representative of the subject's diet. Due regard was paid to the frequency, amount and types of food consumed by each subject other than at the usual meals of the day. The method of preparation of each dish was enquired into and estimates made of the amount and type of fat or oil added to a particular foodstuff. The intake of each foodstuff was estimated on the basis of average portions, unless there were obvious deviations.

The composition of the diet in each case as regards protein, fat, carbohydrate and calories was calculated from food tables[(2)(3)]. The composition of the dietary fat consumed by each subject was further determined by reference to standard tables[(4)].

It is recognised that accurate quantitation of diet by means of history taking gives at the best a good approximation of the actual intake.

BODY MEASUREMENTS

The relative degree of obesity was determined by measurement of the skinfold thickness. These measurements were made with skinfold calipers of a type that provide a constant tension regardless of the degree to which the jaw of the caliper is opened. The caliper was standardized to give a tension of 10 gm. per square millimetre of jaw surface. The sites selected were the right posterior arm, midway between the acromion and olecranon, and on the back, just below the inferior angle of the right scapula with the arm adducted. A fold of skin and subcutaneous tissue was lightly picked up between thumb and index finger about 1 inch distal to the point of application of the caliper contact surfaces and the thickness of the skinfold was measured to the nearest millimetre on the caliper scale. Care was taken to avoid the inclusion of any muscle in this fold and measurement was delayed until the muscle deep to the selected site was completely relaxed.

/These sites.....

These sites were selected because of the good correlation that has been shown between each of these sites and specific gravity studies in the assessment of the degree of body fatness in man. This subject has been discussed in Chapter I.

BLOOD SAMPLING

Fasting venous blood was withdrawn from a suitable vein in the antecubital fossa within 24 hours of admission to hospital. Fasting blood specimens were also obtained from the ischaemic heart disease group. The 7 subjects sampled from the out-patient department had either had no breakfast, or a breakfast that did not include fat.

The blood was transferred to a test tube, allowed to clot, and separated by centrifugation. It was then stored at -15°C. until analysed.

DEPOT FAT SAMPLING

Depot fat was sampled by an aspiration technique described by Hirsch et al.[5]. All samples were taken from the upper and outer quadrant of the buttock. There is no special reason for selection of this site other than its safety and convenience for both operator and subject, as well as the fact that there is usually a relative abundance of fat deposited at this site.

Method

The selected site is cleansed with spirits and an area of skin ½ - 1" in diameter infiltrated with 2 - 3 ml. of 2% Novocaine. An ordinary No.18 venepuncture needle attached to a 50 ml. syringe containing about 1 ml. of normal saline is passed through the anaesthetised area to a depth of about ½". Care is taken to ensure that the needle point does not penetrate the underlying muscle. This is done by picking up a fold of skin and subcutaneous tissue and gauging the thickness of the subcutaneous layer. With the needle *in situ* the plunger of the syringe is then withdrawn to ensure that a blood vessel has not been pierced, and the normal saline

/injected.....

injected into the subcutaneous tissues. The needle is then vigorously rotated, maintaining the point of the needle at the same depth at which the saline was injected. Fibrous bands are felt and heard to be broken down during this rotatory manoeuvre. While the needle is being rotated, suction is maintained by continual retraction on the syringe plunger. The needle is then withdrawn from the site while maintaining suction on the syringe. It is usual in this way to withdraw about half of the injected saline and with it a few droplets of fat which can be seen by holding the syringe up to the light. (See Fig. 1)

Comments

If this procedure is carefully followed it is most unusual to draw any blood into the syringe even in subjects on anticoagulants. There is occasionally a slight ooze of blood, apparently from the skin, on removal of the needle. This is readily controlled by applying moderate pressure with a cottonwool swab for a few minutes. If any blood was drawn up into the syringe the specimen was not considered suitable since the contained lipids would include blood lipids. Where this did occur the procedure was repeated on either the same or the opposite buttock.

The amount of fat obtained by this technique varies largely with the degree of adiposity. The difference in the size and number of fat droplets obtained from a fat person as compared with a lean person is obvious to the naked eye. At the commencement of these studies, when the chemical analysis performed was the determination of the iodine value of the fat sample, it was the practice to weigh the amount of fat in the sample. The dry weight of fat obtained was found to vary between about 1 and 25 mg.

In order to obtain an adequate sample from very lean subjects a slight modification in technique was employed. In the very lean subject the length of the needle bevel is greater than the thickness of the subcutaneous layer. In a few cases

/where this.....

where this was found, the needle, after being passed through the skin, was angled so that the bevel was turned towards the under surface of the thin subcutaneous layer and the saline injected. The needle bevel was then rubbed against this surface. It was in this way possible to obtain the necessary amount of fat for fatty acid analysis even in the thinnest of subjects.

The technique is simple and appears to be perfectly safe. All hospitalized cases were seen for some days following the procedure and in not a single case was there any complication. There is, however, always the possibility of introducing infection or producing a haematoma as well as causing damage to local structures. If the simple precautions stated above are followed, there is little likelihood of doing any harm.

The only pain produced in the whole procedure is the momentary sting of the local anaesthetic. Rotation of the needle in the anaesthetized area is completely painless and there is no local pain after return of sensation to the area.

Cleaning of syringe

Special care was taken to ensure that all traces of fat were removed from the syringes and needles, since ordinary washing would not be completely effective. This was done by rinsing out the syringe and needle three to four times with large volumes of a mixture of chloroform and methanol (2 : 1 v/v) prior to washing with water and autoclaving. It is important to do this since only small amounts of fat are aspirated and contamination by the previous aspirate might dilute any possible difference in a subsequent sample collected with the same syringe.

/ CHEMICAL METHODS.....

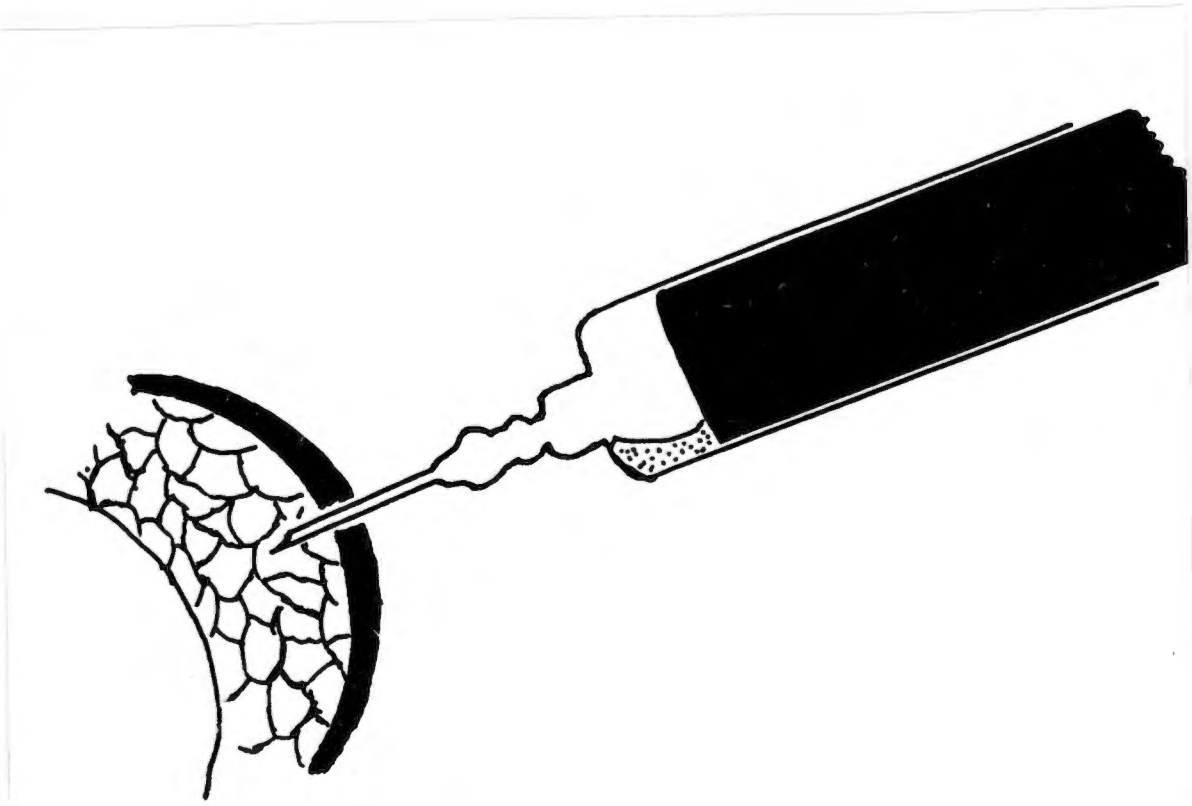


FIG. 1. Showing the needle *in situ* and demonstrating the method of aspiration by this technique.

CHEMICAL METHODS

SERUM CHOLESTEROL

This was determined by the method of Abell et al.[6] All readings were done in duplicate and the value used was the mean of these duplicates which must agree within 5% of each other. The serum cholesterol concentration was expressed in mg./100 ml. (or mg.%). The serum cholesterol concentration of the subjects in this study was determined together with other routine determinations in this laboratory. The reproducibility and standardization of the method is routinely checked.

ANALYSIS OF DEPOT FAT FATTY ACIDS

EXTRACTION OF LIPID

The lipid was extracted by a method recommended by Hirsch[7] based on a principle described by Folch et al.[8] for the isolation and purification of total lipids from animal tissues.

Reagents

1) Chloroform : Methanol 2 : 1 (v/v) reagent grade - redistilled.

2) 0.29% NaCl solution.

Method

The depot fat aspirate in about 1 ml. of saline is transferred to a 20 ml. glass stoppered tube by repeatedly washing out the syringe and needle with small volumes of chloroform : methanol. This solvent system brings the saline and contained lipid into one phase, readily dissolving the suspended lipid globules. The tube is then stoppered and allowed to stand for at least overnight, occasionally inspecting the tube to ensure that there is no phase separation. Phase separation will occur if there is a relative excess of saline. The amount of saline drawn back into the syringe is extremely variable. If there is any phase separation more solvent is

/added.....

added to ensure that everything is in one phase.

The samples were then either stored in this state at 4°C. or further treated and analysed forthwith. Samples may be stored in this condition without evident deterioration in well stoppered containers for several months[9].

Recovery of Lipid

To the mixture of depot fat aspirate, saline and chloroform : methanol is added 5 ml. of 0.29% NaCl. The tube is stoppered and inverted a few times and allowed to stand for half an hour. The added salt solution will result in separation of the phases giving rise to a methanol : water upper phase and a chloroform lower phase. The upper phase will contain the non-lipids and the lower phase the lipids. Protein precipitate or bits of connective tissue often aspirated is usually seen at the interface The upper phase is pipetted off and discarded. The chloroform phase is then filtered through a No.4 sintered glass disc to remove protein precipitate or connective tissue. This is then totally transferred to a 20 ml. quick-fit tube suitable for reflexing. The chloroform is evaporated at 40°C. under water pump vacuum until the remaining lipid is completely solvent-free and dry.

Proof of Recovery By Method Used

In order to demonstrate that the method used is able to give total recovery of lipid the following procedure was carried out.

Glycerol stearate (tristearin), B.D.H., was kept in a dessicator over calcium chloride under vacuum for 24 hours. 14.0 mg. of the dried tristearin were weighed out. To this was added 1 ml. of saline and taken up in chloroform : methanol according to the above method. The lipid was then extracted as above. The lipid in chloroform was transferred in stages to a weighing tube and evaporated under a stream of nitrogen then kept in a dessicator under vacuum over $CaCl_2$ until it attained a constant weight. The lipid recovered weighed 14.1 mg.

/This method.....

This method can therefore be accepted as able to provide a total extraction and transfer of triglyceride lipid.

It might be pointed out that it is not necessary to transfer quantitatively the lipid aspirate dissolved in chloroform. Since it is in a homogeneous solution in chloroform any aliquot that will give an adequate supply of lipid may be taken. It is of no moment to determine the total lipid aspirated. It is only necessary to ensure that all the lipid aspirated has been extracted.

Evidence demonstrating the adequacy of the technique will be presented below.

FATTY ACID ANALYSIS

The depot fat lipid is more than 99% triglyceride[5]. Further fractionation is therefore not necessary. The total lipid aspirate is therefore regarded and will be treated as pure triglyceride.

Prior to fatty acid analysis by gas-liquid chromatography the triglyceride is converted to its constituent methyl esters.

Preparation of methyl esters

A necessary prerequisite to the analysis of lipid mixtures of biological origin by gas-liquid chromatography is the quantitative formation and isolation of the constituent methyl esters[9]. In the original gas-liquid chromatographic separation by James and Martin[10] they noted asymmetry in the eluted peaks. These showed an extended front and sharp tailing of the zones. This they presumed to be due to dimerization in the liquid phase produced by the free acids of the sample. They later[11] used the methyl esters with elimination of this problem.

Principle

This procedure is an interesterification reaction, which involves an interchange between an alkoxy group of an ester and the alkoxy group of an alcohol. This reaction is more specifically referred to as alcoholysis. The depot fat is triglyceride, which is a polyester. This is brought into reaction with methyl alcohol

/and the.....

and the procedure is referred to as methanolysis. Methanolysis can be carried out by heating an excess of the alcohol with the ester in the presence of a catalyst. The catalysts usually employed are the mineral acids, hydrochloric or sulphuric.

The technique used in the preparation of the methyl esters from the depot fat triglycerides was based on a micromethod described by Stoffel et al.[12] This method was chosen in preference to an alternative method of methylation with diazomethane because of certain disadvantages in the latter. Methylation with diazomethane may give poor yields because of pyrazoline formation. Isomerization may occur during saponification and the isolation of soaps from non-saponifiable contaminants is rarely completely satisfactory[12]. It has been verified by ultraviolet and infrared spectrophotometry and by degradation studies, that there is no change in polyenoic acids during methylation with methanol.

Reagents

5% sulphuric acid in methanol which has been dried over $CaCl_2$ and redistilled.
Sodium sulphate - sodium bicarbonate mixture, reagent grade, anhydrous, 4 : 1 (w/w).
Petroleum ether 40° - 60°C. B.P. reagent grade; redistilled.
Benzene, reagent grade, dried over sodium and redistilled.

Apparatus

Round-bottomed 20 ml. test tubes with quick-fit ground-glass joints suitable for refluxing.
Liebig condensers to fit above.
Calcium chloride moisture trap.
Silicone bath.
Conical test tubes.

Method

To the extracted and dried depot fat is added 4 ml. of acidified methanol and 0.5 ml. of dry benzene in the 20 ml. refluxing tube. To this is connected the

/Liebig condenser.....

Liebig condenser fitted with a calcium chloride moisture trap. The mixture is refluxed in a silicone bath, to avoid moisture, for 2 hours at 100° ± 10°C., with frequent shaking for the first half hour to promote solution of the lipid mixture (see Fig. 2).

After refluxing, the tube is lifted out of the bath and allowed to cool to room temperature. Two volumes of distilled water are then added to the refluxed mixture. The methyl esters are extracted by shaking three times with 3 ml. of petroleum ether. The petroleum ether extracts are pipetted off, pooled, and simultaneously neutralized and dried over a knife edge of sodium sulphate-sodium bicarbonate for one hour. The esters are then quantitatively transferred to a conical test tube with petroleum ether and the solvent evaporated at 40°C. in a water bath under a stream of nitrogen. This tube is repeatedly cooled in a beaker of cold water so that the condensed solvent will wash down traces of the sample on the tube wall to the bottom of the tube. The sample is then ready for analysis by gas-liquid chromatography.

COMMENTS

The benzene is added to the refluxing mixture to promote solution of the lipid. If the lipid is incompletely dissolved, interesterification will be slow and possibly incomplete. Solubility of triglyceride in methanol increases, however, with progression of the interesterification reaction[13] and with completion of the process a homogeneous solution is obtained.

Sulphuric acid was used as the catalyst in the methylation procedure on the advice of Lovelock and Lipsky[14]. Stoffel et al.[12] in their description of this method used hydrochloric acid. It is, however, pointed out by the latter authors that anhydrous conditions are mandatory for the successful production of methyl esters. Sulphuric acid has the distinct advantage of being an excellent dehydrating agent, thus ensuring that the methanol in the refluxing mixture is dry. In comparing the results on gas-liquid chromatographic analysis of methyl esters prepared by using hydrochloric acid

/and sulphuric acid.....

and sulphuric acid respectively as catalysts on aliquots of the same sample, very close agreement was obtained, being within the limits of accuracy of the method. Sulphuric acid was however favoured on the above theoretical grounds. Possible oxidation by the use of sulphuric acid was not a factor.

Microsublimation

Stoffel et al.[12] recommended this step following the methylation procedure. Its purpose is to ensure quantitative recovery of methyl esters in pure form, separating them from non-saponifiable material, e.g. cholesterol. This step was not followed as a routine. As has already been pointed out, the content of cholesterol in depot fat is minimal (Chapter 2). Lovelock and Lipsky[14] pointed out that the analysis of fatty acids by gas-liquid chromatography is specific for methyl esters and the traces of cholesterol in depot fat would in any case not be detected. It will be shown later that the proportion of fatty acids in a sublimated aliquot of a single fat sample falls well within those proportions found in unsublimated aliquots of the same sample. This confirms the recommendation that this step may safely be left out.

It might further be pointed out that there is a potential hazard in including the sublimation procedure. The nature of the method does not ensure that methyl esters will sublime in the proportions in which they are present in the total sample if the process is incomplete. This did indeed occur in the early stages of this study while techniques were being developed. It was found that where the process was incomplete, the proportions of fatty acids in the sublimed portion and unsublimed residue differed. The mean of these values agreed with the proportions found in an aliquot of the same sample that was not sublimated. This last observation is not offered as a criticism of the method, but aims to point out a potential pitfall which can be overcome by adequate sublimation.

Since there are no positive virtues in this procedure in the analysis of depot fat fatty acids and since it may in fact have shortcomings, it was not followed.

/FATTY ACID ANALYSIS.....

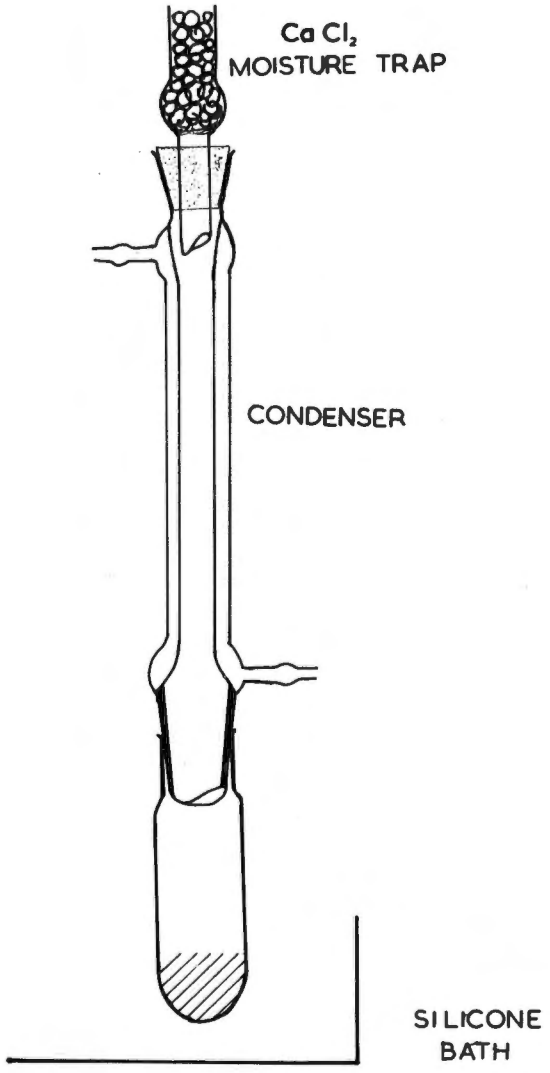


FIG. 2. Showing refluxing apparatus used for methylation of depot fat triglyceride.

FATTY ACID ANALYSIS BY GAS-LIQUID CHROMATOGRAPHY

As this method of analysis is relatively new, its principles and techniques will be described in some detail.

BACKGROUND TO GAS-LIQUID CHROMATOGRAPHY

In 1906 Tswett[15] used a physical method for separating components of plant pigments, involving the elution of these components through a column of solid adsorbing material. As his elutions gave rise to the development of discrete bands of coloured material, he termed the method "chromatography". This term has remained in general use as a collective term for similar methods of separation even when applied to colourless materials.

Keulemans[16] defines chromatography as follows:

> "Chromatography is a physical method of separation, in which the components to be separated are distributed between two phases, one of these phases constituting a stationary bed of large surface area, the other being a fluid that percolates through or along the stationary bed".

All the chromatographic separations involve the transport of a sample of a mixture through a column, or the physical equivalent of a column (e.g. the filter paper strip in paper chromatography). The mixture may be either a liquid or a gas. The column contains the stationary phase, which may consist of either a solid adsorbing agent or a liquid partitioning agent. The transport of the constituents of the sample through the column is effected by the moving phase, a liquid or a gas. Owing to the selective retardation exerted by the stationary phase, the components of the mixture move through the column at different effective rates and thus separate into zones or "bands". The chromatographic procedures are designed to detect, characterize and isolate these bands at some point, usually the exit of the column.

The chromatographic methods are thus founded on the distribution of the components of the sample over two phases and on the subsequent separation of these two phases. Phase separation results from the fact that the fluid phase moves along the stationary phase.

/In 1952.....

In 1952 James and Martin[10] published the basic method of gas-liquid chromatography for the separation and estimation of volatile fatty acids. This has since been refined and developed to a highly sensitive technique with which it is possible to separate and estimate the components of minute amounts of a fatty acid mixture.

THE METHOD OF FATTY ACID ANALYSIS BY GAS LIQUID CHROMATOGRAPHY (G.L.C.)

APPARATUS

The apparatus used in this study was the Pye Argon chromatograph[17] which operates as follows:

The moving gas phase is the inert gas argon, which is supplied by a cylinder of the compressed gas. The gas is fed to a column through a reducing valve, set at a pressure to provide a suitable gas flow rate. The sample is placed on to the column which contains the stationary (liquid) phase. The gas passes through the column into a detector where a signal is generated by each zone or "band" of the sample components. This signal is amplified by an electronic circuit and fed to a recorder. The gas, after leaving the detector, escapes into the atmosphere. (see Fig.3)

OPERATION OF THE INSTRUMENT

There are a number of factors that influence the efficiency of operation of the instrument. It is not possible in practice to prepare two columns that will have identical characteristics for all the variables, although it is usual to achieve close approximations.

Gas Flow

The gas flow rate must be maintained constant for each sample analysis. Where the other conditions remain constant, the gas flow rate is kept constant simply by maintaining the pressure of gas at the same level by means of a reducing valve. In these studies a pressure around 10 lbs. per square inch was usually found to be ideal.

/The most suitable.....

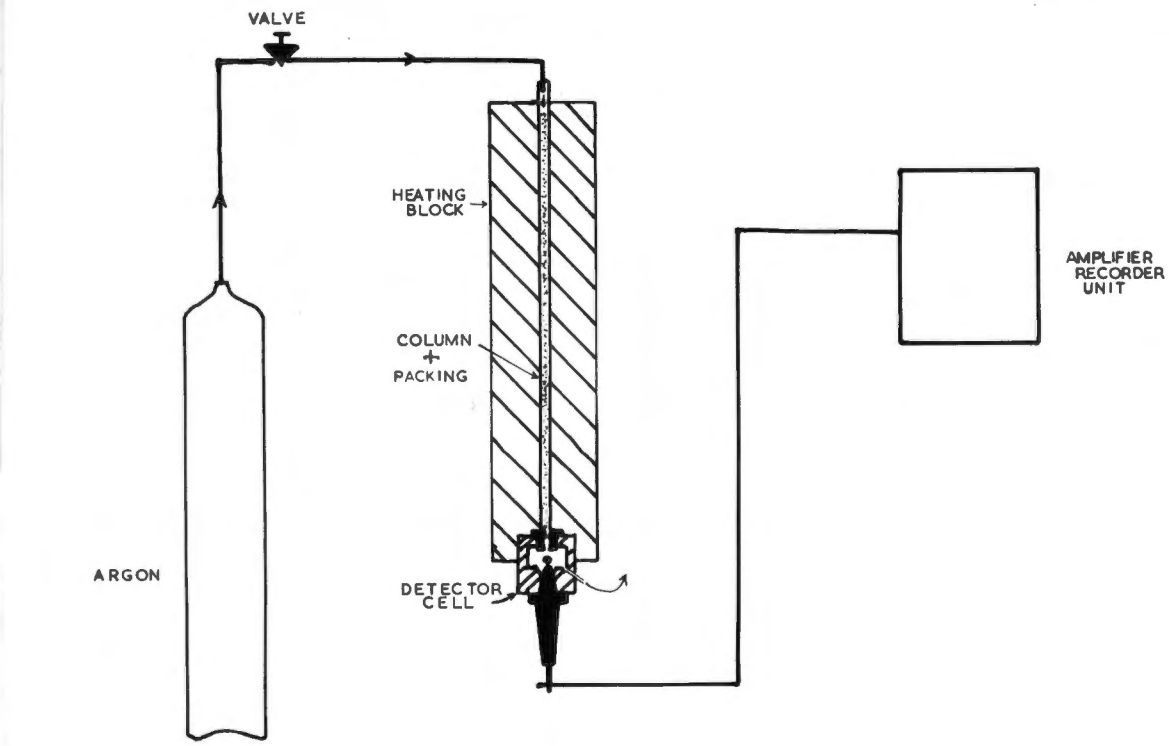


FIG. 3. Schematic diagram of the gas-liquid chromatographic apparatus.

The most suitable rate of flow is determined empirically for each column. There is in practice an ideal flow rate, which under the conditions found suitable for these studies was about 40 - 60 ml. per minute. The most ideal gas flow rate will depend chiefly on the column temperature.

Column temperature

The operating temperature of the column is largely determined by the type of stationary phase used. A temperature on either side of a suitable range will not permit of efficient operation. In general, the higher the temperature, the faster the rate of analysis for a particular column. If the temperature is too high, speed of analysis is gained at the expense of resolution. In addition, the useful life of the column is shortened at excessively high temperatures due to a pronounced increase in the "bleed" of stationary phase.

The temperature at which colums were operated in this study was 185°C. or 197°C. These particular levels were chosen because of reported studies where these temperatures were used (5,9) for the same stationary phase. It was thus possible to make direct comparisons with other studies, which was of help in the identification of unknown peaks. The gas flow rate was balanced against the column temperature to provide separation of components in a reasonable time at the limits of the resolving power of the column.

Preparation of the column

The column used was the standard fitting for the apparatus. It is glass tubing of uniform bore, 4 mm. internal and 6 mm. external diameter, 4 feet long and constricted at one end. The constricted end is tapered and ground to fit a silicone rubber seal inserted in the detector inlet to form a gas-tight joint.

The column is filled with the stationary phase coated on a solid support, slipped into the heating jacket and fitted to the detector. The heating jacket consists of an aluminium bar which is heated electrically and maintained at the set temperature by the

/inclusion.....

inclusion of an adjustable contact thermometer in the heating circuit. The contact thermometer used was not standard equipment, but had the advantage of being adjustable over the whole temperature range.

Solid support

The function of the solid support is to provide a porous bed of even-sized particles which are coated with the stationary phase. A porous support is used in order to obtain an increased surface area per particle. The aim is to use a support that will provide the greatest surface area per unit volume of support at a particle size allowing a convenient rate of flow at a reasonable gas pressure to give good resolution of components.

The support used in these studies was the diatomaceous earth Celite, acid washed and size graded to 80 - 100 mesh[18].

Preparation of stationary phase

The stationary phase used throughout these studies was polyethylene glycol adipate (PEGA). This was prepared according to the method of James[19] and was done as follows.

A mixture of ethylene glycol (32.6 gm.) was heated with adipic acid (73.1 gm.), giving a molar ratio of 1.05/1.0, in a 250 ml. double inlet round-bottomed flask in a silicone bath. When the material had melted, 25 mg. of p-toluenesulphonic acid was added to act as catalyst. The mixture was then brought to 180°C. while passing a stream of nitrogen over the reactants. This polymerization reaction was allowed to continue for 2 hours. The nitrogen stream was then stopped and water-pump vacuum applied for 1 hour at the same temperature.

The excess glycol in the original mixture is used in order to force the reaction to completion. The nitrogen stream prevents the water evolved in the reaction from condensing on the vessel wall. The vacuum that is later applied removes the evolved water and excess glycol.

/The mixture.....

The mixture was then rapidly filtered through filter paper, while hot, and allowed to cool in a wide-mouthed jar, where it solidifies at between 40 - 60°C.

Coating of solid support

The celite is coated with 20% (w/w) of PEGA. The pre-determined amount of PEGA is dissolved in chloroform with warming. When the PEGA is in solution, the proportional amount of celite is added and the chloroform slowly evaporated over a hot plate with constant stirring. The last traces of chloroform are removed on a rotary evaporator. The coated celite can then be stored in a stoppered jar.

Packing of column

The constricted end of the column is plugged with a small quantity of teased-out glass fibre. Uncoated celite is poured into the column to a height of about 1". The coated celite is then poured into the column in small amounts, tapping the end of the column on a clean surface to promote even packing. This is continued until the column is filled to the desired height. The tapping of the column is continued with argon flowing through it and until the upper level of the column remains constant. The upper level of the column must be such that it is below the top of the heating jacket when in situ and yet in reach of the loading pipette. A plug of glass fibre is then placed on top of the coated celite and the column is slipped into the heating jacket and fitted to the detector seat.

Conditioning of the column

The purpose of this step is to remove any remaining traces of chloroform. There is also a variable degree of "bleed" of stationary phase when initially heated, giving temporary base-line instability.

The column heating jacket is brought to the desired temperature and argon flow maintained at about 20 ml./minute. While the column is being conditioned the heating jacket is maintained at a temperature somewhat above that used for analytical runs,

/usually about.....

usually about 200°C. Conditioning is continued until the base-line of the recorder settles down to a constant level. This usually requires 1 - 2 days. The column is then brought to the desired operating temperature (185° or 197°C. in these studies) and is ready for analytical procedures.

LOADING OF SAMPLE

The methyl esters, free of solvent, collected at the bottom of a conical tube are picked up by means of a special micropipette. The pipettes used were of 3 standard sizes, with capacities of 0.025, 0.05 and 0.1 µl. The size of pipette used was generally 0.025 µl. Where however, there was little methyl ester, it was necessary to leave the sample dissolved in a small volume of solvent (petroleum ether B.P. 40° - 60°C.) in order to load the pipette. In these cases the 0.05 or 0.1 µl. pipette was used.

The gas flow through the column is stopped by shutting off the appropriate valve and the pressure in the column allowed to fall. The pipette is loaded and when all argon flow through the column has ceased the gas inlet is disconnected at its junction with the column. The pipette is placed on the top of the column packing and the methyl esters ejected by applying pressure to the rubber tubing attached to the pipette. The gas inlet is immediately replaced and argon flow recommenced at the preset pressure with as little delay as possible. Loading, with practice, can be accomplished in less then 20 seconds.

DETECTION OF COMPONENTS

The methyl ester mixture is carried down the column and separated into its various components according to the physical laws outlined above. As each "band" enters the detector its presence and quantity is recorded.

Principle of detector operation

The sensitivity of the detector in use is the final step in making gas-liquid chromatography as sensitive a method of analysis as it is today. It is able to detect

/2 x 10 -13 moles,.....

2×10^{-13} moles, is insensitive to temperature, pressure and rate of gas flow, and its response is linear with concentration over a considerable range[20].

The detector used is the Argon beta-ray ionisation detector developed by Lovelock[21]. It consists of a small chamber containing a cylindrical radio-active source (radium D in this apparatus) emitting β particles. A high D.C. potential is maintained across the cell giving an ionisation current of 10^{-8} amperes when pure Argon is flowing. The principle of operation of this instrument is described by Lovelock[21] as follows:

> "The absorption of ionising radiation by gases produces ions and excited but unionised (metastable) atoms and molecules. With most gases the lifetime of the metastable atoms is short, c.a. 10^{-9} seconds, and consequently under constant conditions of irradiation the ions, which have a comparatively long lifetime, greatly outnumber the excited but unionised atoms. The rare gases are unusual in their possession of long-lived metastable atoms, and in these gases the concentration of the metastable atoms approaches that of ions during steady irradiation. In the pure gas the metastable atoms eventually decay to the normal state with the emission of radiation. In the presence of small traces of other gases the metastable rare atoms can, during their lifetime, transfer their energy of excitation by collision. If the ionisation potential of the gas molecules is less than the excitation potential of the rare gas atoms, the transfer of energy on collision leads to the ionisation of the added gas. The molecules of most organic compounds have ionisation potentials lower than the excitation potential of helium, neon and argon, and are ionised on collision with the metastable atoms of these noble gases".

It has been shown that with substances of molecular weights greater than 100, the mass sensitivity is constant within the limits of precision of chromatographic analysis and the peak areas of the chromatogram are directly proportional to the mass of substance separated[21]. It is thus possible, by calculating the areas of peaks, to determine the relative proportion of the separated components of a mixture. Substances with molecular weight less than 100 have been shown to produce a response that may not be proportional to mass[22].

SENSITIVITY ADJUSTMENTS ON THE APPARATUS

Detector voltage

Lovelock et al.[20] have shown that increasing the electrical potential applied across the detector produces an increase in the concentration of metastable ions when

/one of the.....

one of the rare gases, e.g. argon, is flowing. An increase in applied voltage from 400 - 1600 increases the concentration of metastable atoms almost 10^4 times. This is one method of varying the sensitivity. There are, however, limits to the useful sensitivity of operation and for these studies the voltage applied across the detector was most usually the 1000 V position on the amplifier recorder unit panel of the instrument. Occasionally the 1250 volt position was used. This is a perfectly legitimate procedure and in no way invalidates comparisons between graphs as the peak response remains linear to concentration of components.

Sensitivity switch

This is built into the apparatus and provides a ready means of adjusting the sensitivity during a run in the ratio 1 : 3 : 10, corresponding to positions "X10", "X3" and"X1" respectively on the instrument panel.

In view of the nature of the fatty acid pattern of depot fat it was found that the most suitable method of operating the sensitivity switch during a run was at the least sensitive ("X10") position. This sensitivity was maintained until C18 : 2 had emerged when the sensitivity was switched to the "X3" position. This manoeuvre does not alter the width of the peak but will increase the height threefold relative to the "X10" position. This is allowed for in calculating the areas by dividing the area of those peaks recorded at "X3" sensitivity by 3. The purpose of altering sensitivity is to facilitate calculations of the areas of the minor components by increasing the height of their peaks. The later the peak appears, the wider the base of the peak. Thus for any given area the height of the peak will be lower, the later it emerges.

Amplifier-recorder unit

The small current flowing across the detector is balanced at the electrical zero of the recorder. The increase in current produced by the appearance of "bands" of components eluted from the column produces an increase in this current. This current

/is electronically.....

is electronically amplified and fed to a potentio-metric recorder which responds in a linear fashion, proportional to the increase in current.

A permanent record of this is produced by the drawing of a graph on a roll of calibrated paper moving at a constant speed. The speed of the chart is adjusted to produce peaks sufficiently widely spaced to show clear separation of components with the peak widths of suitable dimensions. The chart speed found suitable for these studies under the conditions used, was 30" or 45" per hour.

CALIBRATION OF INSTRUMENT

This instrument has the mechanisms of linearity of response built into it according to the principles of the detector operation and amplifier-recorder unit response. The ionization current recorded on the chart is directly proportional to the amount of fatty acid methyl ester applied to and eluted from the column. In practice it is therefore only necessary to determine whether a particular apparatus responds in this manner.

Weighed amounts of pure methyl ester "standards" were made up in known proportion by weight and these proportions are compared with those calculated from the chromatographic recording. Table 2 shows such a comparison, using proportions that give marked variation in peak heights. It can be seen that agreement between the theoretical and chromatographic proportions is very close and within acceptable limits of experimental error.

FATTY ACID	WEIGHT (%)	AREA (%)
12 : 0	5.0	4.7
14 : 0	10.4	10.0
16 : 0	13.3	13.9
18 : 0	27.4	27.9
18 : 1	14.3	14.5
18 : 2	2.5	2.2
18 : 3	27.1	26.7

TABLE 2. The percentage of fatty acid methyl esters in a "standard" mixture by weight compared with the peak areas of each component as found on analysis by gas-liquid chromatography.

The response of this apparatus can be accepted as being linear to the concentration, by weight, of methyl esters.

/METHOD OF CALCULATING.....

METHOD OF CALCULATING THE PROPORTION OF FATTY ACID METHYL ESTERS IN A SAMPLE

The type of recording system used draws a differential curve and the areas of the peaks represent the relative proportions of the separated sample components. It remains then to measure the areas of all the peaks, to total the areas, and to calculate the percentage of the total represented by each peak.

The methods available for determining areas under the peaks of chromatographic tracings are by planimetry, cutting out the curves and weighing the paper, triangulation, or automatic integration. The last of these involves special equipment which was not available. The simplest method is triangulation and was the method used in these studies. It is the method commonly used by workers in this field[5,9,11]. The area under the peak is represented by the product of the height of the peak and the width at half the height. Before heights of peaks are measured the base line of the entire chromatogram is drawn in. This is ideally a straight line but there may be smooth curves which are taken into account in drawing the base line.

FACTORS AFFECTING THE SEPARATION OF LONG-CHAIN SATURATED AND UNSATURATED FATTY ACIDS

> "The relative position of the zones on a gas-liquid chromatogram is controlled by the differences in partial vapour pressure of the substances (solutes) when dissolved in the column stationary phase. The nature and magnitude of the cohesive forces between solute and solvent molecules defines the partial vapour pressure of the solute at constant temperature". (26)

This statement outlines the general principles determining the separation factor for components of mixtures by gas-liquid chromatography. The cohesive forces between solute and solvent decreases with a decrease in molecular weight. This holds for the saturated acids. The behaviour of the unsaturated acids will depend on the nature of the stationary phase, and this depends on whether there is or is not an interaction between the unsaturated fatty acid esters and the stationary phase.

/Where non-polar.....

Where non-polar stationary phases are used, e.g. the saturated paraffin hydrocarbon greases, such as Apiezon, there is no interaction between unsaturated acids and stationary phase. The unsaturated acid, being of lower molecular weight than its saturated homologue, will therefore emerge before the latter. The greater the degree of unsaturation the earlier the time of emergence relative to the saturated acid with the same number of carbon atoms.

The polar stationary phases show different characteristics. Here there is a specific intermolecular attraction between the polarizable double bonds of the unsaturated acids and the ester groups of the stationary phase. The unsaturated fatty acids will therefore emerge after the saturated acid with the same number of carbon atoms. Further, the more saturated the fatty acid, the greater the degree of interaction between solute and solvent and consequently the longer the time of emergence. Polar stationary phases allow good separation between acids of the same number of carbon atoms but with an increasing degree of unsaturation[23,24,25]. Polar stationary phases have the advantage of giving better separation between the 18 carbon atom fatty acids, particularly between linoleic and linolenic acid. The resolving power of the non-polar phases for these two acids is poor. The non-polar phases, like Apiezon, however, can separate isomers of oleic acid which cannot be achieved on polar phases[23]. James[23] has described a method by means of which it is possible to get the best from both types of separation. Basically this involves an analysis of each sample on both columns.

The stationary phase used throughout these studies was polyethylene glycol adipate (PEGA). This is a polar stationary phase and has the characteristics of polar phases as described above. It has the disadvantage of being unable to resolve the isomers of oleic acid; oleic acid and its possible isomers thus appear as a single symmetrical peak. While it has the above disadvantage it provides in general a better analysis, certainly for comparative studies, because of the excellent resolution between the 18 carbon fatty

/acid series.....

acid series. Most interest at present seems to be in the essential fatty acid, linoleic acid, and this acid is clearly separated on this stationary phase.

Methods Used In The Identification Of Peaks

Plotting log retention volume, or retention volume relative to a standard substance, against the number of carbon atoms in the molecule for members of a homologous series produces a straight line[10]. The retention volume is calculated by multiplying the flow rate by the time taken for the component to emerge. Time of emergence is calculated from the commencement of the time of loading the sample to the centre of the relevant peak. Where the gas flow is interrupted in order to load the sample on the column, as in the apparatus used here, the retention time is measured from the centre of the air "dip" on the graph. This is known as the adjusted retention volume. All retention volumes on this type of apparatus are calculated from this position[10].

It may happen that this position is not clearly shown on the graph. Peterson and Hirsch[27] have evolved a calculation for locating this point which is dependent upon the relative retention volumes of familiar components.

Since the chart speed is constant, distance is directly proportional to time. As the gas flow rate is constant, one merely measures the distance from the air dip, or its calculated point, to the centre of the relevant peak, giving a value directly proportional to adjusted retention volume. By plotting log distance, or log distance relative to a standard substance against the number of carbon atoms in the molecule, peaks are identified. The log retention volumes of the unsaturated acids if so plotted do not fall on the same straight line as do the saturated acids of a homologous series. The unsaturated acids can, however, be identified by applying the same principle. All the mono-unsaturated acids of a homologous series fall on a straight line if plotted against log retention volume. Similarly with the di, tri, tetra, etc. unsaturated acids. In practice, identification of unsaturated components is made by comparing the retention volume of the

/unknonw

unknown unsaturated acid relative to the retention volume of an identified component against the relative retention volumes of known components in a standard fatty acid mixture.

e.g. if $\frac{\text{Retention volume A}}{\text{Retention volume B}} = \frac{\text{Retention volume C}}{\text{Retention volume D}}$

and if B, C and D are known, A is the same as C. Similar comparisons with published tables of chromatographic separations on the same stationary phase, operated at the same temperature, will also be helpful in the identification of unknown components.

Most valuable methods in identifying unsaturated components are to either brominate or hydrogenate the sample of natural methyl esters[9]. During bromination, bromine becomes bound at the double bonds of the unsaturated fatty acid esters. This increases their molecular weight and they become virtually non-volatile[11]. The chromatogram of the brominated sample will then show only the saturated components of the original mixture. Hydrogenation will convert the unsaturated fatty acid esters to their respective saturated compounds. By comparing chromatograms of such mixtures before and after hydrogenation it is possible to deduce which components of the original mixture were unsaturated. Moreover, the chain length of the unsaturated acids can then be conclusively established[9].

Branched chain saturated fatty acids are in principle identified in the same manner as the straight chain acids. In general, branched chain saturated acids have been found to have smaller retention volumes than their saturated straight chain homologues[9,23]. The positive identification of many branched chain acids by gas-liquid chromatography is still to be achieved. One can imagine the potential difficulties in this regard when considering the combination of forces that might be operative in the chromatographic separation of unsaturated branched chain fatty acids.

EFFICIENCY OF THE COLUMN UNDER SPECIFIC OPERATING CONDITIONS

The efficiency of chromatographic separation is determined on the basis of the number of theoretical plates in the column. This is calculated according to the

/formula[9].....

formula[9]

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

where n = number of theoretical plates.

tR = retention time (or distance).

W = base width of component in the same units as tR.

In deciding on the best operating conditions for a particular column, adjustments can be made to the gas flow rate and temperature of operation to give the highest possible number of theoretical plates. It was usual in the column prepared for these studies to obtain theoretical plates of 2000 - 2400, and occasionally higher, calculated at methyl stearate (C18 : 0). Theoretical plate value may be calculated at any peak, but a suitable peak somewhere in the middle of a run is recommended. These columns were all able to give adequate resolution of components for accurate calculation of component peak areas. Fig. 4 illustrates the method of determining the values tR and W, and in this average column n = 2366.

Indications for changing column

All columns age with time and usage. The basis used in these studies for judging when the useful life of a column was nearing its end was on the degree of resolution between methyl stearate (C18 : 0) and oleate (C18 : 1). When the commencement of the oleate peak took off at half the height of the stearate peak, the column was considered to have served its limit of satisfactory operation and was changed.

/APPLICATION OF THE.....

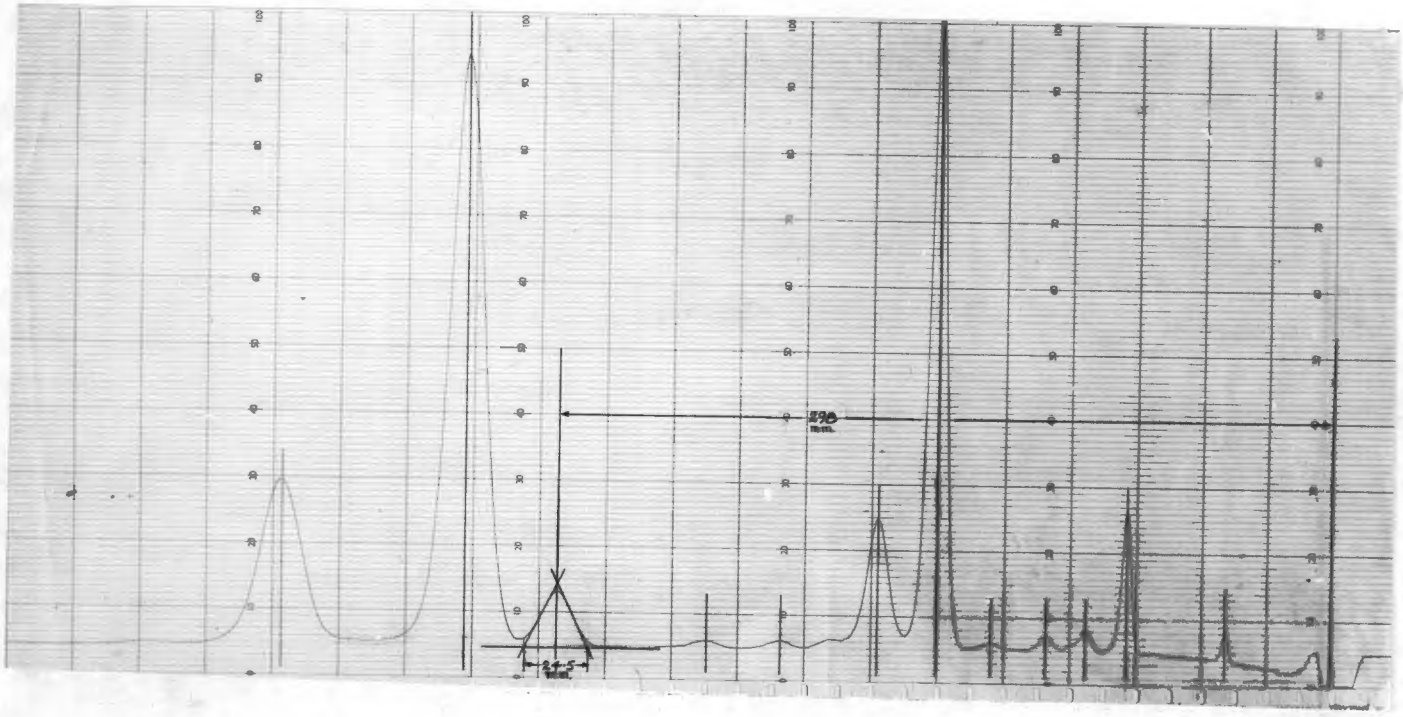
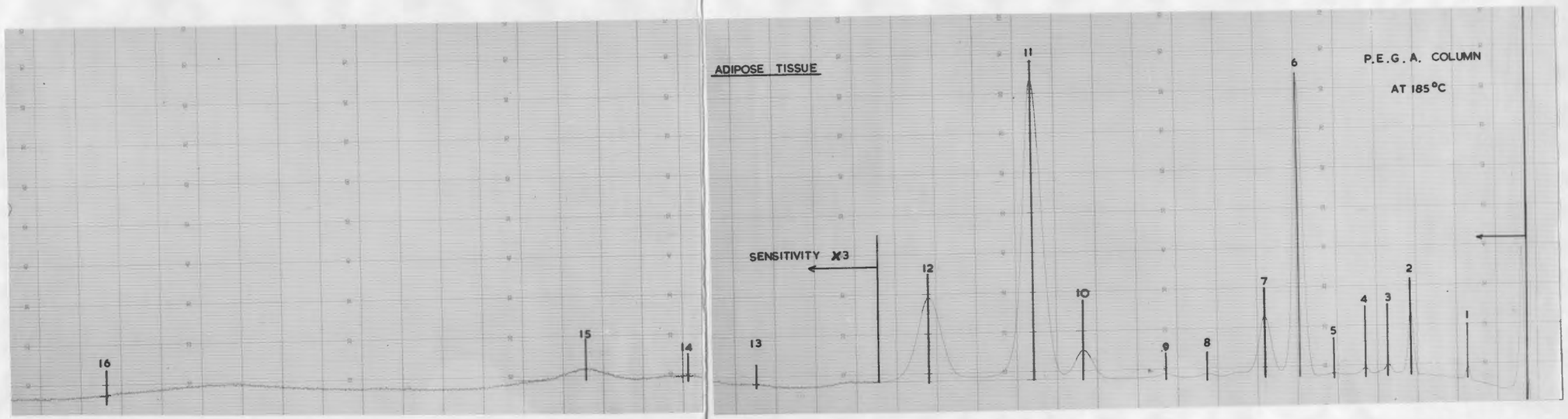


FIG. 4. Chromatogram of a sample of adipose tissue showing the measurements made in calculating the theoretical plate value in assessing the efficiency of a column. Note that the peak widths become progressively broader the later they emerge.

APPLICATION OF THE ABOVE METHODS IN THE IDENTIFICATION OF THE FATTY ACIDS IN HUMAN ADIPOSE TISSUE

A typical chromatogram of human adipose tissue fatty acid methyl esters is shown in Fig. 5. The distance from the air "dip" to the centre of all the peaks is then measured (Table 3). A "standard" fatty acid ester mixture was prepared and chromatographed in the same column at the same temperature (Fig.6). In the latter case the components were identified on the basis of the quantitative amounts known to be present, being guided in addition by the knowledge that retention time will increase with the chain length of the saturated components. Further, since a polar stationary phase was used, the unsaturated acids emerge later than the saturated acids with the same number of carbon atoms. Also, increasing unsaturation causes a progressive increase in retention time. Table 3 shows the distance at which each peak of the standard fatty acid mixture emerged. The retention times of the various components relative to C18:0 in the standard mixture are shown in Table 3. These can be seen to give the same indices as in the adipose tissue sample when peak 10 (Fig. 5) is taken as being C18:0 (Table 3). In this way, by comparing both the absolute and relative retention times of unknown peaks in the adipose tissue sample with the known fatty acids in the standard sample, peaks 1, 2, 6, 10, 11, 12 and 13 have been identified as fatty acids C12 : 0, C14 : 0, C16 : 0, C18 : 0, C18 : 1, C18 : 2 and C18 : 3 respectively.

/ TABLE 3.....



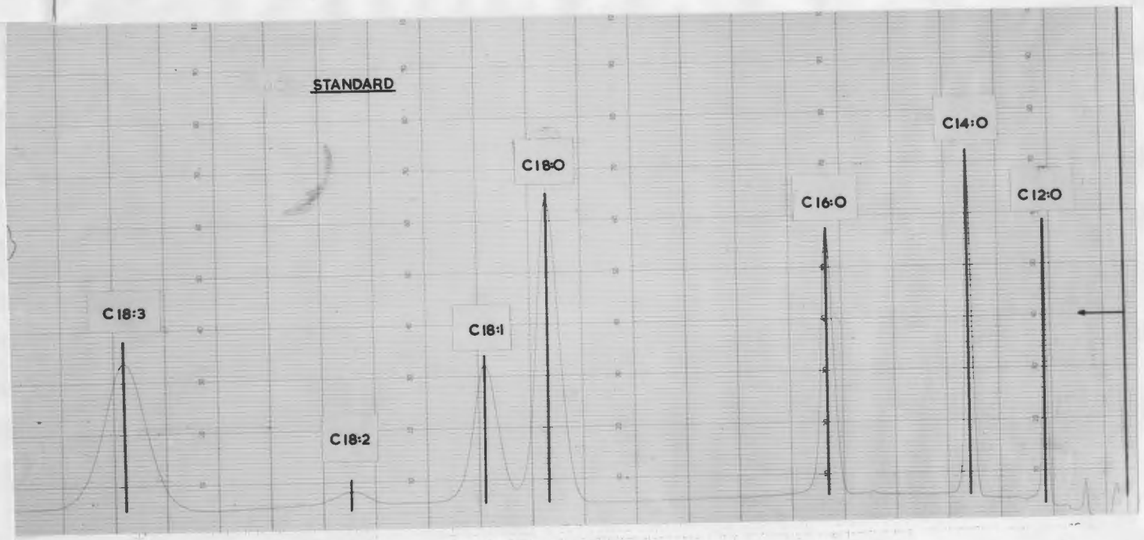


FIG. 5. Adipose Tissue. A typical chromatogram of human adipose tissue showing all the usual peaks appearing during analysis on a P.E.G.A. column.

FIG. 6. Standard. A mixture of methyl esters made up of pure methyl ester standards in known proportion by weight.
Note low peaks in the adipose tissue sample coincide in time of emergence with peaks in the standard mixture.

STANDARD FATTY ACID MIXTURE			ADIPOSE TISSUE FATTY ACIDS		
FATTY ACID	d	d/C18 : 0	Peak	d	D/Peak 10
C12 : 0	38	0.136	1	38	0.135
C14 : 0	75	0.268	2	74	0.263
			3	88	0.313
			4	104	0.370
			5	123	0.438
C16 : 0	144	0.514	6	146	0.520
			7	166	0.591
			8	201	0.715
			9	229	0.815
C18 : 0	280	1.00	10	281	1.00
C18 : 1	311	1.11	11	312	1.11
C18 : 2	377	1.35	12	378	1.35
C18 : 3	486	1.74	13	487	1.73
			14	528	1.88
			15	587	2.09
			16	891	3.17

TABLE 3. Showing the retention time, measured as distance (d) in m.m. and the retention time relative to C18 : 0 (d/C18 : 0) in a standard fatty acid mixture compared with the distance of the peaks in a sample of adipose tissue analysed on the same column at the same temperature. Note how closely both the total distance and the distance relative to C18 : 0 of peaks 1, 2, 6, 10, 11, 12 and 13 in the adipose tissue sample agree with the fatty acids C12 : 0, C14 : 0, C16 : 0, C18 : 0, C18 : 1, C18 : 2 and C18 : 3 respectively in the "standard" mixture.

Plotting the log distance of the peaks in the adipose tissue chromatogram it is found that the saturated acids C12 : 0, C14 : 0, C16 : 0 and C18 : 0 corresponding to peaks 1, 2, 6 and 10 fall on a straight line. In addition peaks 4, 8 and 14 also fall on the same straight line. These correspond to the fatty acids C15 : 0, C17 : 0 and C20 : 0 respectively (Fig.7). Peak 11 in the adipose tissue sample is seen to have the same absolute and relative retention characteristics as C18 : 1 in the standard sample (Table 3). By plotting the log distance of peak 11 it is found that the peaks 3, 7 and 15

/fall onto.....

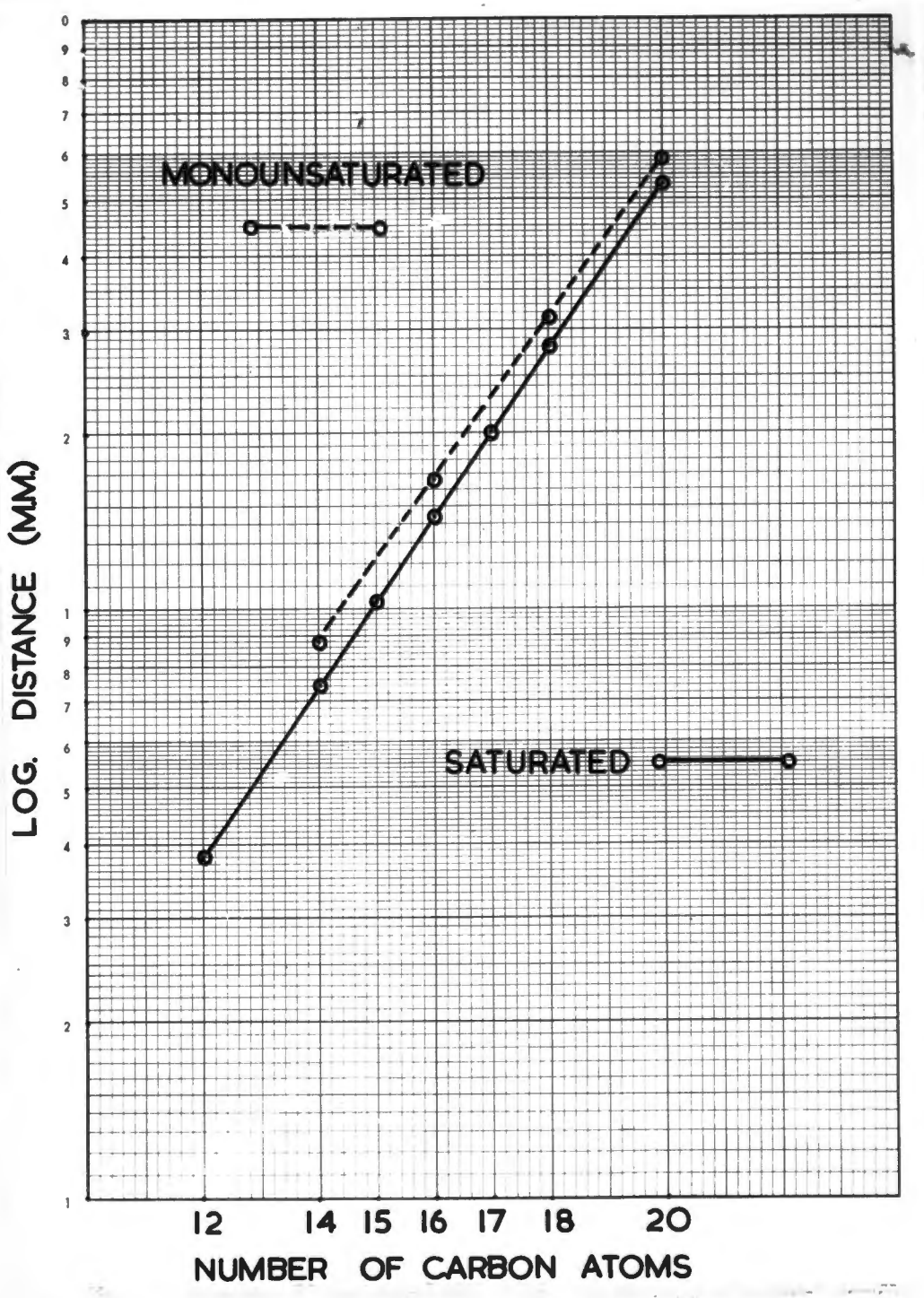


FIG. 7. Plotting the log distance of the centre of each peak against the number of carbon atoms in the chromatogram of adipose tissue shown in Fig. 5.
Note that the saturated fatty acids fall on to a straight line; the monounsaturated fatty acids fall on to a separate straight line.

fall onto this same straight line. The peaks 3, 7, 11 and 15 therefore represent the monounsaturated acids C14 : 1, 16 : 1, 18 : 1 and 20 : 1 respectively (Fig. 7). There is no peak in the adipose tissue sample at position 16 (Fig.5). It is however the calculated position for C20 : 4, based on its retention time relative to C18 : 0 in another standard fatty acid mixture (Fig.8 Table 4).

The peaks thus identified, on the basis of retention time, relative to C18 : 0, correspond with the relative retention times found by Farquhar et al.[9] using the same stationary phase at the same temperature as in this analysis. Peaks 5 and 9 constituting a very small percentage of the total fatty acids have not been positively identified. Hirsch et al.[5] appear to consider them to be branched chain fatty acids with 15 and 17 carbon atoms respectively. This is not acceptable because, as will be remembered from above, branched chain fatty acid esters as a class emerge before the straight chain saturated acid esters with the same number of carbon atoms. They have been identified as C15 : 1 and C17 : 1[28]. Reference to Table 3 and Fig.7 shows that both of these peaks do in fact fall onto the monounsaturated fatty acid line. While peak 9 (Fig.5, Table 3) probably is C17 : 1, peak 5 is not an unsaturated acid. This can be seen from Fig.10 where normal adipose tissue fatty acid esters have been brominated and hydrogenated. It can be seen that peak 9 has disappeared in both cases but not peak 5. Both of these peaks however are very minor components, constituting less than 1% of the total fatty acids and, quantitatively, are not important.

/To confirm.....

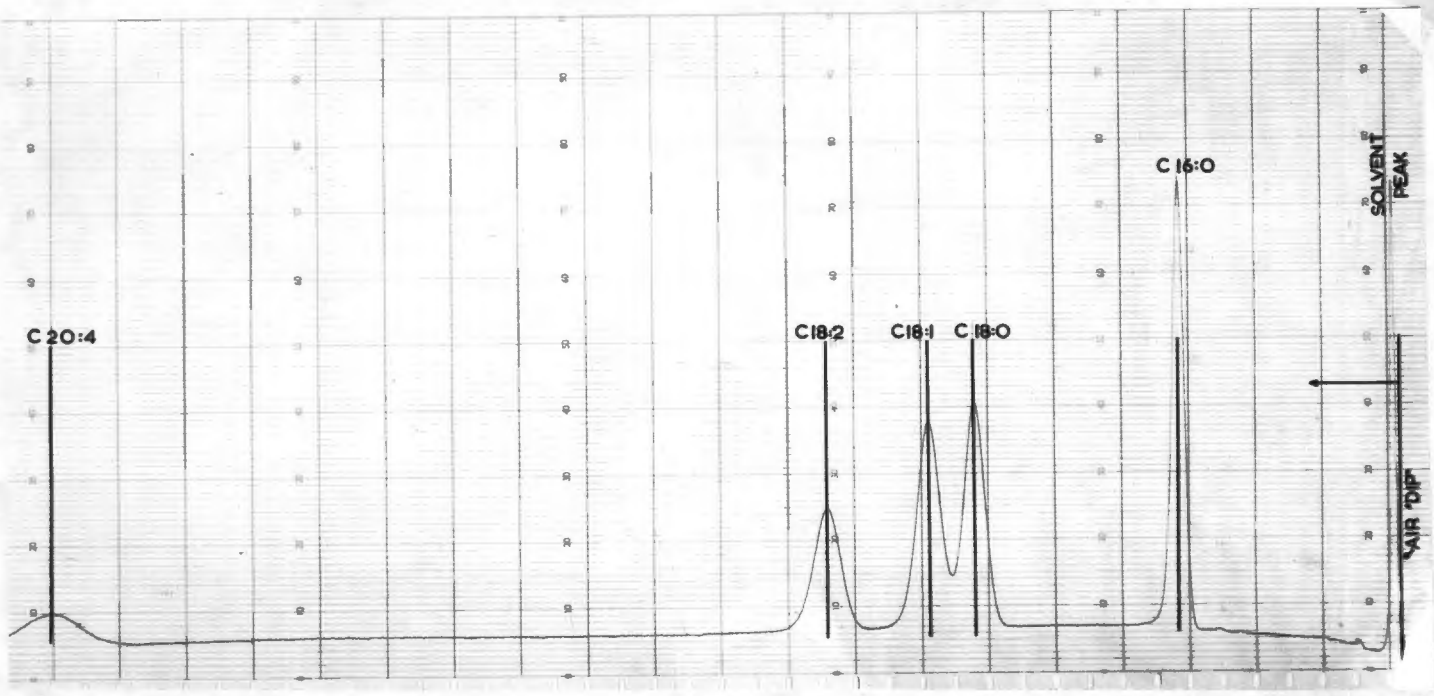


FIG. 8. Chromatograph of a standard fatty acid ester mixture containing the fatty acids palmitic, stearic, oleic, linoleic and arachidonic.

FATTY ACID	d (m.m.)	d/C18 : 0
C16 : 0	84	0.519
C18 : 0	162	1.00
C18 : 1	180	1.11
C18 : 2	218	1.35
C20 : 4	513	3.17

TABLE 4. Standard fatty acid ester mixture showing retention time measured as distance (d) and relative to C18 : 0 (d/18:0). Note that although the peak absolute distances are much shorter for components common to those in Table 3, this column having been operated at a faster gas flow rate, the relative retention times d/C18:0, agree because both separations were done at the same temperature (185°C.)

To confirm that the peaks identified as representing unsaturated components are in fact so, aliquots of the same sample of adipose tissue lipid have been brominated and hydrogenated.

Microbromination method[9]

About 5 mg. of the methyl esters were dissolved in a few ml. of diethyl ether in a conical tube. The tube was then chilled to -15°C. and a 2% solution of liquid bromine in diethyl ether added dropwise to the ester solution until the yellow colour persisted. Only a few drops were required. The excess bromine and ether were then completely evaporated under a stream of nitrogen at room temperature. The conical tube was centrifuged to separate the crystallized brominated unsaturated acids. The supernatant was then chromatographed.

Microhydrogenation of fatty acid methyl esters[17]

About 5 mg. of the methyl esters were dissolved in 25 ml. of ethanol and transferred to a 50 ml. quick-fit Erlenmeyer flask. To this was added 10 mg. of platinum oxide to act as catalyst in the hydrogenation reaction. The flask was attached over a magnetic stirrer, to a microhydrogenation apparatus (Fig.9). Hydrogen from a cylinder was allowed into the apparatus and the water displaced. The air in the apparatus was in this way "washed out" ten times. The magnetic stirrer was then switched on and the water level in the burette seen to rise. When the water level in the burette became constant, the reaction was considered to have gone to completion. The fatty acids in the ethanol were then filtered, through filter paper that had been washed with ethanol, in order to remove the platinum oxide, and transferred to a conical tube. The ethanol was evaporated under nitrogen at 40°C. and the esters collected at the bottom of the tube. These were solid at room temperature. The sample was brought to solution by adding a small amount of petroleum ether (B.P. 40 - 60°C.) and warming. The hydrogenated sample was then chromatographed.

/ Fig.10.....

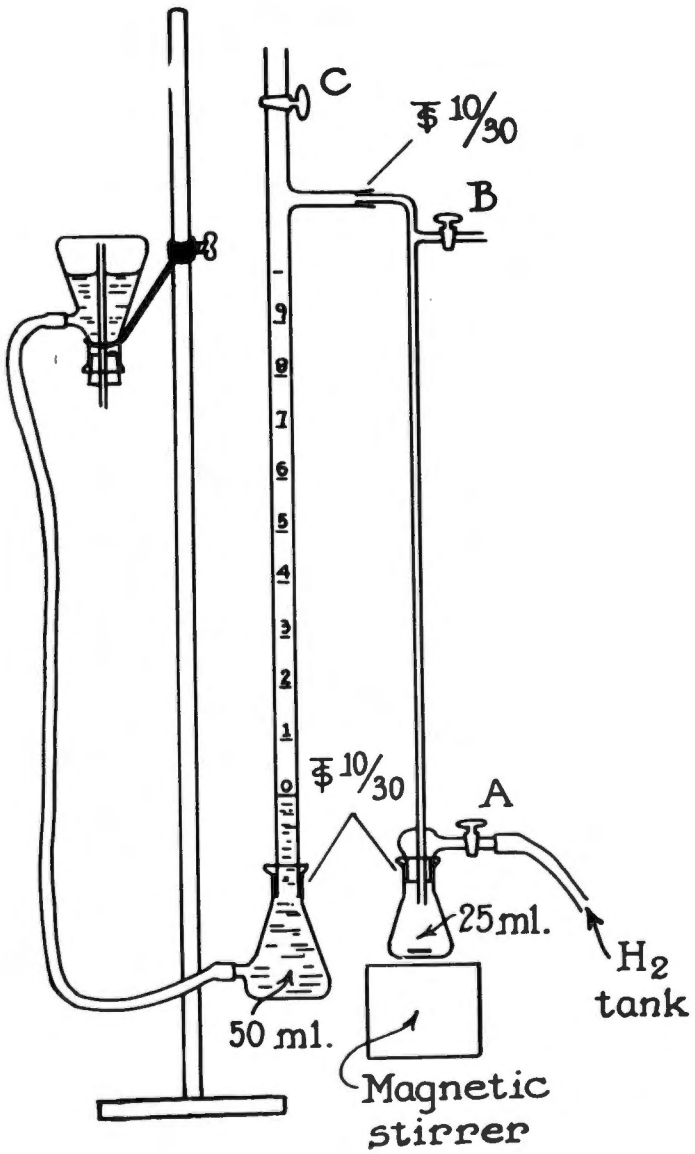


FIG. 9. Microhydrogenation apparatus taken from Farquhar et al.(9). A prototype was built and used for the hydrogenation of fatty acid esters in this study.

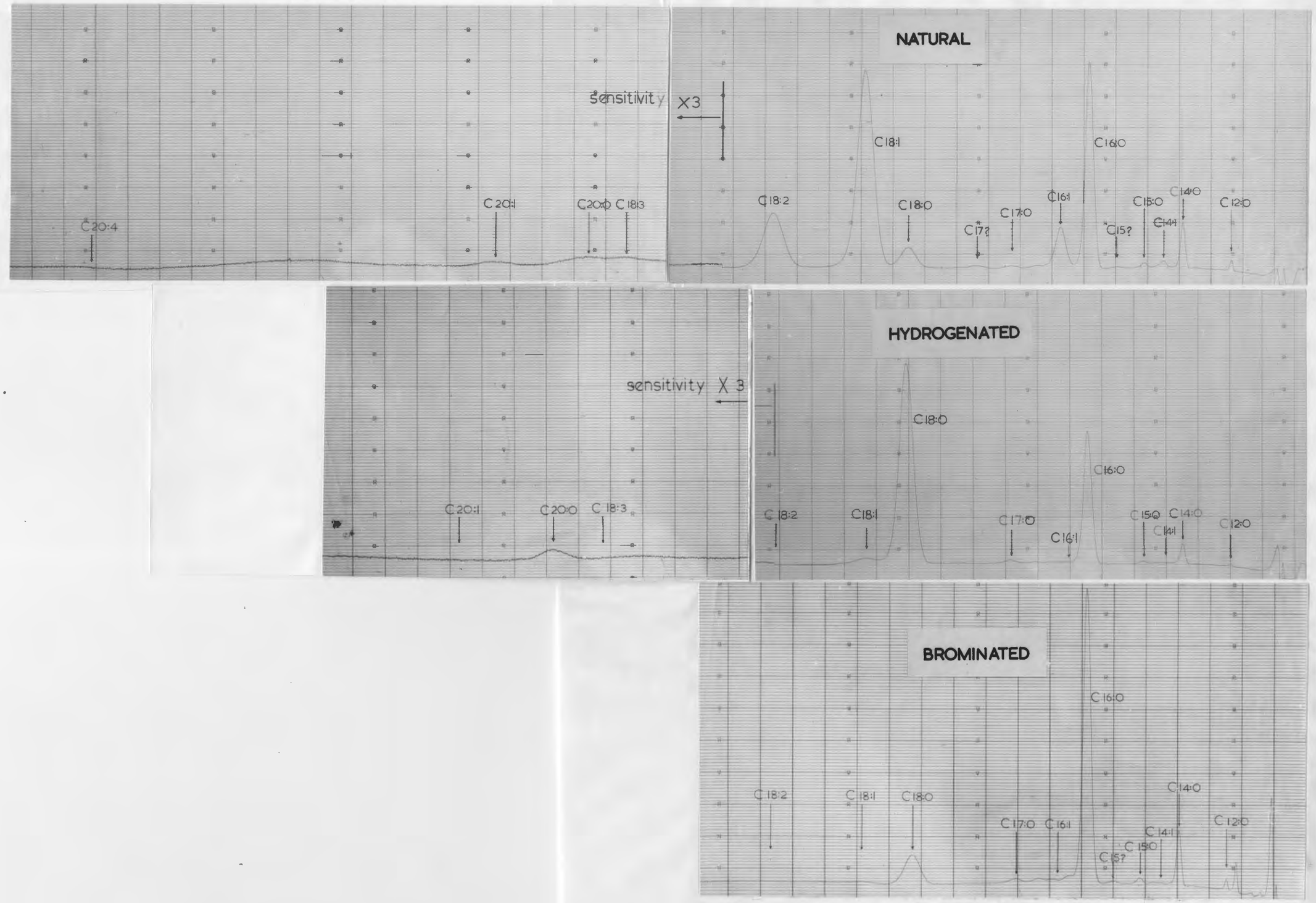


FIG. 10. Chromatograms of the natural, hydrogenated and brominated preparations of aliquots from a single sample of adipose tissue.

Fig.10 shows the chromatograms of the natural, brominated and hydrogenated esters. It can be seen from the brominated sample that the unsaturated components are no longer present and that the remaining saturated components show the same quantitative relations to each other. In the hydrogenated sample the unsaturated components have also not appeared, the saturated components however have in this case altered their quantitative relationships one to the other. This is the result of the unsaturated acids having been converted to the saturated acid with the same number of carbon atoms. To confirm that the peaks in the hydrogenated chromatogram are indeed all due to saturated components the retention time of these peaks, as well as their retention time relative to C18 : 0 have been plotted on a semi-log scale (Table 5, Fig.11) and can be seen to fall on a straight line. The C18 : 1 component has not been entirely eliminated as seen by the small peak still present in the chromatogram of the hydrogenated sample (Fig.10).

FATTY ACID	d	d/C18 : 0
C12 : 0	40	0.140
C14 : 0	77	0.269
C15 : 0	107	0.374
C16 : 0	147	0.513
C17 : 0	205	0.716
C18 : 0	286	1.00
C20 : 0	544	1.90

TABLE 5. Hydrogenated sample of fatty acid methyl esters from adipose tissue showing the retention time, measured as distance (d) and relative to C18:0 (d/C18:0).

/Farquhar et al.[9]....

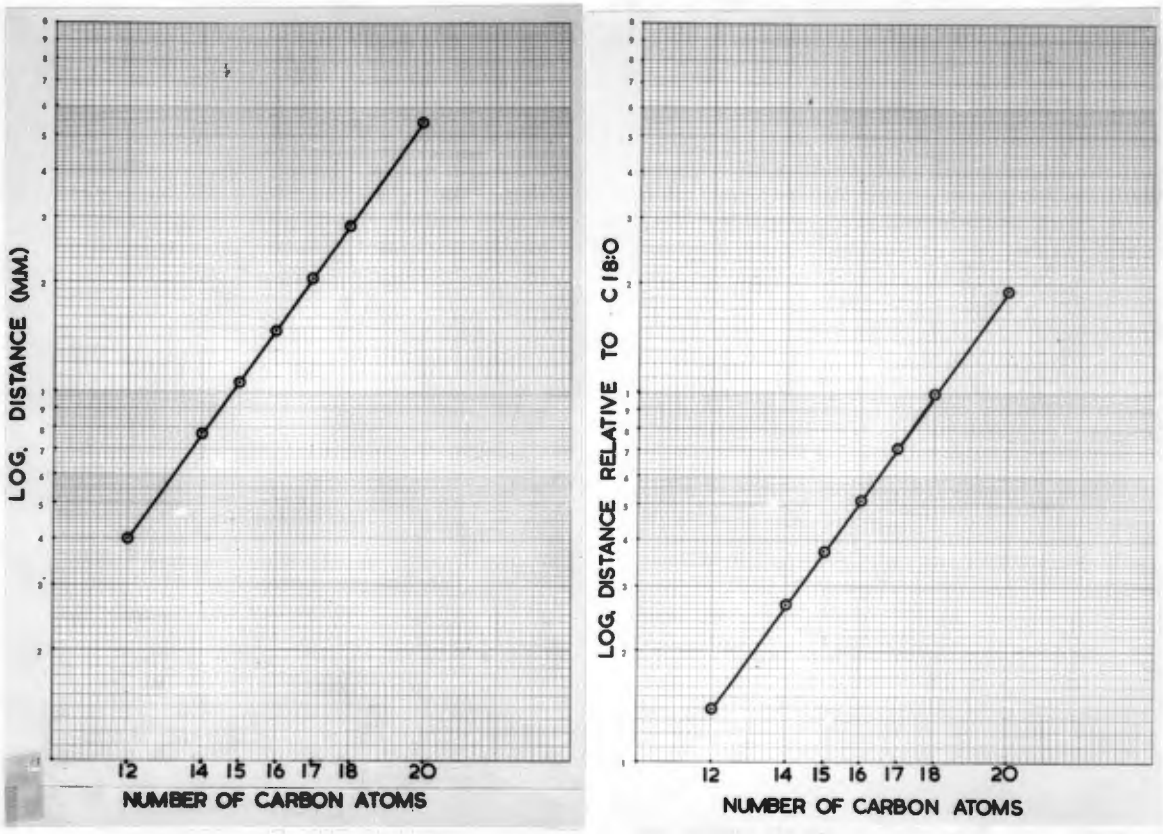


FIG. 11. The retention time, measured as distance as well as the retention time of each peak relative to C18:0 (see Table 5), of the hydrogenated sample, have been plotted on a semi-log scale. Note that all the peaks fall on the same straight line in each case.

Farquhar et al.[9] have further analysed some of the unsaturated components found on gas-liquid chromatographic analysis with specific retention times. They have positively identified C18 : 2 with a retention time relative to C18 : 0 of 1.35 on polyethylene glycol adipate at 184.5°C. as having the double bonds in the 9 and 12 position from the carboxyl end. This therefore represents linoleic acid. It can be seen from Table 3 that the peak identified as C18 : 2 in human depot fat has an identical relative retention factor on the same type of column at the same temperature. Further, this factor for C $\frac{18 : 2}{18 : 0}$ in the adipose tissue sample is the same as that found in the standard mixture which contained a sample of pure methyl linoleate. This further confirms that this peak represents linoleic acid. Farquhar et al.[9] have shown that a C18 : 2 fatty acid with the double bonds at positions 6 and 9 has a relative retention factor of 1.43 under the same conditions. This would mean that on the chromatogram in Fig.5 the centre of the latter peak would appear at 402 mm. instead of 378 (Table 3). This difference of 24 mm. is clearly too large for the peaks to be confused.

The peak with the retention characteristic of C18 : 2 as above is universally referred to as linoleic acid by all who have done fatty acid analyses by G.L.C. on either blood, atheromatous plaques or depot fat.

It will however be recalled from above that the polyethylene glycol adipate column is unable to separate isomers of C18 : 1. It is similarly possible that the column is unable to resolve isomers of linoleic acid. "Linoleic acid" can theoretically occur in 4 different forms, viz: cis - cis, cis - trans, trans - cis and trans - trans [13]. Only cis - cis octadecadienoic acid with the double bonds in the 9 and 12 positions is linoleic acid and is the only form that occurs in nature. It is however possible that industrial processes used in hardening oils could give rise to one or other of these geometric isomers and that the ingestion of fats such as margarines could lead to the deposition of these isomers in human depot fat. It has not been established that the

/fatty acid.....

fatty acid in human depot fat (C18 : 2) which has here been identified as linoleic acid is in fact natural or cis - cis linoleic acid. Such an analysis has been carried out by Böttcher[29] on the C18 : 2 fatty acid in the cholesterol ester lipid fration found in human atheromatous plaques. By means of ozonolytic degradation and infra-red spectrophotometry he has established that this fraction is 98% cis. This of course does not mean that the same holds for human depot fat but possibly serves to indicate that unnatural isomers of linoleic acid are rare. Thus while C18 : 2 will often be referred to as linoleic acid in the text the possible limitations of this reference must be borne in mind.

As had already been stated above, the poly-ethylene glycol adipate stationary phase does not separate oleic acid (C18 : 1) from its possible isomers. As can be seen from Table 3 the relative retention factors for C18 : 1 in the adipose tissue sample and standard mixture is identical (1.11). While this peak will often be referred to as oleic acid, it must be borne in mind that the percentage area represented by this peak includes the possible isomers of oleic acid. Hirsch et al.[5] have shown, on Apiezon columns, that isomers of oleic acid are present in human adipose tissue and that these constitute about 4 - 6% of the total fatty acids; i.e. about 10% of C18 : 1. Isomers of oleic acid are commonly produced by incomplete hydrogenation of natural oils in industry.

/REPRODUCIBILITY OF FATTY.....

REPRODUCIBILITY OF FATTY ACID ANALYSIS BY GAS-LIQUID CHROMATOGRAPHY

The first factor to be determined is the degree of reproducibility in determining the proportion of fatty acids as analysed by the gas-liquid chromatographic technique. In order to determine this a "standard" mixture of methyl esters was made up and submitted to eight repeated analyses. The percentage of each component was then determined and the coefficient of variation for each component calculated (Table 6).

Sample	14 : 0	16 : 0	18 : 0	18 : 1	18 : 2
1	2.9	7.2	18.8	64.3	6.7
2	2.6	7.0	19.4	64.0	7.0
3	2.9	6.7	19.0	64.5	6.9
4	2.6	6.9	19.4	64.6	6.5
5	2.5	7.1	19.7	64.1	6.6
6	3.0	7.1	18.9	64.4	6.6
7	2.6	7.0	19.8	63.9	6.7
8	2.8	7.3	19.8	63.8	6.3
Mean	2.7	7.0	19.4	64.2	6.7
+S.D.	0.1	0.2	0.4	0.3	0.2
++C.V. (%)	5.1	2.6	2.1	0.5	3.1

TABLE 6. The percentage of the component fatty acids of a standard fatty acid mixture as found on repeated analysis by GLC showing good agreement between samples.

+S.D. = 1 standard deviation.

++C.V. = The coefficient of variation expressed as %.

From Table 6 it can be seen that G.L.C. gives a high degree of reproducibility in the fatty acid analysis of methyl esters. Farquhar et al.[9] have claimed that on a similar repeated analysis of 4 components of a "standard" fatty acid methyl ester mixture they were able to achieve reproducibility where the coefficient of variation

/for any single.....

for any single component was less than 1.5%. Such a degree of reproducibility was not attained here. As can be seen from Table 6 the smaller components give the greater error. This error is probably largely the result of inaccuracies in measuring the peak areas of small components rather than an inherent defect in the instrument. It is probably possible to achieve the accuracy that has been claimed[9] by making up a standard mixture that will give approximately equal peak heights. This however would not reflect the accuracy with which natural fatty acid methyl ester mixtures can be determined since they are not present in that proportion in the depot fat triglyceride.

/ VALIDITY OF THE.....

VALIDITY OF THE ASPIRATION TECHNIQUE AS A METHOD OF SAMPLING SUBCUTANEOUS FAT AND REPRODUCIBILITY OF FATTY ACID ANALYSIS OF ADIPOSE TISSUE TRIGLYCERIDE BY GAS-LIQUID CHROMATOGRAPHY

In sampling adipose tissue by the aspiration technique several variables are introduced. (1) It has been shown in some animals that the composition of the depot fat alters with increasing depth from the skin surface (see later). In sampling adipose tissue by this technique the depth at which tissue is sampled is largely arbitrary. It is generally at about half the distance between skin and muscle, but this will vary with the thickness of the subcutaneous layer and this varies markedly between subjects. (2) It must be established whether the fat obtained by aspiration has the same composition as that excised from the same site. (3) The amount of fat obtained from different subjects as mentioned above varies between about 1 and 25 mg. That the larger sample may not be completely extracted or methylated by the methods used is a distinct possibility.

The limits of accuracy for all these variables must therefore be established.

Other variables were that columns were operated at either 185 or 197°C. during the course of these studies. Also a number of different columns were used. Although these latter factors should theoretically show no variation in analysis this needs to be confirmed.

In order to establish the overall degree of reproducibility of all the above variables the following procedure was carried out.

In a subject from whom a keloid was to be excised from the buttock region, a sample of adipose tissue was obtained from the region of the buttock by the aspiration technique. The surgical specimen of the excised keloid included the full thickness of skin and subcutaneous tissue. Seven additional samples were excised from this frozen surgical specimen at increasing depth from the surface, extending from the superficial to the deep layers and varied in wet weight from about 5 to 50 mg. Each sample, the aspirated and excised portions, was then submitted to separate extraction, methylation and fatty

/acid analysis.....

acid analysis in the identical manner to that routinely used. Four samples were analysed on one column at 185°C. and four on another at 197°C. The results are presented in Table 7.

Microsublimation

The question of the necessity for microsublimation of samples was also considered. Another excised sample from the same surgical specimen was separately extracted, methylated and in addition submitted to microsublimation prior to fatty acid analysis. The results are shown in Table 7.

RESULTS

Table 7 shows the percentages of the major fatty acids (see Chapter 5) found on repeated analysis of separate samples of subcutaneous adipose tissue from the buttock region; comparing an aspirated (sample I). with 7 excised samples (2 - 8), taken at progressively greater depth from the surface, analysed on 2 columns operating 185 and 197°C.

Also shown in Table 7 is the percentage of the major fatty acid components as found on a sample of subcutaneous tissue obtained from the same subject that had in addition been submitted to microsublimation.

TABLE 7.....

		FATTY ACID (%)					
Column	Sample No.	14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2
A	1	2.8	22.5	6.0	4.3	43.7	16.4
A	2	2.8	22.9	6.0	4.3	44.8	15.3
A	3	2.9	23.3	6.1	4.5	44.2	15.5
A	4	3.0	23.6	6.4	4.0	44.2	15.2
B	5	2.8	23.7	6.4	4.0	45.0	14.8
B	6	3.0	23.5	6.4	4.0	45.5	14.3
B	7	2.9	24.0	6.3	4.5	43.6	14.8
B	8	2.9	23.0	6.3	4.3	42.1	16.0
	MEAN	2.9	23.3	6.2	4.2	44.1	15.3
+	S.D.	0.1	0.5	0.2	0.2	1.0	0.2
++	C.V. (%)	2.9	2.1	2.9	5.0	2.4	3.0
	Sublimed specimen	2.9	23.6	6.3	4.0	45.7	14.6

\+ = 1 Standard deviation.

++ = coefficient of variation.

TABLE 7. The percentage of the major fatty acid components from the subcutaneous adipose tissue obtained from one subject. Sample number 1 was obtained by needle aspiration from the buttock region. Samples 2 - 8 were obtained from a surgical specimen of the full thickness subcutaneous adipose tissue overlying the buttock, excised at progressively increasing depth from the surface. The samples were analysed on two columns, operating at 185°C. (Column A), and 197°C. (Column B). The findings on the specimen submitted to microsublimation are also shown.

From Table 7 it can be seen that there is very close agreement between the aspirated sample (No.1) and 7 other excised samples (2 - 8) at increasing depth from the surface. The greatest coefficient of variation for any fatty acid is that of C18 : 0 which is 5%. The sublimated sample agrees very closely with the unsublimed samples.

/CONCLUSIONS.....

CONCLUSIONS

It is concluded that: (i) The aspiration technique provides a highly representative sample of the fatty acids present in the adipose tissue of the buttock region. (ii) There is no variation in the fatty acid composition related to the depth from the skin surface at this site. (iii) The methods of lipid extraction methylation and fatty acid analysis used are highly reproducible in determining the major fatty acid components of subcutaneous depot fat triglyceride over a large range of sample size exceeding that normally obtained. (iv) The microsublimation procedure is not a necessary prerequisite to the analysis of depot fat triglyceride methyl esters by gas-liquid chromatography.

EXTRACTION OF LIPID FROM MALE AND FEMALE ADIPOSE TISSUE

Certain trends were found on comparing the fatty acid composition of depot fat between males and females. It was suggested by Kench (30) that before concluding that these were true differences in fatty acid composition it would be desirable to establish whether the extraction procedure is equally effective for male and female adipose tissue triglyceride. A method of examining this point does not spring readily to mind. Gas-liquid chromatography measures the relative proportion of the fatty acids. The method of testing the effectiveness of lipid extraction from adipose tissue in males and females would therefore have to demonstrate that one or other of the tissues does not selectively retain any specific fatty acid. It was agfeed that this point be tested by adding a fatty acid mixture of known composition to adipose tissue and determining the proportional recovery of the fatty acid components in the added lipid. This was done as follows :

If there is some substance in male or female adipose tissue giving rise to incomplete extraction it would be necessary to add the known fatty acid mixture to unextracted adipose tissue. Samples of excised adipose tissue from a 23 year old

/ female.....

female and 30 year old male were obtained from subjects undergoing minor surgical procedures. The sample from each subject was then treated as follows. One portion was extracted and analysed in the usual manner. To another portion was added some glycerol stearate (tristearin) B.D.H., the fatty acid composition of which had been determined in the usual manner. These latter samples of adipose tissue plus glycerol stearate were then extracted and analyzed in the routine manner. The results are presented in Table 8.

	F. o.		F +			TRISTEARIN (%)	
FATTY ACID	%	x/18 : 1	%	x/18 : 1	diff.	Rec.	Original
14 : 0	5.7	0.135	5.2	0.184	0.049	4.2	3.9
16 : 0	31.0	0.733	35.4	1.251	0.518	44.8	43.8
16 : 1	9.0	0.213	6.4	0.226			
18 : 0	4.4	0.104	19.6	0.693	0.589	50.9	52.3
18 : 1	42.3	1.000	28.3	1.000	--		
18 : 2	7.7	0.182	5.1	0.180			
	M. o.		M +				
14 : 0	4.4	0.109	4.4	0.150	0.041	4.4	3.9
16 : 0	22.4	0.554	27.2	0.928	0.374	39.8	43.8
16 : 1	5.0	0.124	3.9	0.133			
18 : 0	8.7	0.215	21.7	0.741	0.526	55.8	52.3
18 : 1	40.4	1.000	29.3	--			
18 : 2	19.1	0.473	13.5	0.461			

TABLE 8. The common fatty acid in unadulterated female (F.o.) and male (M.o.) adipose tissue have been expressed in proportion to each other (%). The ratio of each fatty acid to C18 : 1 (x/18 : 1) has been calculated. The samples F + and M + represent the findings in the female and male adipose tissue extracts respectively to which the tristearin has been added. It will be noted that the tristearin contained C14 : 0, C16 : 0 and C18 : 0 in certain proportions. The difference between the unadulterated samples and the samples to which the tristearin has been added is shown in the column "diff." This represents the relative proportion of these fatty acids (C14 : 0, C16 : 0 and C18 : 0) accounted for by the difference between the pure and adulterated samples of adipose tissue. These latter have been expressed as a percentage and compared with the original composition of the tristearin. It can be seen that in both cases there is very close agreement between the recovered (Rec.) and original composition of tristearin

/It can.....

It can be seen from Table 8 that the recovery of the fatty acids found in a preparation of tristearin is essentially the same when extracted together with the lipid from both male and female adipose tissue. It may thus be concluded that the finding of possible differences in the fatty acid composition of male and female adipose tissue is not likely to be due to a difference in the effectiveness of the extraction procedure from male and female tissue.

/GENERAL COMMENTS.....

GENERAL COMMENTS ON FATTY ACID ANALYSIS BY G.L.C.

LIMITATIONS OF THE APPARATUS USED IN THESE STUDIES

The sensitivity adjustments that may be made during a single run on the "Pye" chromatograph are limited to a range of from 1 - 10 fold. The proportions of the various fatty acids in human depot fat is such that a number of minor components emerge late in the analysis. In order to detect these components it was necessary to operate the instrument at its maximum sensitivity. Unfortunately the temperature regulating mechanism operating through a contact thermometer is not as constant as it might be and varied by $\pm$ 2°C. This does not materially interfere with the analysis at minimal sensitivity, at which the instrument is operated until the emergence of C18 : 2. The components emerging after this however are present in very small amounts and the peaks at these positions are relatively wide (see Fig. 5). In order to detect them in measurable amounts it is necessary to operate the instrument at higher sensitivity (X3). This gave some base-line cycling (see Fig.5) and at times very erratic peaks. As can be seen from Fig.5 the components C18 : 3, C20 : 0 and C20 : 1 are very minor even at X3 sensitivity. In order to adequately detect and measure C20 : 4 which emerges last in this analysis (see Fig.5) it would be necessary to operate the instrument at higher sensitivity still. Operating at maximum sensitivity ("X1") for a given detector voltage gave rise to considerable base-line cycling and an erratic and unsatisfactory peak. C20 : 4 could therefore not be sufficiently accurately quantitated to be useful for comparative purposes. It was therefore the practice not to include it in determining the proportion of the various components once it was established that it constituted no more than 0.5% of the total fatty acids. The error that may be introduced by not including this component is at the most 0.5% for the total fatty acids measured and represents a very small potential error in determining the percentage of each of the major fatty acid components. Hirsch[5] has shown that other

/ long chain.....

long chain components emerging between C20 : 1 and C20 : 4 are also present in trace amounts. These acids were not found on routine analysis in these studies. It is very likely that they are always present, and have in fact been found here when a deliberate overload had been placed on the apparatus. In order to determine their presence and quantitate them with the apparatus used in these studies it would have been necessary to first determine the proportions of the more common components; then to analyse a deliberate overload in order to obtain fair sized peaks for these minor components; then to calculate the percentage of these minor components by proportion. All of these extra manoeuvres would serve only to decrease the reliability of the final figures. Interest has thus been centred on the major components as determined by analytical runs done in the way described above. The fatty acids representing the common components will be demonstrated in Chapter 5.

VALIDITY OF GAS-LIQUID CHROMATOGRAPHY AS A METHOD OF ANALYSIS

There are in some circles chemists who do not accept gas-liquid chromatography with the enthusiasm evident among the pioneers and users of this method of analysis. Some of the criticisms are possibly valid. The chief criticism is that analyses by gas-liquid chromatography do not give the same results with respect to polyenoeic acids as are found with alkali isomerization; particularly with regard to the highly unsaturated fatty acids containing 5 and 6 double bonds.

The exact characterization of a substance and its distinction from steric and structural isomers is by no means always possible by G.L.C. and other methods have to be applied for complete characterization. G.L.C. has, however, the advantage that these components of mixtures can be isolated in pure form and then subjected to further analysis either by re-chromatographing directly on other colums[23] or after

/further treatment,.....

further treatment, e.g. oxidation to split the component and then rechromatographing. This will give precise information of chemical structure[31]. Further, these pure components can then be characterized by the use of other analytical techniques.

The method has virtues for analysis of common and natural fatty acids that are most important both from the analytical and comparative standpoints.

Reproducibility

The degree of reproducibility has been shown above (Tables 7 and 8) and is of an order not readily achieved by other chemical methods applied to this type of analysis.

Linearity of response

This has been shown to be directly related to unit mass for each component of a mixture of fatty acid methyl esters with a molecular weight above 100. The response is not linear with mass for substances below this molecular weight[22]. Since the fatty acid with the shortest carbon chain found in the depot fat is C12 which has a molecular weight well above 100, this criticism does not apply to these studies.

Possible effects of high temperature

An operating temperature near 200°C. can conceivably produce changes in the double bond structure of the highly unsaturated esters. This possibility has been investigated by Stoffel, Insull and Ahrens[32] on columns of the non-polar phase, Apiezon M, at 197°C. and the polar, Reoplex 400, at a temperature of 200°C. They found that there was:

(1) Quantitative recovery of the fatty acid esters applied - within 1%.

(2) There were no changes in retention volume after cold trapping and rechromatographing the sample. This indicates that there was no change in the solute solvent relationships and therefore presumably no structural change.

/(3) No changes.....

(3) No changes either qualitative or quantitative in the specific extinctions after isomerization in alkali.

(4) Infra-red spectroscopy showed no change for any of the unsaturated fractions greater than 1 - 2% in investigating the possible production of trans double bonds.

(5) There was no interruption in double bond structure, nor any shift in the double bond position along the chains, as determined by the identification of fragments produced by ozonolytic degradation.

They concluded that methyl esters of highly unsaturated long-chain fatty acids are not significantly altered in chemical structure during gas-liquid chromatographic analysis.

Comparison with other methods of analysis

James[26] has compared the results found by alkali isomerization with those by G.L.C. for linoleic plus linolenic and arachidonic acids over a wide range and got very close agreement. Comparisons have also been made with these 2 techniques on the cholesterol ester fatty acids with good agreement[33]. There is thus adequate evidence to confirm the accuracy of G.L.C. in the detection and quantitation of polyunsaturated acids up to tetraenoic. The penta- and hexa-enoic acids usually measured by the alkali isomerization technique are identifiable and can be measured by G.L.C. as has been shown on analysis of fish oils[9]. The difficulty of obtaining samples of penta- and hexa-enoic acids of adequate purity seems to be the major obstacle to settling this possible criticism as to the accuracy of G.L.C. in the quantitation and detection of these highly unsaturated fatty acids.

On the basis of the above evidence it may be concluded that G.L.C. is an accurate reproducible and reliable method of analysis for fatty acid methyl esters and that the substances detected are derived from the parent sample. The criticisms with regard to the penta- and hexa-enoic acids have yet to be disproved.

/The great advantage.....

The great advantage of this analytical tool is the micro-scale on which it is possible to make analyses. Certainly this study on the depot fat of living man, where the total sample is frequently not much more than a mg. of lipid, could not have been done by any other method. The information obtained by this technique is indeed remarkable.

STATISTICAL METHODS.

The following statistical formulae were employed in this study.

Mean : $\bar{X} = \Sigma X/N$ . . . . . (1)

where X = each observation and N = number of observations .

Standard deviation (S.D.) for a variate :

$$S.D = \sqrt{\Sigma x^2/N-1} \quad . . . . . (2)$$

where $\Sigma x^2 = \Sigma X^2 - (\Sigma X)^2/N$.

The difference between 2 means was analysed by t-test , t being calculated as :

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{\Sigma x_1^2 + \Sigma x_2^2}{\frac{(N_1 \times N_2)(N_1 + N_2 - 2)}{(N_1 + N_2)}}}} \quad . . . . . (3)$$

The significance of the difference between two means was determined by reference to standard tables* for the distribution of t .

Differences were considered to be highly significant when $p < 0{\cdot}001$ significant when $p < 0{\cdot}01$ and of probable significance when $p < 0{\cdot}05$.

The correlation coefficient between two variates X + Y was analysed as

$$r = \frac{N.\Sigma XY - \Sigma X.\Sigma Y}{\sqrt{N.\Sigma X^2 - (\Sigma X)^2}\ \sqrt{N.\Sigma Y^2 - (\Sigma Y)^2}} \quad \ldots\ldots\ldots (4)$$

The significance of r was similarly determined by reference to standard tables* .

Regression lines were calculated by the method of least squares , the correlations having been assumed to be linear , $Y = a + bx$.

$$b_{YX} = \frac{N.\Sigma XY - \Sigma X.\Sigma Y}{N.\Sigma X^2 - (\Sigma X)^2}$$

$$\ldots\ldots\ldots\ldots\ldots (5)$$

$$a_{YX} = \frac{\Sigma Y - b.\Sigma X}{N} \quad .$$

To test the variability of repeated analyses the coefficient of variation (C.V) was calculated as :

$$C.V. = \frac{S.D \times 100}{\bar{X}} \quad \text{and expressed as a percentage...(6)}$$

(* Fisher, R.A. and Yates, F. Statistical Tables for Biological, Agricultural and Medical Research, 5th Ed. Revised and Enlarged. 1957. Oliver and Boyd, London).

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CHAPTER 4

SERUM CHOLESTEROL, RELATIVE OBESITY AND DIET OF THE SUBJECTS SAMPLED

It will be recalled from Chapter I that there are trends in various aspects of lipid metabolism with respect to age, sex and diet. The subjects sampled for this study were drawn from a selected hospital population. It is therefore of importance to establish whether the sampled subjects show the expected trends with these other variables. If they do follow these trends with respect to some aspects of lipid metabolism the data on the depot fat will be more meaningful. For example the findings by Hirsch et al.[1] that there was no change in depot fat composition with age would have been of greater interest if they had data on some other lipid parameter that did show a change where it would be expected on the basis of previous or parallel studies on the same group.

Data on the serum cholesterol concentration, the relative degree of obesity and the dietary details in each group of subjects according to age, sex and race have been determined by the methods described in Chapter 3 and are presented below.

RESULTS

SERUM CHOLESTEROL

The Effect of Age

The mean concentration of serum cholesterol in the subjects sampled have been compared at the two age levels in each race and sex (Fig. 1). Table 1 shows the mean, 1 standard deviation (S.D.), the difference between means (diff.) and the significance of this difference (P.) between age groups in each race and sex.

TABLE I.....

	MALES						FEMALES					
	20 - 29		40 - 49				20 - 29		40 - 49			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	240.0	(42.3)	283.1	(44.6)	43.1	<0.05	211.7	(27.9)	275.0	(65.5)	63.3	<0.0
C	211.1	(35.3)	253.9	(36.5)	42.8	<0.01	209.3	(55.8)	241.8	(41.7)	32.5	N.S.
B	209.9	(32.6)	196.6	(51.6)	13.3	N.S.	206.0	(24.2)	192.2	(39.5)	13.8	N.S.

TABLE 1. The mean serum cholesterol concentration (mg.%) shows a significant rise with age in the White (W) males and females and in the Coloured (C) males. The rise with age in the Coloured females was not significant. There is no change with age in either the Bantu (B) males or females.

It can be seen from Table 1 that the White subjects, both males and females show a significant rise in serum cholesterol concentration with age, as is the case in the Coloured males. The rise in the mean level with age among the Coloured females (Fig. 1) was not significant. The Bantu subjects show no significant change with age among either the males or females.

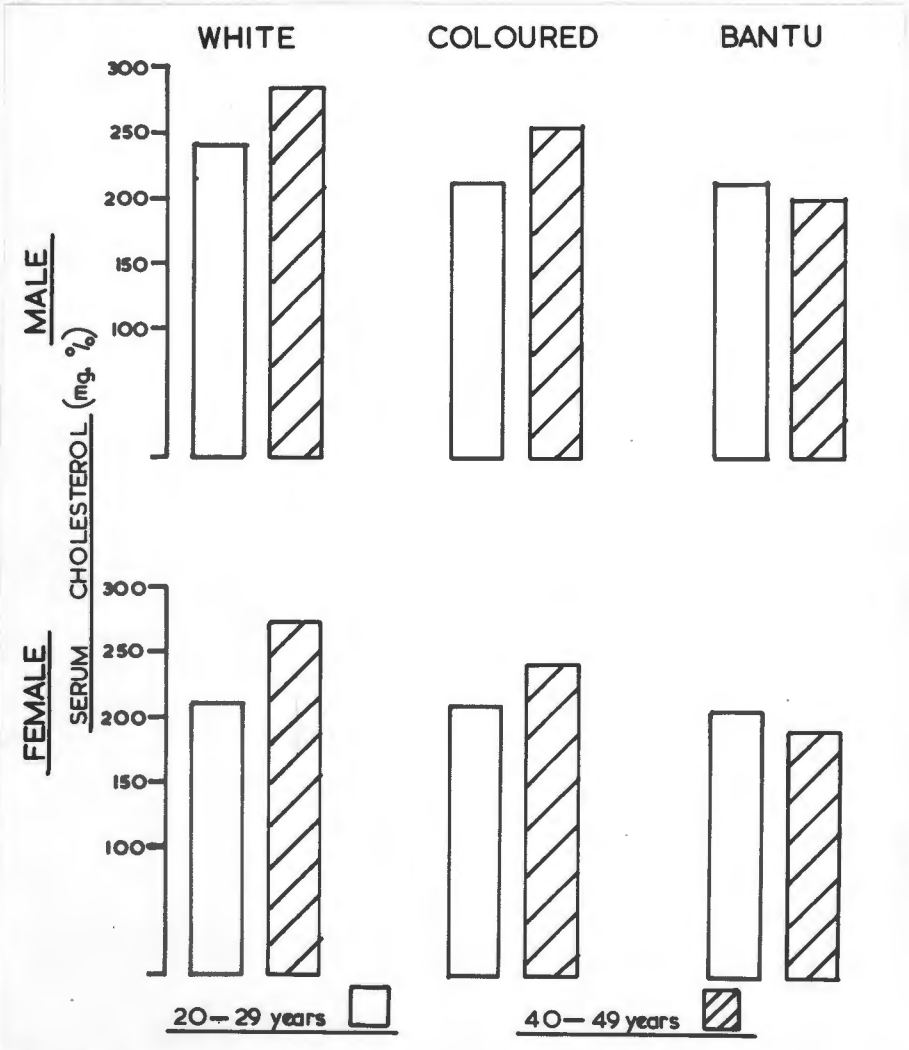

FIG. 1.

/The Effect of Sex.....

The Effect of Sex

The serum cholesterol concentration has been compared between males and females at each age level in the three racial groups (Table 2, Fig. 2).

AGE	20 - 29 years						40 - 49 years					
SEX	Males		Females				Males		Females			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	240.0	(42.3)	211.7	(27.9)	28.3	N.S.	283.1	(44.6)	275.0	(65.5)	13.1	N.S.
C	211.1	(35.3)	209.3	(55.8)	1.8	N.S.	253.9	(36.5)	241.8	(41.7)	12.1	N.S.
B	209.9	(32.6)	206.0	(24.2)	3.9	N.S.	196.6	(51.6)	192.2	(39.5)	4.4	N.S.

TABLE 2. Comparing males with females at the same age level showed no significant difference between sexes in any racial group.

From Table 2 it can be seen that there is no significant difference between the sexes at the same age level in any of the racial groups. The higher mean value in the 20 - 29 year old White males than in the females of the same age range (Fig. 2) was not significant.

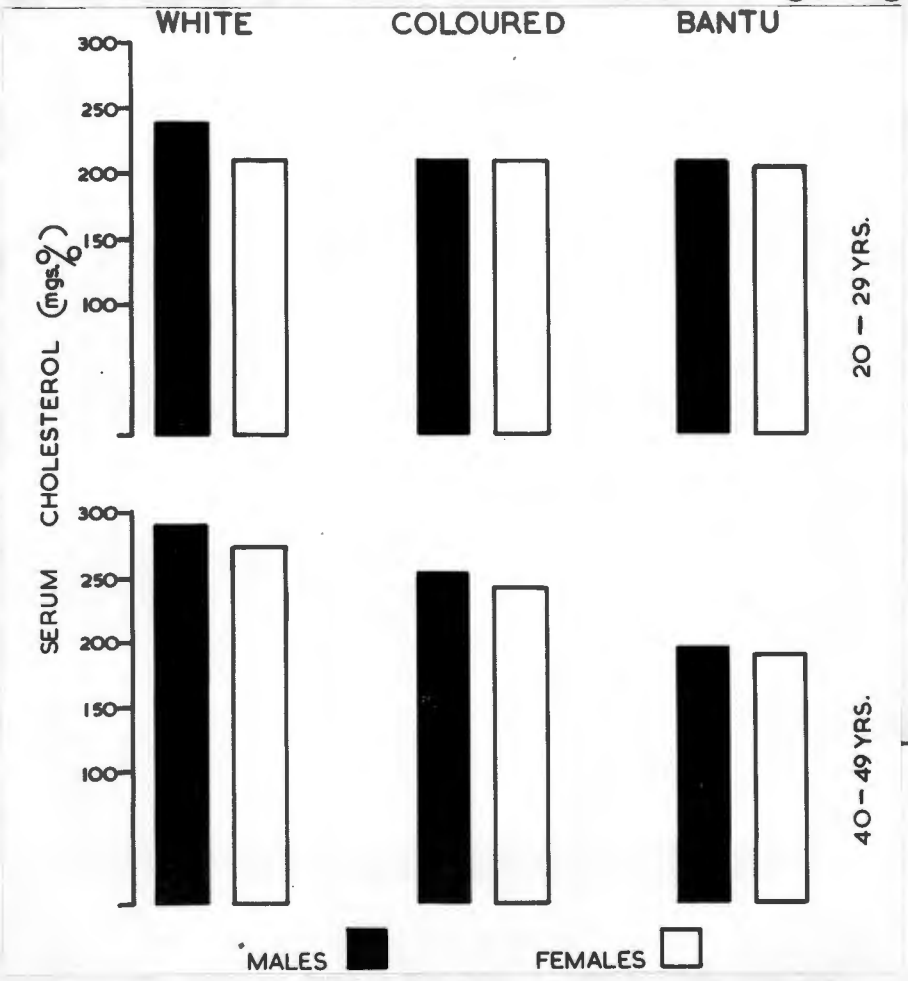

FIG. 2.

Differences.....

Racial Differences

The serum cholesterol concentration has been compared in Tables 3 and 4 between White and Coloured (W vs.C), White and Bantu (W vs.B) and Bantu and Coloured (B vs.C) at each age level, taking account of sex. The mean values for the 20 - 29 year age groups are shown in Table 3 and for the 40 - 49 year age groups in Table4.

AGE	20 — 29 years									
SEX	Males						Females			
RACES	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W vs. C	240.0	(42.3)	211.1	(35.3)	28.9	N.S.	211.7	(27.9)	209.3	(55.8)	2.4	N.S.
W vs. B	240.0	(42.3)	209.9	(32.6)	30.1	N.S.	211.7	(27.9)	206.0	(24.2)	5.7	N.S.
B vs. C	209.9	(32.6)	211.1	(35.3)	1.2	N.S.	206.0	(24.2)	209.3	(55.8)	3.3	N.S.

TABLE 3. There is no significant difference in serum cholesterol concentration between any of the racial groups in either sex at the age level 20 - 29 years.

AGE	40 — 49 years									
SEX	Males						Females			
RACES	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W vs C	283.1	(44.6)	253.9	(36.5)	29.2	N.S.	275.0	(65.5)	241.8	(41.7)	33.2	N.S.
W vs B	283.1	(44.6)	196.6	(51.6)	86.5	<0.001	275.0	(65.5)	192.2	(39.5)	82.8	<0.01
B vs C	196.6	(51.6)	253.9	(36.5)	57.3	<0.01	192.2	(39.5)	241.8	(41.7)	42.6	<0.05

TABLE 4. The difference in serum cholesterol concentration between White and Coloured is not significant among either the males or females. The Bantu males and females however show a significantly lower level than both the White and Coloured groups.

/While there is.....

While there is a trend among the younger males for the White subjects to show a higher mean concentration of serum cholesterol than both the Coloured and Bantu groups, the differences were not significant (Table 3). No trend is evident among the females (Fig. 3) in this age group. In the 40 - 49 year age group the Bantu subjects, both males and females (Table 4) show a significantly lower level than the White and Coloured groups. The higher mean concentration in the White subjects (Fig. 3) was not significantly greater than in the Coloured subjects.

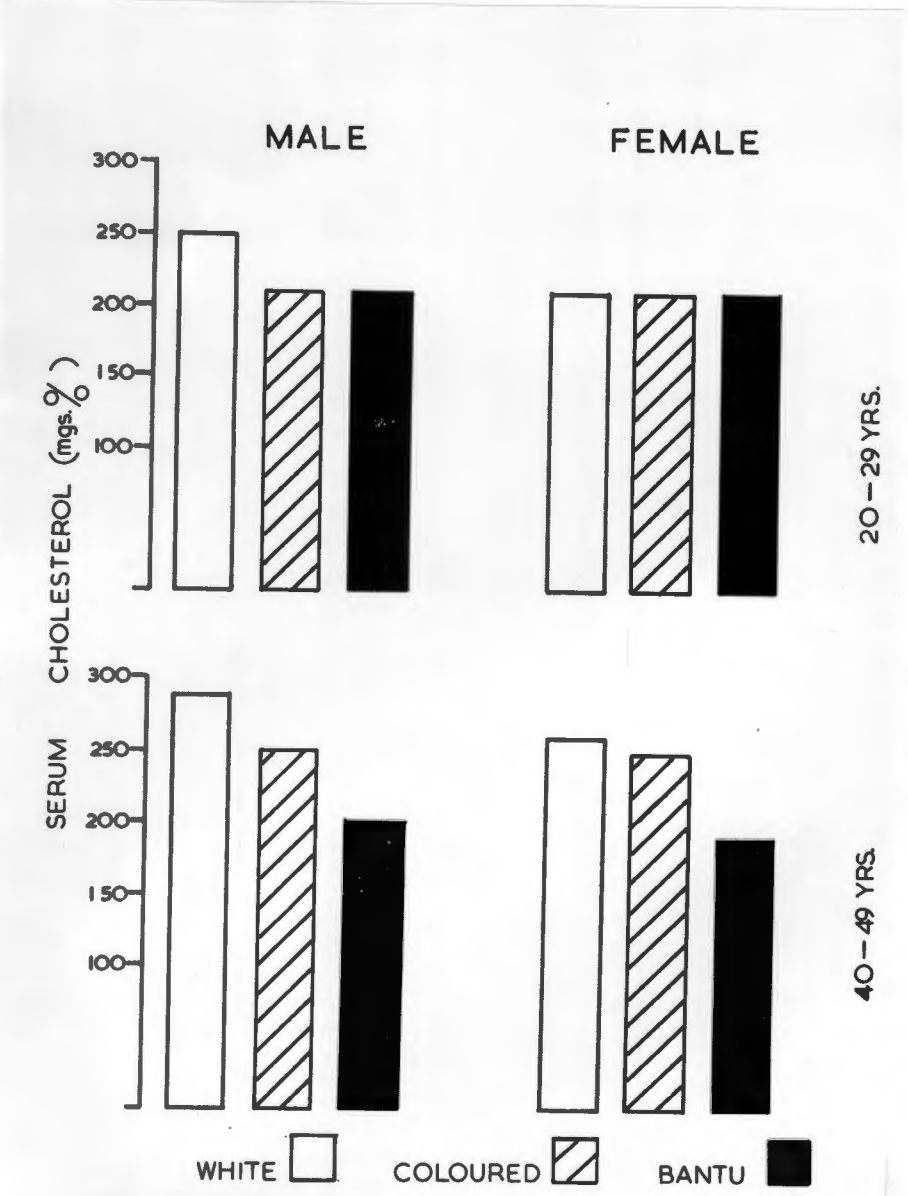

FIG. 3.

/RELATIVE OBESITY.....

RELATIVE OBESITY

The degree of body fatness - leanness has been determined by skinfold measurements. The trends with age, sex and race have been assessed.

Effect of Age

The trend for the adipose organ to increase in size with age was discussed in Chapter 2. The subjects of this study show this trend among both the males and females (Fig. 4). Subdividing the groups according to race reveals that while the same trend is obvious amongst the females in each race (Fig. 5), this trend is not so striking among the males (Fig. 6).

Effect of Sex

The trend for females to be more obese than males (Chapter 2) is evident at each age level in all the racial groups (Fig. 7).

Racial Differences

The distribution of obesity has also been compared between the racial groups in both males and females. It can be seen from Fig. 8 that the White males as a group tend to be more obese than both the Coloured and Bantu males. The distribution among the females does not appear to follow a pattern.

/ DIETARY DETAILS.....

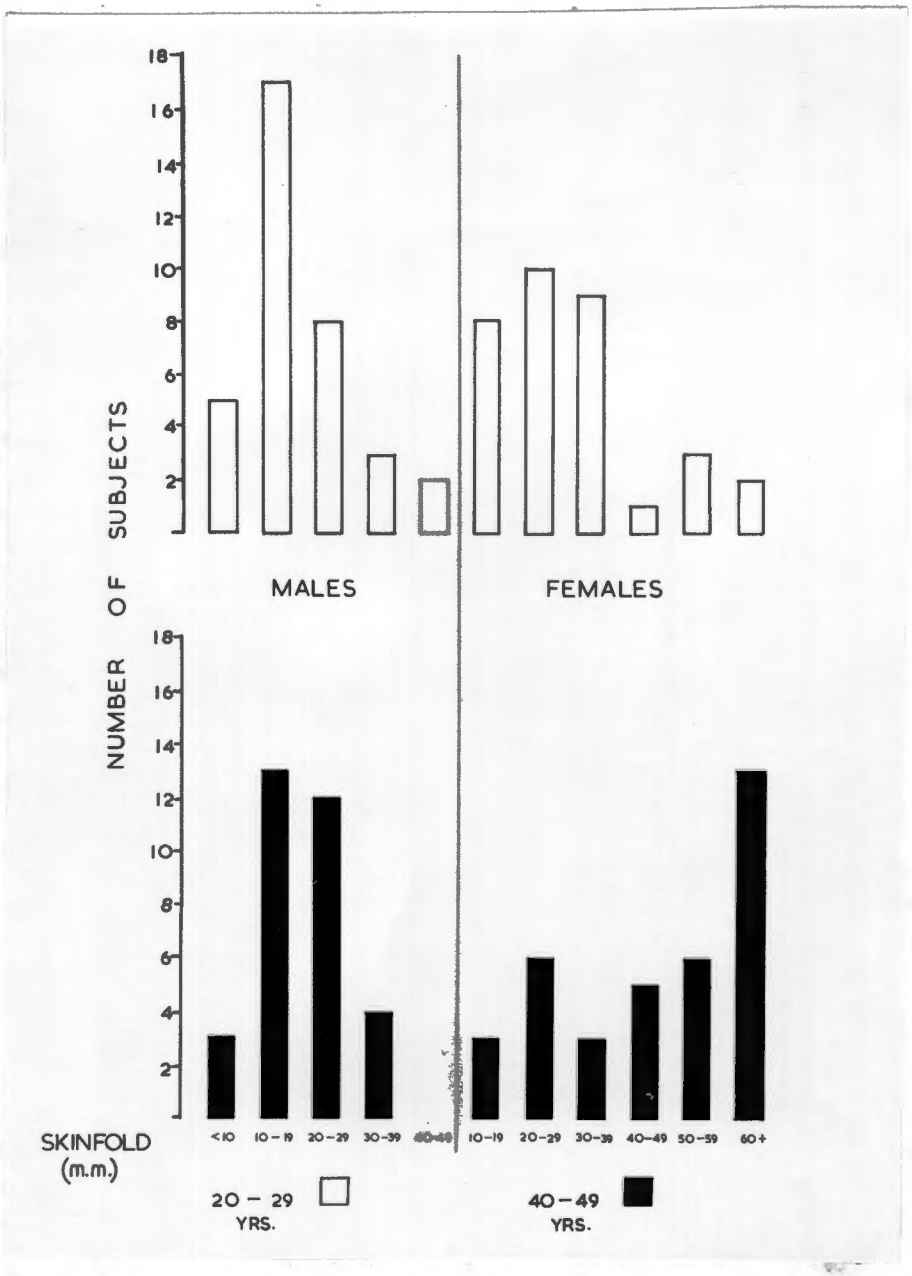

FIG. 4. The distribution of obesity among the males and females sampled, compared according to age groups. Note the trend for a larger proportion of subjects in the older age groups to be more obese than in the younger age groups. This is particularly marked among the females.

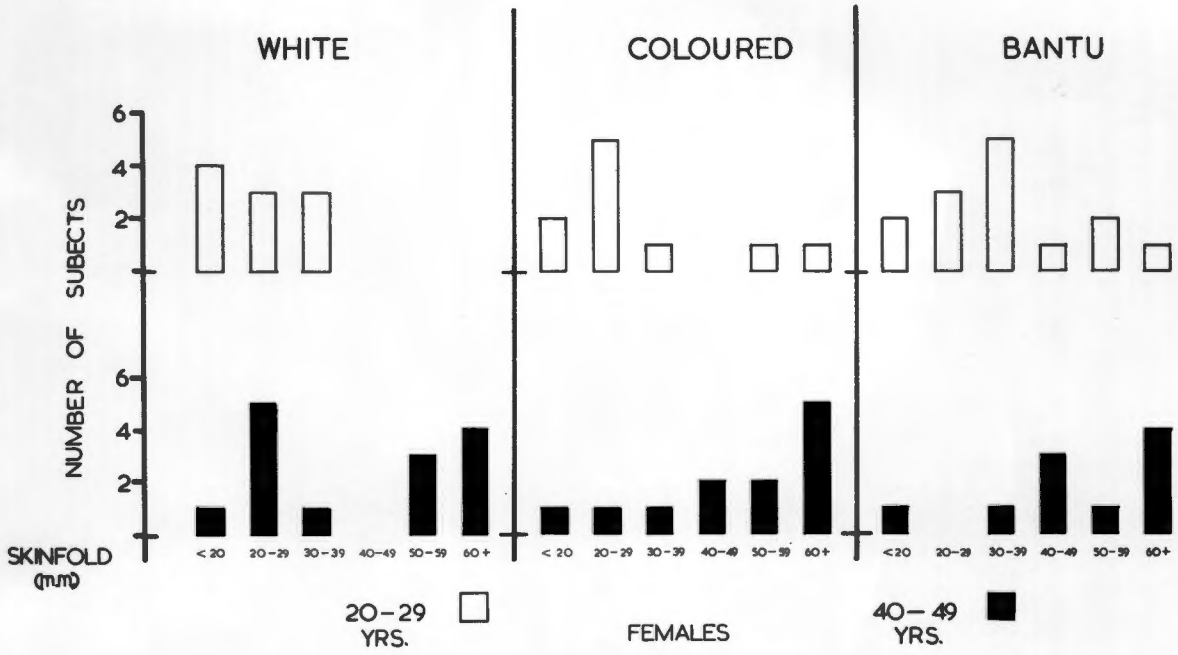
WHITE
COLOURED
BANTU
NUMBER OF SUBECTS
6
4
2
SKINFOLD (mm)
< 20
20-29
30-39
40-49
50-59
60+
20-29 YRS.
FEMALES
40-49 YRS.

FIG. 5

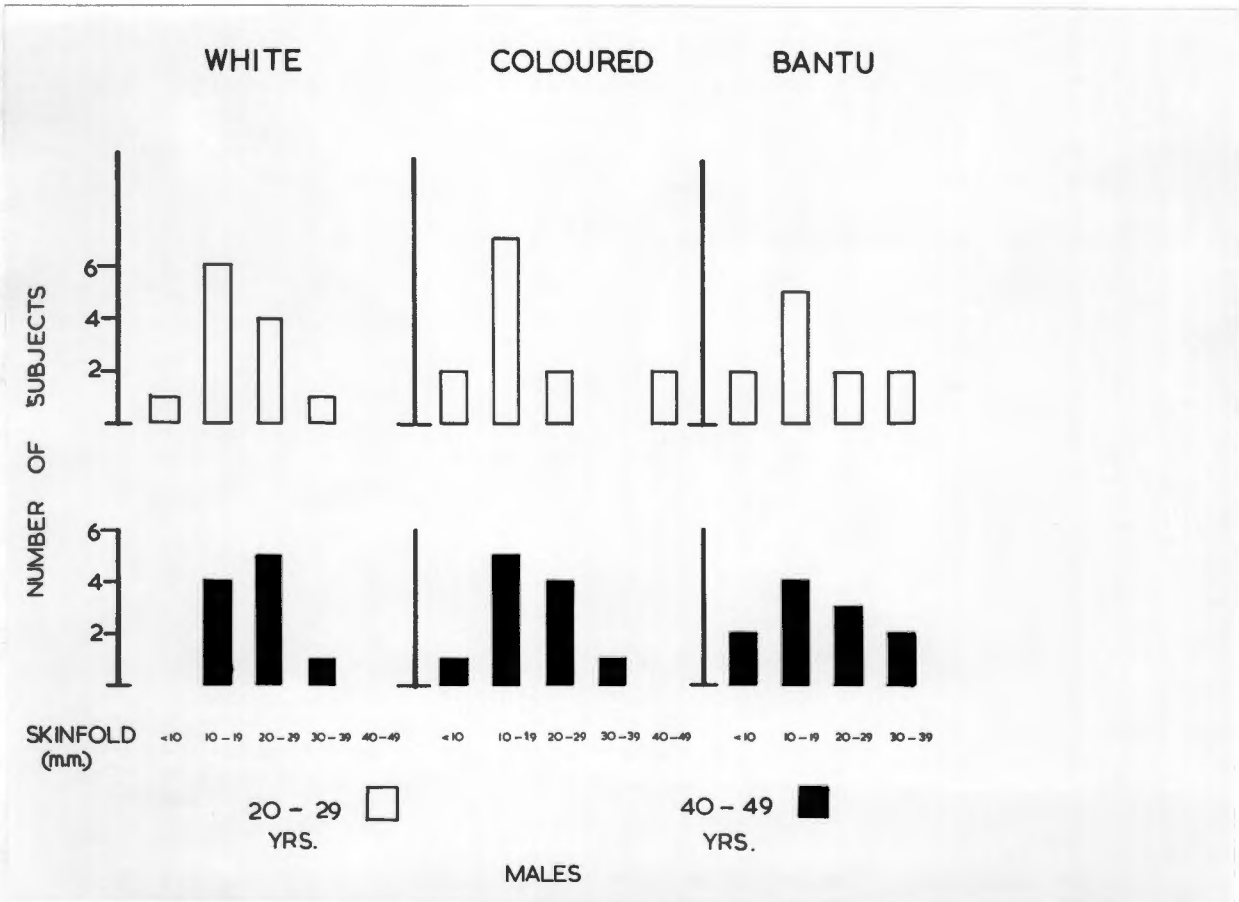
WHITE
COLOURED
BANTU
NUMBER OF SUBJECTS
6
4
2
SKINFOLD (mm.)
<10
10-19
20-29
30-39
40-49
20 - 29 YRS.
40 - 49 YRS.
MALES

FIG. 6

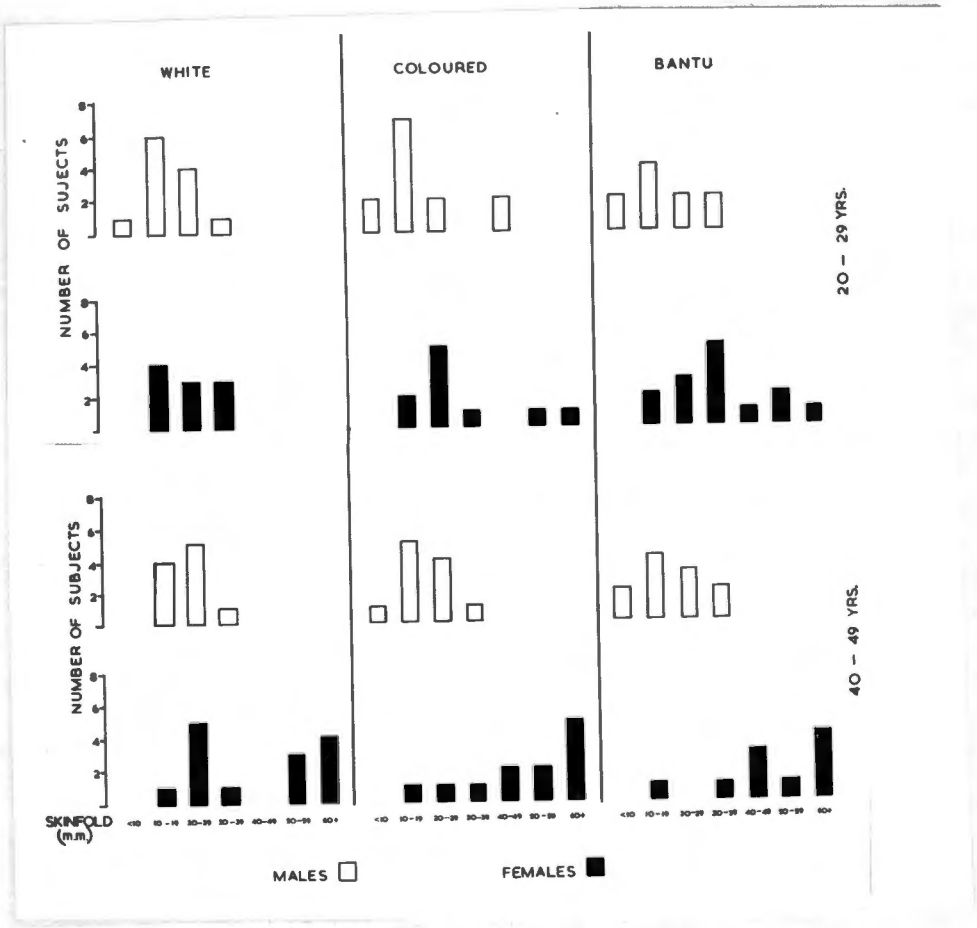

FIG. 7. Comparing the distribution of obesity between males and females at each age level in each race.

Note the well marked trend for females to be more obese than males in each race and at each age level.

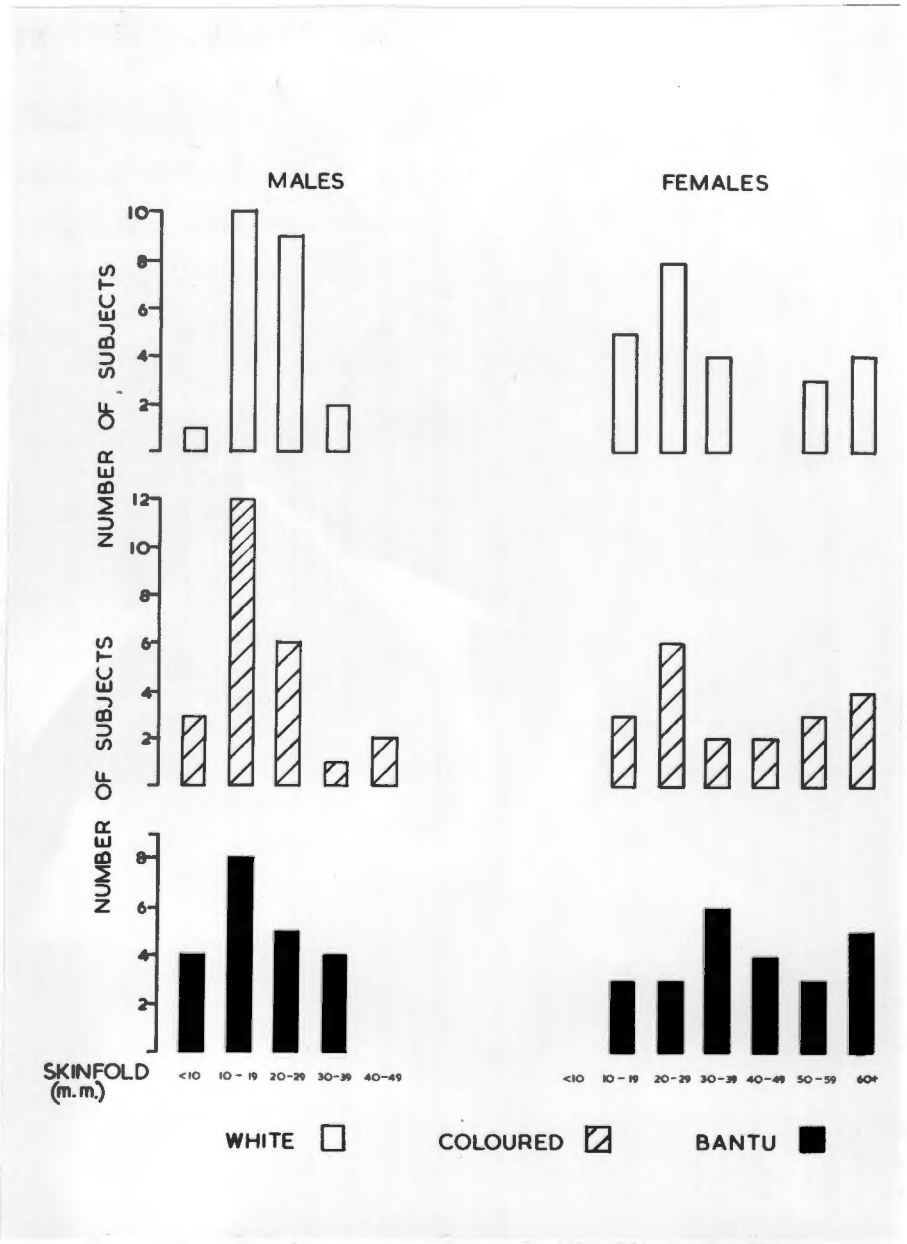

FIG. 8. The distribution of obesity, comparing the males and females according to race.

Note the trend for the degree of body fatness to decrease from White, through Coloured to Bantu among the males. The females do not appear to show differences between the races.

DIETARY DETAILS

The percentage of daily calories derived from the basic foodstuffs, carbohydrates, proteins and fat in each racial group has been quantitated by the methods described in Chapter 3.

The proportion of daily calories derived from each of these foods in both males and females is shown as a mean in Table 5 and Fig. 9.

SEX	Males			Females		
RACE	W.	C.	B.	W.	C.	B.
Fat	47.5	38.5	19.7	44.9	42.0	22.2
Carbohydrate	33.3	42.9	63.1	42.2	42.1	64.6
Protein	18.8	17.8	16.4	14.2	15.8	13.3

TABLE 5. The mean percentage of calories derived from each of the basic foodstuffs by the males and females in each racial group; White (W), Cape Coloured (C) and Bantu (B).

From Table 5 the racial differences in the proportion of calories derived from fat and carbohydrate is most striking. The protein moiety does not vary strikingly between the races among the males or females.

The fat intake has been further subdivided with regard to its origin, namely vegetable fat and animal fat. This is shown in Table 6 and Fig.10.

SEX	Males			Females		
RACE	W.	C.	B.	W.	C.	B.
Animal	103.3	76.5	31.3	78.8	70.7	29.6
Vegetable	13.7	15.9	15.1	20.0	20.0	14.0

TABLE 6. The mean daily intake in gm. of fat from animal and vegetable origin in the males and females of each racial group.

/From.....

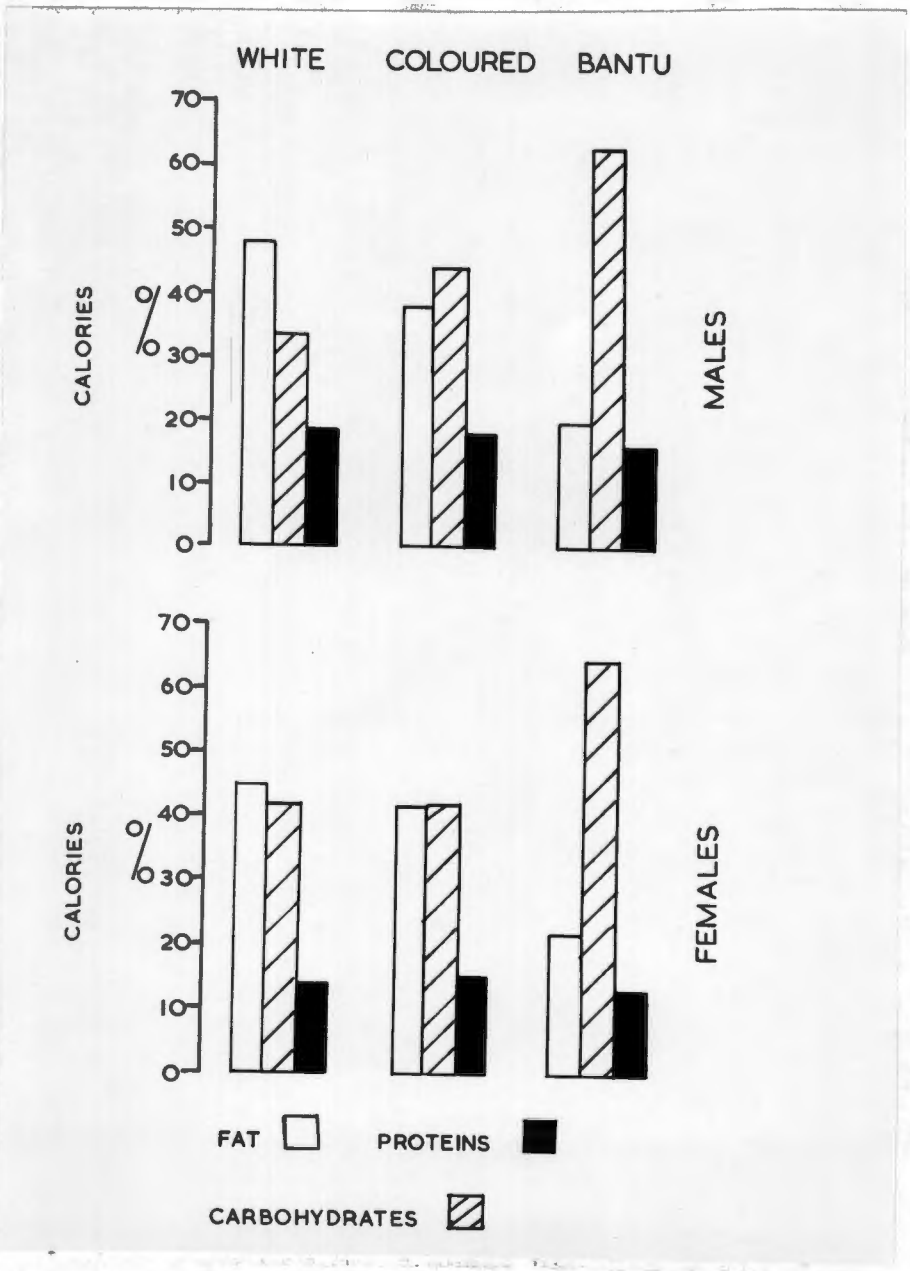

FIG. 9. The percentage of daily calories derived from fat, proteins and carbohydrate in each racial group among both the males and females. Note that the bulk of calories are provided by carbohydrate in the case of the Bantu; the White subjects derive the majority of their calories from fat.

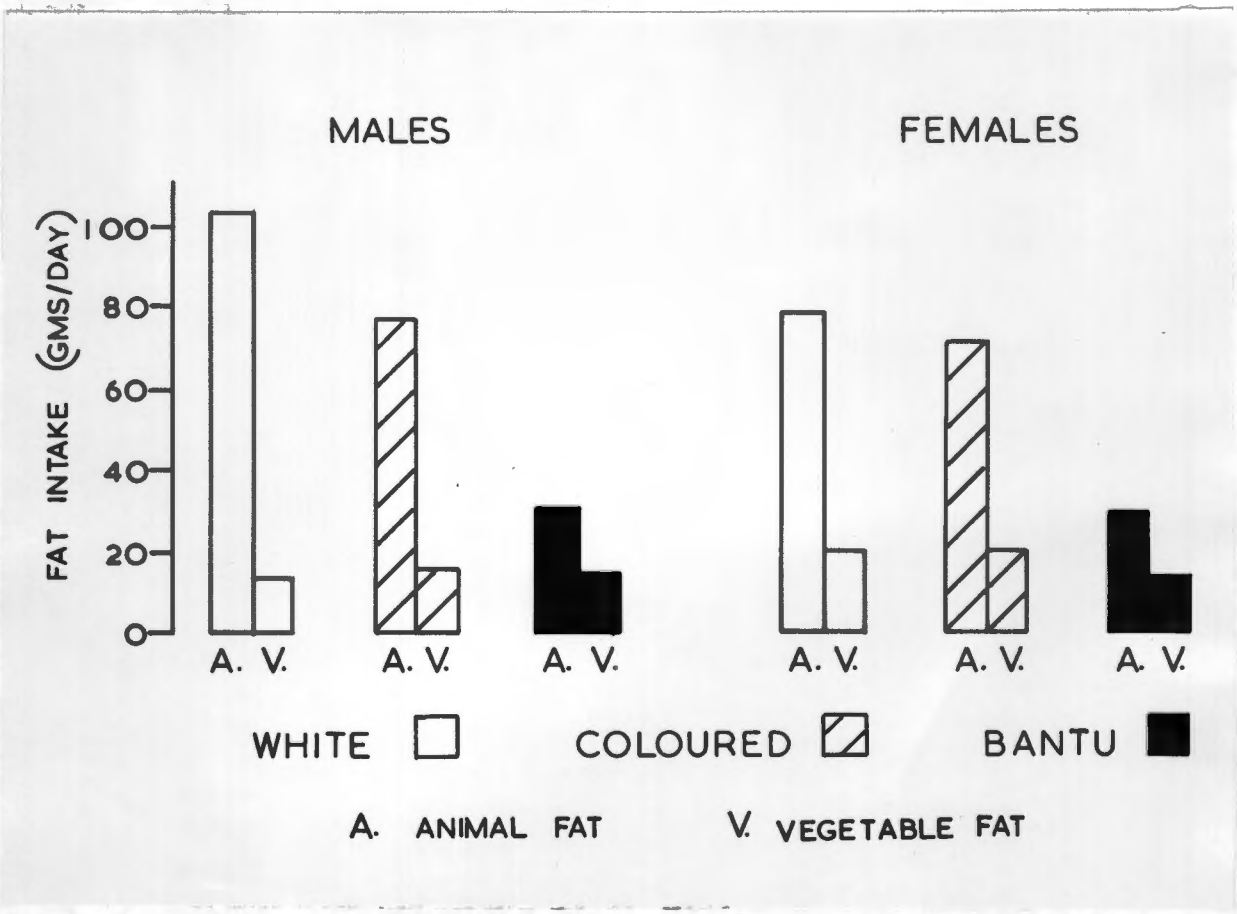

FIG. 10 The daily intake of fat in gm. from animal and vegetable sources among the males and females of each racial group shown as a mean.

Note the fall in animal fat intake from White, through Coloured to Bantu which is most striking amongst the males.

From Table 6 it can be seen that the relative intake of vegetable fat to animal fat decreases from Bantu through Coloured to White. This is most striking among the males. The difference between the Coloured and White females is not so striking. It will also be noted that the daily total fat intake falls from White, through Coloured to Bantu. This is most striking amongst the males. The Bantu and Coloured females have approximately the same intake as the males of the same race, but the intake of fat among the White females is lower than in the White males.

DISCUSSION

It is apparent that there are trends in serum cholesterol concentration and relative obesity with age and sex. These trends are different between the racial groups. The dietary differences between the racial groups is most striking.

SERUM CHOLESTEROL

(i) Effect of Age. White males and females both show a significant increase in serum cholesterol concentration with age (Table 1, Fig. 1). This trend has been established among White males in Cape Town, sampled from the general population, at the same age levels as the subjects of this study[2]. No levels have been established among local White females. It has been shown in a single American community, where the males showed an increase in serum cholesterol concentration with age, the females also had a rise over the same age range[3].

The Cape Coloured males also show a significant rise in mean cholesterol concentration with age (Table 1, Fig. 1). The increase with age in the Coloured females is not significant (Table 1, Fig. 1). The Coloured males sampled for this study conform to the findings previously reported for this race at these age levels[2]. There are no similar studies on Coloured females.

/The Bantu.....

The Bantu subjects show no significant change in serum cholesterol concentration with age; neither the males nor the females. The males again conform in this regard to the previously reported findings on this racial group in Cape Town[2]. There are again no similar studies on Bantu females.

The findings in these small groups of subjects show the trends with age reported in a previous study at the same age levels on the local racial groups. These findings serve to confirm that the trend for serum cholesterol concentration to increase with age is not a function of age but, as has been discussed in Chapter 1, is probably related to other factors. The different racial groups show different trends with age in both males and females.

(ii) Effect of Sex. There is no significant difference in serum cholesterol concentration between males and females at the same age levels in any of the racial groups (Table 2, Fig. 2). The White males in the 20 - 29 year age group show an impressively higher mean value mean value than the White females of the same age range, but this difference was not significant. It is worthy of note that the difference between means in these latter groups is considerably reduced on comparing White subjects in the 40 - 49 year age groups (Table 2). This serves to confirm the studies reported by Adlersberg et al.[3], who in a study on subjects ranging from 3 - 67 years showed that the rise with age in males occurs steeply at about 30 years with a levelling off soon after this age. Females continue to climb at a steady rate from the mid-twenties reaching the male values at about 50 years of age. It must again be emphasized that these are not general trends (viz. local Bantu), but would appear to occur where subjects show an increase with age. This serves to stress the relationship between nutritional and endocrine factors in the regulation of lipid metabolism (Chapter 1).

/(iii) Racial.....

(iii) Racial Differences. From Table 3 and Fig. 4 it can be seen that there are no significant differences between the racial groups among either the males or females in the age range 20 - 29 years. Inter-racial differences become manifest in the 40 - 49 year age groups (Table 4, Fig. 3), due entirely to an increase in the cholesterol concentration in the White and Cape Coloured subjects (see Table 1).

The mean serum cholesterol concentration falls in both males and females from White through Cape Coloured to Bantu (Fig. 3). This inter-racial pattern among the local groups has been demonstrated at the same age level[2] and at the age range 40 - 58 years[4] in males only. The females in this study (Table 4, Fig. 3) showing an identical pattern further serve to emphasise the dietary factors implicated by Bronte-Stewart et al.[4] in defining differences between the local racial groups.

RELATIVE OBESITY

(i) Effect of Age. It is clear from Fig. 4 that there is a trend in the subjects sampled, for the degree of obesity to increase with age in both males and females; a greater proportion of subjects having larger skinfolds in the 40 - 49 than in the 20 - 29 year age groups. These findings are in keeping with other studies (Chapter 2), on the trends with age in the size of the adipose organ[5,6]. This trend is well shown in the females sampled for this study, both in general (Fig. 4) and in each race (Fig.5). No other local study has been done on females, either in general or according to race. It is therefore not known whether these results are representative of the racial groups. It is however a common subjective impression that Bantu females over the age of 40 years are seldom lean.

The males show a less striking general increase with age (Fig. 4). Subdivision according to race makes the trend even less apparent (Fig. 6) although it appears to be there. No similar study on males has been reported but available data[7] on males in the local racial groups support these findings on small numbers of subjects.

/(ii) Effect of Sex.....

(ii) Effect of Sex. It is apparent from Fig. 7 that females in general tend to be more obese than males. The fact that these differences are present in each race and at both age levels emphasizes that this trend is a true biological variant, focussing attention on the possible role of sex hormones in determining the deposition of depot fat.

Racial Differences

It appears from Fig. 8 that White males,regardless of age, tend to be more obese than both Coloured and Bantu males. The difference in the distribution of obesity between White and Cape Coloured subjects has been reported(8). It was found that White men tend to be more obese than Coloured men. These latter differences were more marked than those found here, but this may be due to a sampling difference. No comparable data are available on Bantu men.

There are no striking trends between the races amongst females (Fig. 8).

DIETARY DETAILS

The very striking differences in the dietary habits of the local racial groups is readily apparent from Table 5 and Fig. 9. The Bantu derive the majority of their daily calories from carbohydrate at the expense of fat when compared with the White and Cape Coloured groups. The protein intake does not vary strikingly between the racial groups. The proportion of dietary calories provided by fat decreases from White through Coloured to Bantu. The racial differences in fat intake is more apparent when the total fat intake in the form of animal and vegetable fat is compared between the racial groups (Table 6, Fig.10). These differences between males are very marked and parallel the findings previously reported(4). The difference between the White and Coloured females is not as great as between the males in these racial groups; the White females showing a lower animal fat intake than the White males. It is not known whether this is a true difference in the dietary habits of White males and females in general as no similar study has been done on the general White population.

/ CONCLUSIONS.....

CONCLUSIONS

The subjects selected for this study show trends in serum cholesterol concentration with regard to both race and age that have been established for White, Cape Coloured and Bantu males in Cape Town. The females show similar trends according to race and age.

From what is known of the factors that influence serum cholesterol concentration (Chapter 1) it is not unexpected that females of the same race would in general follow the same trends as the males, allowing for the additional fluctuations introduced by the sex variable.

Males, and particularly females, show a trend for an increase in the size of the adipose organ with age. This is clearly evident among the females, regardless of race, but not so marked among the males in each race. The sexual difference in the size of the adipose organ is clearly evident among the subjects of this study; females in each race and at both age levels tend to be more obese than males. This then appears to be a true biological variable.

White males in general appear to have a greater tendency to obesity than both Coloured and Bantu males. The females do not appear to follow this racial pattern.

The dietary differences established for the local racial groups are closely reflected by the males in particular, but also among the females with respect to the quantity and quality of dietary fat. The proportion of dietary calories provided by the basic foodstuffs also show the traditional differences known to exist among these racial groups.

SUMMARY

The serum cholesterol concentration and the degree of body obesity in the subjects sampled for this study have been compared according to age, sex and race. The diets of

/ the subjects.....

the subjects in the racial groups in both males and females have been analysed. The subjects in general show the same trends with age, sex and race as has been established in local and other studies where similar studies have been made.

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CHAPTER 5

THE FATTY ACID COMPOSITION OF HUMAN DEPOT FAT

The data on the fatty acid composition in the depot fat of all the apparently healthy 137 subjects selected for this study have been combined. In Chapter 4 the different trends with age in serum cholesterol concentration among both the males and females in the racial groups was demonstrated. The marked qualitative differences in the dietary habits of the different racial groups and the variations in the relative degree of obesity according to age, race and sex was also shown in Chapter 4. These subjects between them therefore represent extremes in the degree to which lipid metabolism may be influenced by the interactions of age, sex and diet. It is therefore of interest to determine the qualitative and quantitative variations in the type and proportion of fatty acids found in the depot fat in this cross-section of man.

RESULTS

Table I shows the fatty acids found in human depot fat as well as the percentage of each fatty acid expressed as a mean, with 1 standard deviation and the range. In the case of C20 : 4, only a range is given as the accuracy with which this component could be quantitated was poor (see Chapter 3).

/ TABLE I.....

FATTY ACID	MEAN	S.D.++	RANGE
C12 : 0	0.4	0.2	0.1 - 1.3
C14 : 0	3.4	1.2	0.8 - 8.6
C14 : 1	1.0	0.1	0.2 - 3.2
C15 : 0	0.6	0.3	0.2 - 1.8
C15 ?	0.4	0.1	0.1 - 1.2
C16 : 0	22.7	2.6	13.0 - 30.1
C16 : 1	8.4	2.2	2.4 - 15.1
C17 : 0	0.6	0.3	0.2 - 1.9
C17 ?	0.8	0.3	0.4 - 1.9
C18 : 0	4.3	1.6	1.6 - 9.8
C18 : 1	45.35	4.1	33.6 - 56.3
C18 : 2	9.7	3.6	3.8 - 20.7
C18 : 3	0.7	0.4	0.2 - 2.8
C20 : 0	0.9	0.5	trace - 2.6
C20 : 1	0.9	0.5	trace - 2.2
C20 : 4			0 - 0.5

TABLE I. The fatty acid composition of human depot fat. The percentage of each fatty acid based on the analysis of samples from 137 apparently healthy subjects is shown.

++S.D. = 1 standard deviation.

The underlined components represent 93.8% (± 1.6%) of the total fatty acids found.

A feature that is apparent from Table I is the consistency with which each of the first 15 components invariably appeared in every sample analysed although some were present in only trace amounts. This was the case in every subject regardless of age, race or sex.

THE COMMON FATTY ACIDS

The greatest interest has been centred on the major components: C14 : 0, C16 : 0, C16 : 1, C18 : 0, C18 : 1 and C18 : 2. These 6 fatty acids are the more abundant components in every subject and constitute 93.8% (± 1.6%) of the total fatty acids found (Table 2).

/The reproducibility.....

The reproducibility of the method in determining the percentage of these 6 components has been shown in Chapter 3. They therefore provide a sound basis for comparative analyses and will be the fatty acids used for assessing various other factors in the subsequent chapters.

	MALES		FEMALES		ALL SUBJECTS
	20 - 29	40 - 49	20 - 29	40 - 49	
RACE	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
W	93.2 (1.6)	93.8 (1.4)	92.8 (2.2)	92.4 (1.7)	93.8 (1.6)
C	93.1 (1.5)	93.8 (1.4)	94.0 (1.7)	94.3 (1.2)	
B	94.3 (1.1)	94.8 (0.8)	94.5 (0.9)	94.9 (1.1)	

TABLE 2. The percentage of the total fatty acids in human depot fat represented by the sum of the 6 common components: C14 : 0, C16 : 0, C16 : 1, C18 : 0, C18 : 1 and C18 : 2, in White (W), Cape Coloured (C) and Bantu (B) groups at each age level and each race and sex.

The frequency distribution for each of these major components in the subjects studied is shown in Fig. 1. These closely follow normal distribution curves.

/ DISCUSSION.....

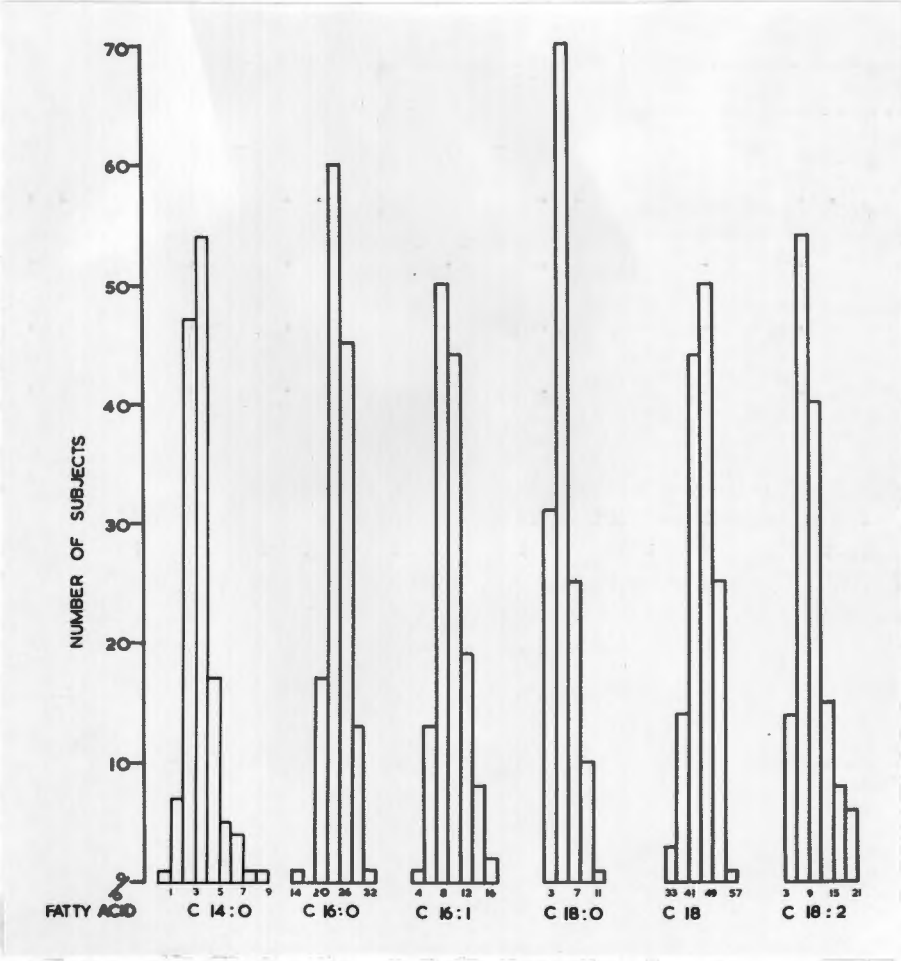

FIG. 1. The frequency distribution for the common fatty acids among all the apparently healthy subjects studied.
Note the normal distribution curves for each component with the possible exception of C14:0 and C18:2 which show slight skewing to the right. This slight skewing is due to differences in the proportion of these fatty acids between the racial groups (see Chapter 9).

DISCUSSION

The most abundant unsaturated fatty acid in human depot fat is obviously oleic acid (C18 : 1) and the most abundant saturated component is palmitic acid (C16 : 0). Hilditch[1] points out that oleic acid (C18 : 1) is undoubtedly the most widespread of all natural fatty acids. It is commonly an abundant fatty acid and at the time of his writing (1956) had been found absent from no natural fat. The characteristic saturated fatty acid in natural fats is undoubtedly palmitic acid (C16 : 0) and like oleic acid is usually present in considerable proportion, being absent in very few, if any, of the natural fats[1]. Human depot fat, as can be seen from Table 1 is no exception to this. The other unsaturated fatty acids in depot fat, linoleic (C18 : 2) and palmitoleic (C16 : 1), are also commonly found in other natural fats and oils. The saturated acids myristic (C14 : 0) and stearic (C18 : 0) are similarly common constituents of other natural fats and oils. Human depot fat is obviously not characterised by a distinct type or very unusual distribution of fatty acids different from that found in nature in general among land and sea animals and vegetables.

The reported work to date on the composition of human body fat is still scanty. Jaeckle[2] in 1902 reported quantitative values for palmitic, stearic and oleic acids. His methods, however, did not reveal the presence of many other acids now known to be present in fair proportion and his findings must be regarded as qualitative only. Eckstein[3] in 1925 made a more detailed analysis. He reported the presence of 26.6% saturated fatty acids and 63.6% of unsaturated acids. In addition to confirming the presence of palmitic, stearic and oleic acids he reported finding traces of lauric acid, 1% of myristic acid and less than 1% each of linoleic, linolenic and arachidonic acids. The presence of linoleic and arachidonic, but not of linolenic acid were soon thereafter confirmed[4].

/Subsequent reports.....

Subsequent reports on the composition of human body fat[5,6,7,8] did not add to the earlier knowledge. The earliest report that provided accurate analytical data comparable to that found by gas-liquid chromatography was made by Cramer and Brown[9]. These authors made studies on depot fat obtained at autopsy of 5 subjects (1 female, 3 males and 1 not stated) ranging in age from 53 - 74 years, who died from causes which these authors considered to be of no significance in the composition of the fat specimen. (It is of interest that 2 of their subjects are recorded as having "arterio-sclerosis"). In addition, they were not particular about the site sampled. These included the "abdominal regions, pannicular, mesenteric, perirenal, etc." The specimens were analysed by ester fractionation together with low temperature crystallization. Their figures show good agreement for the percentage of the component fatty acids between the 5 subjects studied. Two later reports cited by Hilditch[1] were in good agreement with regard to the type of fatty acid found by Cramer and Brown, although there was marked variation in the proportion of some of the components. Two other reports have appeared to date[10,11]. These latter analyses of depot fat were made by gas-liquid chromatography.

COMPARISON OF DATA

The data of Hirsch et al.[10], Gellhorn and Marks[11] and those of Cramer and Brown[9] have been compared with the results obtained in this study (Table 3). There is good agreement between the 4 sets of data. The variations between the mean values of each component in the 4 sets of data are certainly within the range found for the major components in this study. This can be confirmed by reference to Table 1.

/TABLE 3.....

FATTY ACID	(10) n = 12	(11) n = 9	(9) n = 5	n = 137
12 : 0	0.7	0.4	0.5	0.4
14 : 0	3.3	2.4	3.5	3.4
14 : 1	0.6	-	0.4	0.1
16 : 0	19.5	24.6	25.0	22.7
16 : 1	6.9	5.6	6.4	8.4
18 : 0	4.2	6.0	7.0	4.3
18 : 1	46.3	49.9	45.9	45.3
18 : 2	11.4	9.5	9.6	9.7
20 : 4	0.2	-	0.7	about 0.2
other C20 acids	1.7	-	1.2	1.8
TOTAL	94.8	98.4	100.2	96.3

TABLE 3. Mean fatty acid composition (%) of human adipose tissue. Only those components found by Cramer & Brown have been shown.
The percentage of the fatty acid components in human adipose tissue as reported by Hirsch et al.(10), Gellhorn and Marks (11) and Cramer and Brown (9) are compared with the findings in this study.

The more detailed information provided by gas-liquid chromatography as reported by Hirsch et al.(10) have been compared with the findings in this study (Table 4). It can be seen that some minor components found by Hirsch et al. were not detected here. These other components constitute a very small percentage of the total fatty acids. Their non-detection in this study is probably technical, the sensitivity arrangements on the apparatus used here being inadequate (Chapter 3). Gellhorn and Marks(11) found that the 7 fatty acids shown in Table 3 accounted for about 98% of the total fatty acids in their study. These same fatty acids constituted 94.2% of the findings in this study. Gellhorn and Marks found that on analysis depot fat fatty acids, by means of a capillary column, a further 26 fatty acids were detectable. All of these latter components however comprised less than 2% of the total fatty acid composition. It is therefore clear that fatty acid analysis by gas-liquid chromatography using apparatus more sensitive than was available here

/discloses.....

discloses the presence of a great number of fatty acids not detected in this study. Quantitatively however these additional components contribute little to the fatty acid composition of depot fat.

FATTY ACID	(10) MEAN ± S.D. n = 12	(11) MEAN ± S.E.++ n = 9	MEAN ± S.D. n = 137
12 : 0	0.7 ± 0.1	0.35 ± 0.025	0.4 ± 0.2
14 : 0	3.3 ± 0.1	2.40 ± .069	3.4 ± 1.2
14 : 1	0.6 ± 0.05		1.0 ± 0.1
14 : 0 branched	0.1 ± 0.1		-
15 : 0	0.6 ± 0.1		0.6 ± 0.3
15 : 0 ?	0.3 ± 0.3		0.4 ± 0.1
16 : 0	19.5 ± 2.1	24.60 ± .267	22.7 ± 2.6
16 : 1	6.9 ± 0.1	5.60 ± .253	8.4 ± 2.2
17 : 0	0.2 ± 0.2		0.6 ± 0.3
17 : 0 ?	1.0 ± 0.3		0.8 ± 0.3
18 : 0	4.2 ± 1.1	6.0 ± .267	4.3 ± 1.6
18 : 1	41.2 ± 4.4	+++ 49.9 ± .325	+++ 45.3 ± 4.1
18 : 1 isomers	5.1 ± 1.0		-
18 : 2	11.4 ± 1.4	9.5 ± .143	9.7 ± 3.6
18 : 3	0.4 ± 0.1		0.7 ± 0.4
19 : 0 branched	0.5 ± 0.2		-
20 : 0	0.6 ± 0.1		0.9 ± 0.5
20 : 1	0.6 ± 0.3		0.9 ± 0.5
20 : 2	0.1 ± 0.1		-
20 : 2 (unidentified)	0.1 ± 0.1		-
20 : 3	0.2 ± 0.1		-
20 : 4	0.2 ± 0.1		0 - 0.5

TABLE 4. The percentage of the fatty acid components in human depot fat as reported by Hirsch et al.(10) and Gellhorn and Marks(11) are compared with the findings in this study. The underlined components represent 91.6% of the total fatty

++ = Standard Error.

+++ = Represents oleic acid and its isomers.

/acids in the.....

acids in the data presented by Hirsch et al., 98% of the total in the study by Gellhorn and Marks and 93.8% in this study.

++ = Standard Error.
+++ Represents oleic acid and its isomers.

The isomers of oleic acid were not chromatographically separated in this study, nor by Gellhorn and Marks.

This study will be concerned only with the common fatty acids. The relative proportion of these components, C14 : 0, C16 : 0, C16 : 1, C18 : 0, C18 : 1 and C18 : 2, agrees very closely with the findings by Hirsch et al. and Gellhorn and Marks (Table 4). The differences possibly worthy of comment are the higher percentages of C16 : 0 and C16 : 1 and the lower percentage of C18 : 2 in the local data. Also the standard deviation for the values of C14 : 0, C16 : 1 and C18 : 2 given by Hirsch et al. are considerably smaller than in this study. The findings by Gellhorn and Marks show a remarkable homogeneity, much greater even than that of Hirsch et al. Their mean values however again agree well with the findings in this study. A possible explanation for the greater variations in the fatty acids found here may be related to the subjects sampled. The subjects sampled by Hirsch et al.[10] appear to have been drawn from a fairly homogeneous population with regard to diet and totalled 12. The local subjects were deliberately selected from different racial groups whose dietary habits are markedly different (Chapter 4) and totalled 137. Gellhorn and Marks however sampled subjects regardless of age, sex or ethnic origin, coming from all economic and national groupings within Metropolitan New York. They therefore assumed differences in dietary habits. Such an assumption is not readily justified. It has often been shown that immigrants from one country to another take on the dietary patterns and reflect the trends in serum cholesterol as is the trend in the adopted country. This is evident where immigrants have migrated to a country with socio-economic conditions more highly developed than in their homelands[12]. It is therefore likely that the dietary habits of their subjects were more homogeneous than they have assumed. They further do not state the numbers of

/people.....

people from different ethnic groups, what the different groups were nor the number of males and females sampled. They studied a total of 20 subjects and give the values for 9 only. It will subsequently be shown that there are some differences between the various groups of subjects sampled for this study. This probably accounts for the relatively greater variation in the percentage of the various fatty acid components found here.

In general the most impressive feature of this study, on such a diversity of subjects as are available here, is the homogeneity in the depot fat fatty acid composition, both qualitatively and in their relative proportion. This serves to emphasize the ability of the adipose organ to regulate its fatty acid composition, a conclusion reached by Hirsch et al. and Gellhorn and Marks. Dietary factors, particularly the fat intake, would therefore appear to be of relatively minor importance in determining the depot fat composition. Such a statement however needs to be qualified. It will be recalled from above that the depot fatty acids common in human adipose tissue are also common in other natural fats. In a study such as this, which reflects only a static state, it is not possible to readily evaluate the relative contribution made by diet and depot fat regulatory mechanisms in maintaining a characteristic fatty acid composition. This question will be considered more fully in subsequent chapters.

CONCLUSIONS

The depot fat fatty acids of man are remarkably uniform. This is evident both in regard to the type of fatty acid present and the proportion of each fatty acid. Six fatty acids, C14 : 0, C16 : 0, C16 : 1, C18 : 0, C18 : 1 and C18 : 2 represent more than 90% of the total fatty acids and the frequency distribution of the percentage of each of these 6 fatty acids among the subjects sampled closely follows a normal distribution curve. This uniformity is noteworthy because of the subjects sampled for this study; each racial group subsisting on a diet different from the other. These findings would appear to confirm the view that the adipose organ is able to maintain a fairly constant fatty acid composition despite considerable dietary variations.

/SUMMARY.....

SUMMARY

The fatty acid composition of human depot fat in 137 apparently healthy subjects has been determined. These subjects were drawn from males and females, at 2 age levels, in White, Cape Coloured and Bantu adults. The findings show a remarkable degree of uniformity, both qualitatively and quantitatively for the fatty acids found. The findings have been compared with similar reports of human depot fat fatty acid analyses and show close agreement in the mean values. The common fatty acids, their nature and relative proportion, have been identified on the basis of this and other similar studies.

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CHAPTER 6

THE COMPOSITION OF FAT IN THE BUTTOCK REGION COMPARED WITH THAT AT OTHER SITES

These studies were based on an analysis of fat aspirated from the buttock region. If any conclusions are to be drawn from this work it is necessary to know whether fat sampled at this site is representative of the whole adipose organ. It has been shown in animals that the composition of depot fat varies with increasing depth from the skin surface. In man, the greatest accumulation of adipose tissue is subcutaneous (Chapter 2). A considerable proportion of depot fat is however found in the abdominal mesentery and omentum, the perinephric and pericardial regions.

It has already been shown that depot fat does not vary in composition with increasing depth from the surface in the region of the buttock in man (Chapter 3). It would therefore appear that in man the subcutaneous layer of fat does not vary in composition with increasing depth from the surface. It must however be determined whether the fatty acid composition in the buttock region is representative of other subcutaneous sites. It must also be determined whether the considerable fat deposits in the body cavities differ in composition from the subcutaneous layers. Fat was therefore sampled from several subjects by aspiration from the buttock and excision from one or two other sites during surgical procedures and their composition compared.

RESULTS

The major fatty acid components expressed as a percentage of the total fatty acids found in the adipose tissue taken from 5 subjects at 2 or 3 sites is shown in Table 1.

/TABLE 1.....

		FATTY ACID (%)					
SUBJECT	SITE	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
1	Buttock	2.7	20.3	8.5	3.4	49.2	10.3
(female)	Omentum	3.2	21.5	6.7	5.7	46.0	10.6
	Abdominal Wall	3.2	21.2	8.0	4.6	48.1	9.6
2	Buttock	2.3	22.4	8.7	6.3	47.1	8.0
(male)	Pericardial	2.9	24.7	8.9	6.8	42.5	9.4
	Chest wall	2.7	26.3	7.6	6.8	44.7	8.3
3	Buttock	2.8	27.7	8.7	4.8	44.5	7.1
(female)	Breast	3.3	30.9	8.6	5.2	41.6	6.6
4	Buttock	3.2	20.8	8.8	4.3	47.7	8.6
(female)	Thigh	2.7	18.7	9.8	3.9	48.6	9.9
5	Buttock	3.8	23.7	5.4	6.9	45.7	10.3
(male)	Perirenal	3.2	23.4	5.6	8.0	45.9	8.4

TABLE 1. The percentage of the major fatty acid components of adipose tissue obtained from the buttock region by needle aspiration and from other superficial and deep sites obtained by excision, are compared.

From Table 1 it can be seen that there are only small differences in the fatty acid composition of the adipose tissue obtained from different sites in each subject despite considerable differences for some of the components between subjects.

DISCUSSION

It has been shown in animals that the composition of adipose tissue varies somewhat at different sites. Spaeth[1] in 1893 reported that the degree of unsaturation of hog depot fat decreases with increasing depth from the surface. Lummert[2] in 1898 reported similar trends in dogs. Henriques and Hansen[3] in 1901 reported that the fat from the back of the pig has a melting point related to the environmental temperature. They found that in a pig kept at a room temperature of 30 - 35°C. for 2 months the

/iodine number.....

iodine number of the fat of the back was 69.4. In a litter mate maintained on the same diet and kept at a temperature of 0°C., the fat from this site had an iodine number of 72.3. Fat from this site in a third litter mate, also on the same diet and maintained at a temperature of 6°C. but protected by a fur coat, had an iodine number of 67.0. They concluded that fat was laid down differently in regions of different temperature. Low environmental temperature tended to result in the deposition of low-melting fat (high iodine number) while higher environmental temperatures resulted in the deposition of higher-melting fats, with a lower iodine number. These findings on the degree of unsaturated related to temperature have been confirmed by Dean and Hilditch. (4)

This adaptation to cold would appear to be directed at ensuring that the body fat did not solidify. In birds, who are apparently able to protect their skin from external temperature changes, the composition of the superficial and deep layers of body fat is reported to be fairly uniform(5).

Fishes too, show a similar phenomenon(6), those living in cold environments tending to have a more unsaturated body fat than those living in warmer waters.

This apparent rule has its exceptions. The rat, for example, does not show this stratification in the type of fat laid down(7). There are also studies reported on other animals,cited by Deuel(5), like the Indian dugong or sea-cow and the porpoise, both with a high body temperature, but with body fat similar to that of the cold-blooded fishes. It would appear that although environmental temperature may be a factor influencing the type of body fat found in some animals, this is not a universal phenomenon. The changes that do occur appear to be relatively minor and do not seem able to radically alter the depot fat fatty acids in those animals who show the phenomenon. The information provided by iodine value determinations does not indicate the specific fatty acid changes brought about by environmental temperature alterations. There is therefore no precise information on the type of change produced in the fatty acids.

/In the case.....

In the case of man, environmental temperature appears to play a very minor role, if any, in determining the fatty acid composition of depot fat. Cuthbertson and Tompsett determined the iodine value of samples of human adipose tissue obtained from the superficial and deep layers of a very thick anterior abdominal wall and found them to be the same. They also sampled omental perinephric and pericardial fat. Again there was little variation, except that the perinephric fat was slightly more saturated than that at other sites. They concluded that the differences were probably not significant. Hirsch et al.[9], using gas-liquid chromatography, compared the fatty acid composition of fat obtained from the superficial and deep abdominal panniculus; from the buttock, abdomen, thigh, arm and interscapular area; and the buttock, psoas muscle region, omentum, perinephric area and pericardium. The differences in composition between superficial and deep sites were small and they considered that these were probably not of significance. Moore and Cook[10] also reported small differences between subcutaneous and deep sites, the former tending to have a slightly higher iodine value.

The data reported here also show small differences between sites of the order reported by Hirsch et al. There does not appear to be any difference of note between the sample aspirated from the buttock and other subcutaneous sites in subjects 1, 2 and 4 (Table 1). In subject 3, the mammary gland fat is slightly more saturated, but whether this would be a consistent trend is yet to be established. In subjects 1, 2 and 5, superficial and deep sites have been compared. In subjects 1 and 2 there does not appear to be any striking difference in the degree of saturation between subcutaneous and body cavity sites. Subject 5 does show a slightly more saturated fat in the perirenal tissue than over the buttock. Cuthbertson and Tompsett[8] also found that the perirenal fat was slightly more saturated than that obtained at other superficial and deep sites; Hirsch et al.[9] comment only that the pericardial fat was possibly more saturated than that found at other sites. The differences found here between pericardial, buttock and chest wall fat are not striking.

/The data.....

The data available on the uniformity of adipose tissue composition in man is rather limited. It does however appear that only small differences exist. Many more studies would have to be done to confirm the consistency of these apparently small differences before their significance can be assessed.

In view of the reported influence of environmental temperature on the composition of body fat in some animals the findings in man are, on reflection, not surprising. Man is after all able to protect himself from the extremes of environmental temperature and succeeds in maintaining a fairly constant skin temperature. As in the case of the "fur-clad hog", this protection probably ensures the deposition of fat of a constant composition. From the available data in man it appears that the effects of temperature, at most of relatively minor influence in susceptible animals, is not a significant factor in determining the body fat composition in man.

CONCLUSIONS

The composition of the subcutaneous fat sampled at several sites is closely reflected by the composition of adipose tissue obtained by needle aspiration from the buttock region. The composition of the adipose tissue obtained from deep deposits show some small differences which are probably not of significance.

It is concluded that the needle aspirate from the buttock region provides a sample of adipose tissue the fatty acids of which are representative of the depot fat fatty acids throughout the body.

SUMMARY

The composition of the depot fat sampled from the buttock region by needle aspiration has been compared with that at other superficial and deep sites. There is good agreement between the buttock aspirate and other superficial sites. Some deep sites tend to show a slightly higher proportion of saturated fatty acids. These differences however are small and probably not of significance.

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CHAPTER 7.

THE RELATIONSHIP BETWEEN AGE AND DEPOT FAT FATTY ACID COMPOSITION

The changes that have been shown to occur in lipid metabolism with age have been outlined in Chapters 1 and 2. The subjects of this study reflect some of the reported trends.

It will be recalled from Chapter 4 that both men and women show an increase in the size of the adipose organ with age. This is most marked in the females sampled, but also occurs amongst the males; both in general and in each racial group. An increase in serum cholesterol concentration with age was seen in the White subjects in both males and females and in the Coloured males. The Coloured females showed a distinct rise in the mean value which was, however, not significant. The Bantu subjects, males and females, showed no increase in serum cholesterol concentration with age.

The subjects of this study are therefore of particular interest because the racial groups show different trends with age in regard to serum cholesterol concentration. It will now be determined whether there are trends with age in depot fat composition; in general and in each racial group.

RESULTS

The fatty acids to be considered here are the common fatty acids: C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2. The percentage of each of these fatty acids in the 20 - 29 year age group has been compared with that in the 40 - 49 year age group. Because of certain differences that are evident between the sexes the effect of age has been tested separately in each sex; both regardless of race and in each racial group.

/The mean values.....

The mean values for the percentage of each of the fatty acids under consideration, comparing the age groups in both males and females, regardless of race, is shown in Fig.1. The mean percentage of each fatty acid, 1 standard deviation, the difference between means and the significance of this difference is shown in Table 1.

	MALES						FEMALES					
	20-29 yrs.		40-49 yrs.				20-29 yrs.		40-49 yrs			
FATTY ACID	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
C14:0	3.7	(1.4)	3.3	(1.1)	0.4	N.S.	3.3	(1.1)	3.1	(1.1)	0.2	N.S.
C16:0	23.0	(2.6)	23.7	(2.6)	0.7	N.S.	21.9	(2.0)	22.2	(2.7)	0.3	N.S.
C16:1	7.1	(1.6)	7.8	(1.8)	0.7	N.S.	9.5	(2.2)	9.1	(2.5)	0.4	N.S.
C18:0	5.4	(1.9)	5.3	(1.4)	0.1	N.S.	3.3	(1.1)	3.6	(1.0)	0.3	N.S.
C18:1	44.4	(3.8)	44.7	(4.2)	0.3	N.S.	45.3	(3.6)	46.9	(3.7)	1.6	N.S.
C18:2	9.9	(3.4)	9.8	(3.2)	0.1	N.S.	10.4	(3.7)	8.9	(3.7)	1.5	N.S.

TABLE 1. The mean value, 1 standard deviation (S.D.), the difference between the means (diff.), and the significance of this difference (P.), for the percentage of each fatty acid, comparing the 20 - 29 with the 40 - 49 year age groups in each sex, regardless of race, is shown. There is no significant difference between age groups for any fatty acid in either males or females.

It can be seen that there is no significant difference between age groups in either males or females for any of the fatty acids. Age, as such, has therefore no effect on the depot fat fatty acid composition in either the males or the females studied here.

/The effect.....

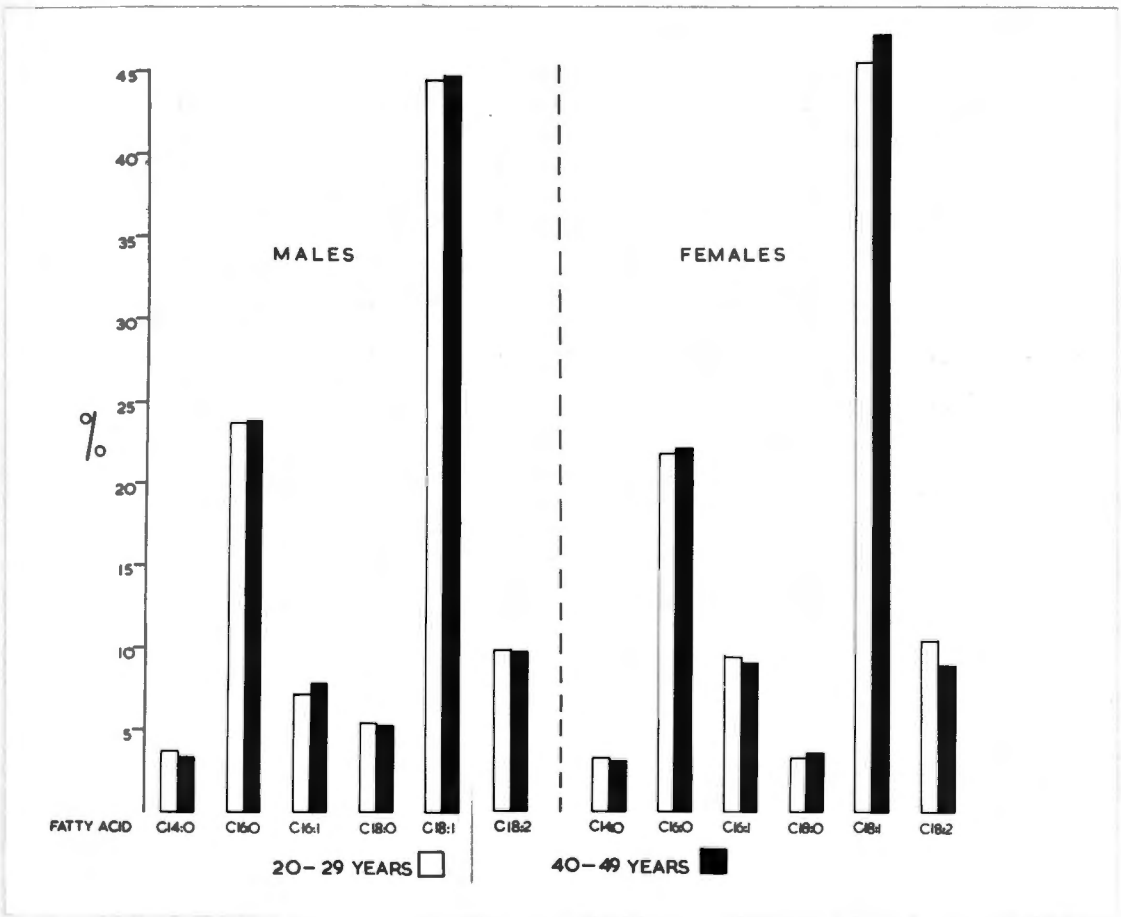

FIG. 1. The mean values for the percentage of the common fatty acids comparing the 20 - 29 with the 40 - 49 year age groups in the males and females.

The effect of age on the depot fat fatty acid composition will now be considered in the males and females within each racial group. Tables 2 - 7 show the mean percentage, 1 standard deviation (S.D.), the difference between means (diff.), and the significance of this difference for White (W), Cape Coloured (C) and Bantu (B) males and females for the fatty acids C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2 respectively.

C14 : 0 (%)

	MALE						FEMALE					
AGE(Yrs)	20 - 29		40 - 49				20 - 29		40 - 49			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	4.3	(1.5)	3.9	(1.4)	0.4	N.S.	4.2	(0.9)	3.8	(1.2)	0.4	N.S.
C	3.8	(1.6)	3.5	(0.8)	0.3	N.S.	3.5	(1.2)	3.0	(0.6)	0.5	N.S.
B	2.8	(0.3)	2.5	(0.5)	0.3	N.S.	2.6	(1.2)	2.3	(0.9)	0.3	N.S.

TABLE 2. There is no significant difference between age groups among either the males or females in any of the racial groups in the percentage of C14:0.

C16 : 0 (%)

	MALE						FEMALE					
AGE(Yrs)	20 - 29		40 - 49				20 - 29		40 - 49			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	23.6	(1.4)	24.8	(2.3)	1.2	N.S.	21.3	(0.9)	22.1	(2.9)	0.8	N.S.
C	23.3	(2.8)	23.9	(3.0)	0.6	N.S.	20.8	(1.8)	22.9	(2.6)	2.1	<0.05
B	22.1	(3.5)	22.4	(2.2)	0.3	N.S.	23.0	(2.2)	21.7	(2.7)	1.3	N.S.

TABLE 3. The Coloured females show a difference with age which is probably significant. No other group shows a significant difference.

/C16 : 1(%).....

C16 : 1 (%)

	MALES				FEMALES			
AGE(Yrs)	20 - 29	40 - 49			20 - 29	40 - 49		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W	8.1 (1.1)	8.2 (1.4)	0.1	N.S.	9.2 (2.4)	9.9 (1.5)	0.7	N.S.
C	6.5 (1.5)	6.7 (1.8)	0.2	N.S.	7.7 (1.1)	7.0 (1.5)	0.7	N.S.
B	6.7 (1.7)	8.5 (1.7)	1.8	<0.05	11.0 (1.7)	10.5 (2.9)	0.5	N.S.

TABLE 4. The Bantu males show a difference with age which is probably significant. No other group shows a significant difference.

C18 : 0 (%)

	MALES				FEMALES			
AGE(Yrs)	20 - 29	40 - 49			20 - 29	40 - 49		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W	4.7 (1.3)	4.3 (1.2)	0.4	N.S.	3.4 (1.0)	3.2 (0.9)	0.2	N.S.
C	5.5 (2.0)	4.9 (1.6)	0.6	N.S.	3.7 (1.0)	4.1 (0.8)	0.4	N.S.
B	6.1 (2.1)	4.9 (1.4)	1.2	N.S.	3.0 (1.1)	3.5 (1.2)	0.5	N.S.

TABLE 5. There are no significant differences between age groups in any of the racial groups, male or female.

C18 : 1 (%)

	MALES				FEMALES			
AGE(Yrs)	20 - 29	40 - 49			20 - 29	40 - 49		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W	44.5 (2.7)	45.7 (3.2)	1.2	N.S.	46.6 (4.9)	46.2 (4.1)	0.4	N.S.
C	42.3 (3.9)	41.7 (4.3)	0.6	N.S.	43.9 (2.5)	44.9 (2.2	1.0	N.S.
B	46.8 (3.5)	47.1 (2.8)	0.3	N.S.	45.5 (3.1)	50.1 (2.0)	4.6	<0.001

TABLE 6. The difference between Bantu females is highly significant. There is no difference between other racial group

/C18:2 (%).....

C18 : 2 (%)

	MALES					FEMALES				
AGE(Yrs)	20 - 29		40 - 49			20 - 29		40 - 49		
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	8.0	(2.0)	6.9	(1.6)	1.1	N.S.	8.0	(2.2)	7.3	(2.0)	0.7	N.S.
C	11.6	(4.4)	12.9	(3.8)	1.3	N.S.	14.3	(3.3)	12.4	(4.0)	1.9	N.S.
B	9.8	(2.2)	9.3	(2.0)	0.5	N.S.	9.4	(2.5)	6.8	(1.6)	2.6	<0.01

TABLE 7. The difference between Bantu females is significant. No other groups show significant differences.

Differences have been found between age groups in the percentage of C16:0 among the Coloured females (Table 3); between Bantu males in the percentage of C16:1 (Table 4). These differences are statistically of probable significance. The difference in the percentage of C18:1 and C18:2 between age groups in the Bantu females (Tables 6 and 7) are statistically significant.

DISCUSSION

Qualitative changes with age in human adipose tissue have excited some interest. As early as 1881 Langer (1) reported that the body fat of the new-born had a melting point of 51 degrees as against 38 degrees in the adult. He analysed the fatty acids and reported that in the new-born oleic, palmitic and stearic acids were present in the proportion of 67.7, 29.0 and 3.3% respectively. In the adult he found these fatty acids to represent 89.8, 8.2 and 2.0%. In view of modern knowledge the data of Langer cannot be accepted as quantitative since there are more fatty acids now known to be present in adipose tissue than were found by him. The trend that he reported probably is acceptable, since, although his methods may not have been as good as are available today, the same error presumably applied to both samples. Jaeckle (2) in 1902 found the iodine number of the body fat in infancy to range between 39 and 49, whereas in the adult the mean value

/was 65.....

was 65. It did thus appear from these 2 studies that there was a trend for body fat to increase in unsaturation from infancy to adult life. An apparently discrepant report indicating the reverse trend was published in 1928[3] in which the body fat at birth was found to have an iodine number of 80, whereas at 35 days of age it was 56. Hirsch et al.[4] have recently shown that marked changes occur at the infantile level in a very short space of time. On comparing the fatty acid composition of adipose tissue in 4 premature with 3 full-term infants, marked differences were apparent. The fat from the premature infants was in fact more unsaturated than that in the full-term subjects. The above apparent discrepant report[3] in the degree of unsaturated based on the iodine number of depot fat may therefore be explained, at least in part, in relation to the maturity of the infants studied.

The adipose fatty acids of children appear to take on an adult pattern at a young age. Hirsch et al.[4] have analysed the adipose fatty acids of 2 children aged 8 and 11 years. The composition of these 2 subjects was very similar and closely resembled that found by them in normal adults. Studies done here on children aged 2 years show quantitatively and qualitatively a fatty acid pattern similar to those of adults[5].

From the data of Hirsch et al.[4] it would appear that the depot fat fatty acid pattern early in intrauterine life is determined by maternal factors. Late in pregnancy this alters, so that the newborn full-term infant exhibits a depot fat fatty acid pattern distinctly different from that in both the immature infant and adult. The young child, however, takes on a fatty acid pattern found in adults by the age of 2 years[5] and possibly sooner.

The relationship between age and depot fat fatty acid composition in the adult has received little attention. Hirsch et al.[4] give the mean percentage values for the 6 common depot fat fatty acids in 12 normal adults with an age range of 20 - 35 years and 3 normal adults aged 46, 56 and 60 years. No differences of note appear to be present.

/They comment.....

They comment, presumably from more extensive data than that given, that they found no variations which could be correlated with age. Gellhorn and Marks[6] also state that they found no changes in depot fat composition with age. The latter authors do not, however, give any data, either of the age of their subjects, nor of the fatty acid proportions found at different age levels.

The findings in this study indicate that age, in general, is not associated with a change in the depot fat fatty acid composition in either men or women at the age levels studied (Table 1). Examining the mean percentage of each fatty acid in each of the racial groups does reveal some differences between age groups among Cape Coloured and Bantu subjects. The White subjects, both males and females, show no significant differences between age groups for any of the major fatty acids (Tables 2 - 7).

The Coloured females show a difference between age groups in the percentage of C16:0 (Table 3). This difference is significant at the 5% level. No similar difference between age groups is seen between the Coloured males. This difference then is not a characteristic of race. The Bantu males similarly show a difference between age groups in the percentage of C16:1 (Table 4). This difference is also significant at the 5% level and is seen only in the Bantu males; again not a characteristic of race. Both of these differences do not appear to be meaningful. The probability that these differences between age groups in the C16 fatty acids are due to chance is not very low and are probably more apparent than real. One would tend to favour the conclusion that these particular findings are not of significance.

The finding in the Bantu females of a difference between age groups in the percentage of C18:1 (Table 6) and C18:2 (Table 7) is more meaningful. The level of significance in both cases is high. The deposition of C18:2 in the depot fat depends upon its availability in the diet (Chapter 2). It would therefore appear that this difference reflects a difference in the relative intake of C18:2 by the two age groups. It is

/doubtful that.....

doubtful that this can be regarded as a general characteristic of the Bantu; this difference is certainly not reflected among the Bantu males (Table 7). The difference between age groups among the Bantu females in the percentage of C18:1 is possibly a consequence of their lower percentage of C18:2. It has been found in this study that the percentage of C18:1 shows a negative correlation with C18:2 in the depot fat fatty acids. This relationship will be discussed in a subsequent chapter.

The difference between age groups in these two particular fatty acids would therefore appear to be the result of a single factor which in this cas may be the difference in linoleic acid intake.

It is apparent that the trends with age seen in serum cholesterol concentration (Chapter 4) bear no relationship to depot fat fatty acid composition. The White males, the White females and the Coloured males who showed a significant increase in serum cholesterol concentration with age show no change in depot fat composition. The Coloured females showed a distinct rise in mean serum cholesterol concentration which however was not significant, and show only a very doubtful difference in the percentage of C16:0. The Bantu males showed no difference in serum cholesterol concentration with age, and they too show a doubtful difference in the percentage of C16:1. The Bantu females are the only group among whom a definite difference is seen in depot fat composition between the age groups. The mean serum cholesterol concentration however showed no change with age. There is clearly no consistent pattern relating a specific change in depot fat fatty acid composition with serum cholesterol trends with age. Similarly the trends with age in relation to the size of the adipose organ (Chapter 4) are also not related to any alteration in depot fat composition.

/CONCLUSIONS.....

CONCLUSIONS

The depot fat fatty acid composition does not in general show a change with age in either males or females at the age levels studied. The differences found between age groups in the percentage of C16:0 and C16:1 among Coloured females and Bantu males are probably not of significance. The difference between age groups in the Bantu females in the percentage of C18:1 and C18:2 is significant. Since both of these fatty acids appear to be negatively correlated they are probably dependent upon a single other factor. In this case it is presumably the dietary intake of linoleic acid. It is unlikely that this difference reflects a general difference between Bantu age groups.

Despite definite trends with age in regard to serum cholesterol concentration and the relative degree of obesity in the groups sampled, the depot fat composition shows no parallel or consistent change.

SUMMARY

The common fatty acids in the depot fat of males and females have been compared at the two age levels studied. There is no general difference between age groups in the percentage of any of the fatty acids in the males or females. The only group in which there was a distinct difference with age was in the Bantu females. The significance of this finding is discussed.

The trends with age in serum cholesterol concentration and the size of the adipose organ are not related to any change in depot fat composition.

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CHAPTER 8

THE EFFECT OF SEX ON THE DEPOT FAT FATTY ACID COMPOSITION

There is much evidence to indicate that there is a difference in fat metabolism between the sexes (Chapters 1 and 2). Certain qualitative and quantitative differences between the sexes in the blood lipid components have been established. The adipose organ is relatively greater in females and there is work to indicate that this organ may have a greater rate of turnover in the female. There has been no satisfactory study comparing the depot fat fatty acid composition between males and females. The findings in such a comparison are presented below.

RESULTS

The percentage of each of the 6 common fatty acids, C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2, in human depot fat have been compared between the apparently healthy men and women. Table 1 shows the mean percentage of each of these fatty acids, one standard deviation (S.D.), the difference between the means (diff.) and the significance of this difference (P.) in the subjects sampled, regardless of race or age. The percentage of each of these fatty acids expressed as a mean is shown in Fig. 1.

/TABLE 1.....

FATTY ACID	MALES Mean	(S.D.)	FEMALES Mean	(S.D.)	diff.	P.
C14:0	3.5	(1.3)	3.2	(1.1)	0.3	N.S.
C16:0	23.4	(2.6)	22.1	(1.3)	1.3	< 0.001
C16:1	7.4	(1.4)	9.3	(2.3)	1.9	< 0.001
C18:0	5.1	(1.7)	3.5	(1.1)	1.6	< 0.001
C18:1	44.5	(4.0)	46.1	(4.1)	1.6	< 0.05
C18:2	9.8	(3.5)	9.6	(3.7)	0.2	N.S.

TABLE 1. The values for the percentage of each of the common fatty acids is shown as a mean, with 1 standard deviation (S.D.), Note that the difference between means, and comparing males with females, in the percentage of C16:0, C16:1 and C18:0 is highly significant and that the difference between the sexes of C18:1 is probably significant.

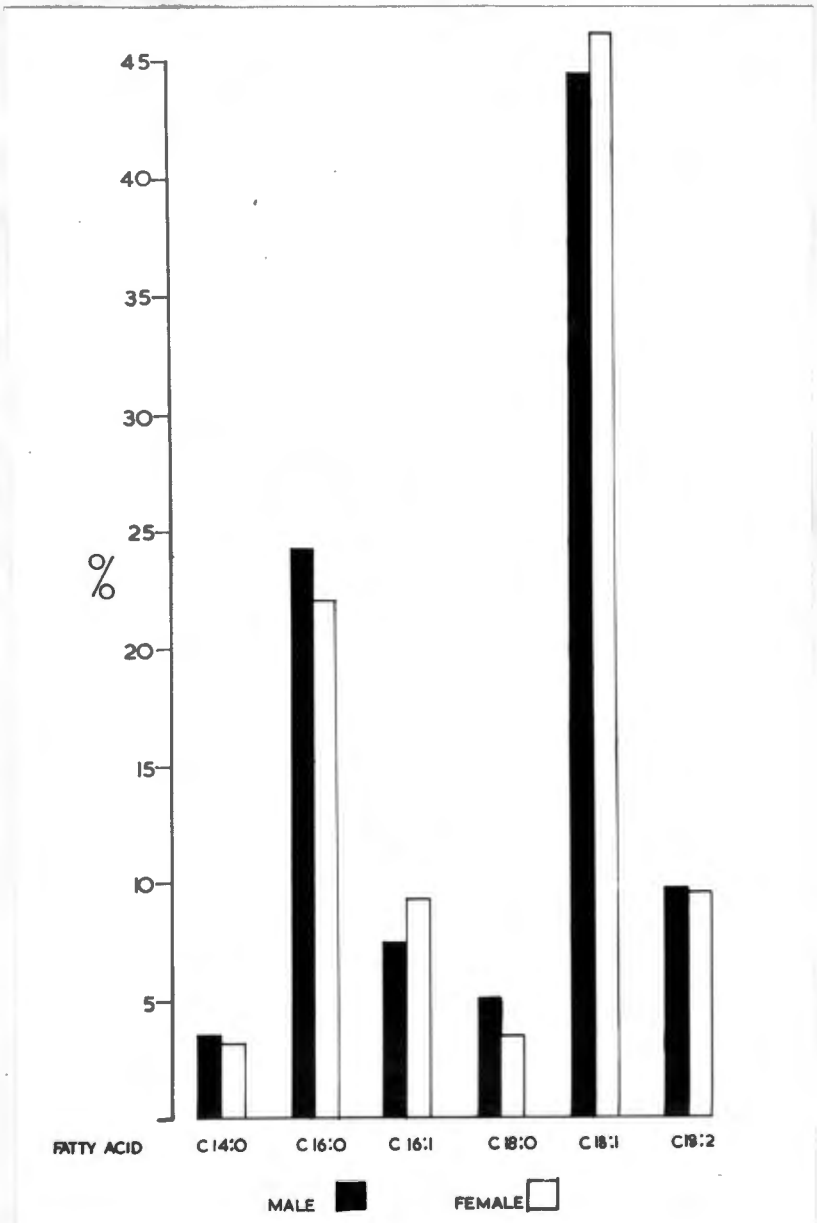


FIG. 1.

The mean value for the percentage of the common fatty acids, comparing males with females.

/It can be.....

It can be seen from Table 1 that there are significant differences in the percentage of some of the fatty acids between males and females. The percentage of C16:0 is significantly higher ($P < 0.001$) in the males than in the females; C16:1 is significantly lower ($P < 0.001$) in the males; C18:0 is significantly higher ($P < 0.001$) in the males and C18:1 is significantly lower ($P < 0.05$) in the males.

The sex differences in the percentage of C16:0, C16:1 and C18:0 are highly significant. The sex differences in the percentage of C18:1 is probably significant ($P < 0.05$). There is no sex difference for either C14:0 or C18:2.

The sex differences in the depot fat fatty acid composition is confined to the 16 and 18 carbon chain-length fatty acids; the saturated acids being higher in the males and the monounsaturated acids higher in the females.

In order to determine whether these findings are general the percentage of each fatty acid has been compared between males and females in each race at each age level (Tables 2 - 13).

There is no significant difference between the sexes at either age level in any of the racial groups for the percentage of C14:0 (Table 2); nor does any difference appear on combining the age groups in each race (Table 3).

C14 : 0 (%)

AGE(Yrs)	20 - 29 Years					40 - 49 Years				
SEX	MALES		FEMALES			MALES		FEMALES		
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	4.3	(1.5)	4.2	(0.9)	0.1	N.S.	3.9	(1.4)	3.8	(1.2)	0.1	N.S.
C	3.8	(1.6)	3.5	(1.2)	0.3	N.S.	3.5	(1.2)	3.0	(0.6)	0.5	N.S.
B	2.8	(0.3)	2.6	(0.7)	0.2	N.S.	2.5	(0.5)	2.3	(0.9)	0.2	N.S.

TABLE 2.

/TABLE 3.....

C14 : 0 (%)

AGE(Yrs)	20 - 29 + 40 - 49			
SEX	MALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.
W	4.1 (1.5)	3.9 (1.1)	0.2	N.S.
C	3.7 (1.3)	3.2 (1.0)	0.5	N.S.
B	2.6 (0.4)	2.5 (0.7)	0.1	N.S.

TABLE 3.

The percentage of C16:0 is higher in the males at each age level with the exception of the young Bantu subjects. The sex differences are however not all statistically significant (Table 4). The White subjects as a whole, as well as the Coloured subjects, show a significant difference between the sexes, but not the Bantu subjects (Table 5).

C16 : 0 (%)

AGE(Yrs)	20 - 29				40 - 49			
SEX	MALES	FEMALES			MALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W	23.6 (1.4)	21.3 (0.9)	2.3	<0.001	24.8 (2.3)	22.1 (2.9)	2.7	<0.05
C	23.3 (2.8)	20.8 (1.8)	2.5	<0.05	23.9 (3.0)	22.9 (2.6)	1.0	N.S.
B	22.1 (3.5)	23.0 (2.2)	0.9	N.S.	22.4 (2.2)	21.7 (2.7)	0.7	N.S.

TABLE 4.

C16 : 0 (%)

AGE(Yrs)	20 - 29 + 40 - 49			
SEX	MALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.
W	24.1 (1.9)	21.8 (2.3)	2.3	<0.001
C	23.6 (2.8)	21.9 (1.7)	1.7	<0.05
B	22.3 (2.9)	22.5 (2.5)	0.2	N.S.

TABLE 5.

/The.....

The percentage of C16:1 is consistently higher in the females at each age level in each race. The difference between the sexes is again not always significant (Table 6). In the White subjects as a whole and in the Bantu, the percentage of C16:1 is significantly higher in the females; but this is not so among the Cape Coloureds (Table 7).

C16 : 1 (%)

AGE(Yrs)	20 - 29					40 - 49				
SEX	MALES		FEMALES			MALES		FEMALES		
RACE	Mean	(S.D.)	Mean	(S.D.)	diff. P.	Mean	(S.D.)	Mean	(S.D.)	diff. P.
W	8.1	(1.1)	9.2	(2.4)	1.1 N.S.	8.2	(1.4)	9.9	(1.5)	1.7 <0.01
C	6.5	(1.5)	7.7	(1.1)	1.2 <0.05	6.7	(1.8)	7.0	(1.5)	0.3 N.S.
B	6.7	(1.7)	11.0	(1.7)	4.3 <0.001	8.5	(1.7)	10.5	(2.9)	2.0 N.S.

TABLE 6.

C16 : 1 (%)

AGE(Yrs)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	8.2	(1.2)	9.6	(1.9)	1.4	<0.01
C	6.6	(1.6)	7.3	(1.4)	0.7	N.S.
B	7.5	(1.9)	10.8	(2.2)	3.3	<0.001

TABLE 7.

The percentage of C18:0 is consistently higher in the males than in the females at both age levels and in each racial group. The differences between the sexes is significant in all but the older Cape Coloured subjects (Table 8). Disregarding age, the differences between the sexes is significant in each racial group (Table 9).

/TABLE 8.....

C18 : 0 (%)

AGE(Yrs)	20 - 29 Years						40 - 49 Years					
SEX	MALES		FEMALES				MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	4.7	(1.3)	3.4	(1.0)	1.3	<0.02	4.3	(1.2)	3.2	(0.8)	1.1	<0.02
C	5.5	(2.0)	3.7	(1.0)	1.8	<0.02	4.9	(1.6)	4.1	(0.8)	0.8	N.S.
B	6.1	(2.1)	3.0	(1.1)	3.1	<0.001	4.9	(1.4)	3.5	(1.2)	1.4	<0.05

TABLE 8.

C18 : 0 (%)

AGE(Yrs)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	4.5	(1.2)	3.3	(0.9)	1.2	<0.001
C	5.2	(1.8)	3.9	(0.9)	1.3	<0.01
B	5.5	(1.8)	3.2	(1.2)	2.3	<0.001

TABLE 9.

The sex difference in the percentage of C18:1 for all subjects (Table 1), with the exception of the young Bantu, is reflected at each age level in the three racial groups (Table 10). Although on combining the age groups in each race the percentage of C18:1 in the females is consistently higher, in no racial group was this difference significant (Table 11).

C18 : 1 (%)

AGE(Yrs)	20 - 29 Years						40 - 49 Years					
SEX	MALES		FEMALES				MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	44.5	(2.7)	46.6	(4.9)	2.1	N.S.	45.7	(3.2)	46.2	(4.1)	0.5	N.S.
C	42.3	(3.9)	43.9	(2.5)	1.6	N.S.	41.7	(4.3)	44.9	(2.2)	3.2	<0.05
B	46.8	(3.5)	45.5	(3.1)	1.3	N.S.	47.1	(2.8)	50.1	(2.0)	3.0	<0.02

TABLE 10.

/TABLE 11.....

C18 : 1 (%)

AGE(Yrs)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	45.0	(2.9)	46.4	(4.3)	1.4	N.S.
C	42.0	(4.0)	44.3	(3.8)	2.3	N.S.
B	46.9	(3.1)	47.4	(3.6)	0.5	N.S.

TABLE 11.

There is no consistent sex trend in the percentage of C18:2 in the race and age groups studies. There is a significant difference between the sexes in the older age group of Bantu subjects (Table 12). This is not reflected in the Bantu subjects as a whole; nor is there a difference between the sexes in the White or Coloured subjects on combining the age groups (Table 13).

C18:2 (%)

AGE(Yrs)	20 - 29 Years						40 - 49 Years					
SEX	MALES		FEMALES				MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	8.0	(2.0)	8.0	(2.2)	0	N.S.	6.9	(1.6)	7.3	(2.0)	0.4	N.S.
C	11.6	(4.4)	14.3	(3.3)	2.7	N.S.	12.9	(3.8)	12.4	(4.0)	0.5	N.S.
B	9.8	(2.2)	9.4	(2.5)	0.4	N.S.	9.3	(2.0)	6.8	(1.6)	2.5	<0.01

TABLE 12.

/TABLE 13.....

C18 : 2 (%)

AGE(Yrs)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	7.5	(1.9)	7.6	(2.0)	0.1	N.S.
C	12.2	(4.1)	13.3	(3.7)	1.1	N.S.
B	9.6	(2.0)	8.3	(2.5)	1.3	N.S.

TABLE 13.

The mean values for each of the major components, in each racial group, comparing males with females, is shown in Fig. 2.

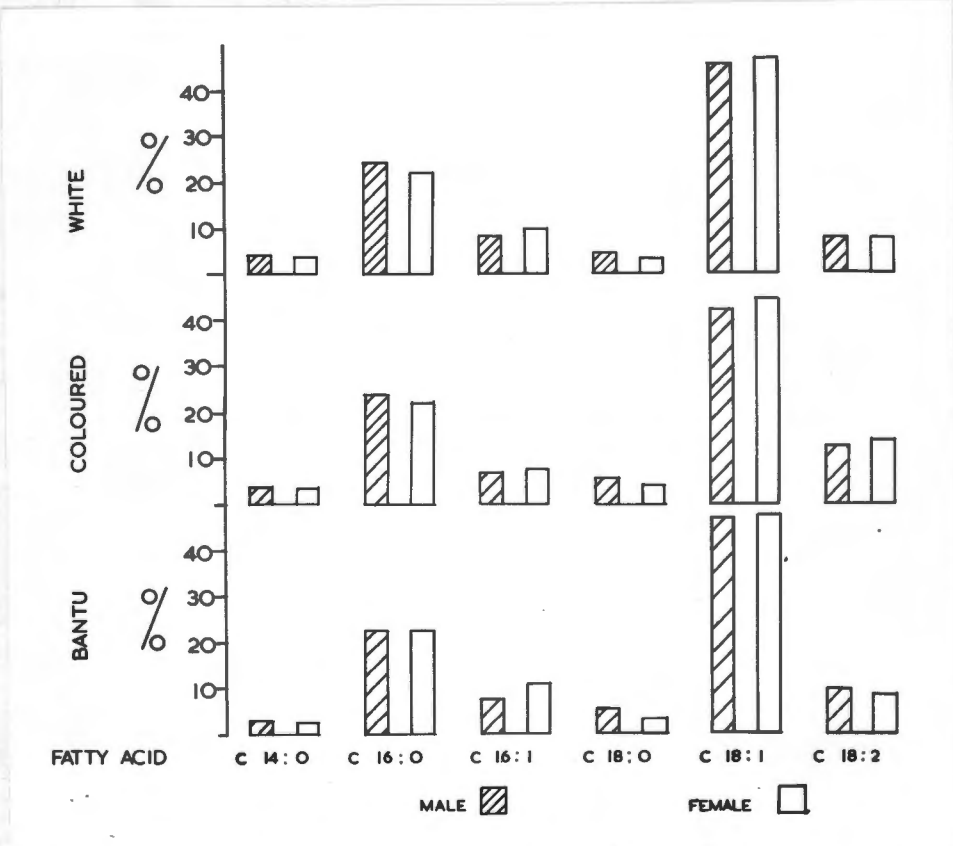


FIG. 2. The mean values for each of the fatty acids comparing males with females in each race. Note that in the Bantu the values for C16:0 and C18:1 are not different between the sexes; C16:1 and C18:0 however appear to show a greater difference than in the White and Coloured subjects.

/DISCUSSION.....

DISCUSSION

On comparing the depot fat composition of apparently healthy males with females certain definite differences have emerged. It is clear that, in general, the females have a lower proportion of C16:0 and C18:0 and a higher proportion of C16:1 and probably C18:1 than the males (Table 1, Fig.1).

The difference between the males and females in the percentage of these fatty acids was significant despite the fact that subjects were drawn from three racial groups whose important distinguishing feature, relevant to a study on depot fat composition, is their different dietary habits (Chapter 4). Despite the dietary differences, sex differences have emerged.

This finding invites the hypothesis that sex hormones influence the metabolism of depot fat. This hypothesis is attractive from two points of view.

1) The particular fatty acids which show differences between the sexes are known to be interconvertible, and can be synthesized from acetate. The diet therefore need not be the determining factor in these sex differences.
2) The fact that these differences are evident between males and females sampled from three groups with markedly different dietary habits tends to further minimise the importance of the diet in determining these particular differences.

Before developing this argument further it is necessary to review the data. It will be recalled that the difference between the sexes of C16:0 in the Bantu subjects (Tables 4 and 5) did not appear to follow the general trend (Table 1). The Cape Coloured subjects, although following the general sex trend for the percentage of C16:1 (Table 1) did not show a significant difference between males and females (Table 7). The sex difference for C18:0 was significant in all groups but for the older group of Cape Coloured subjects (Table 8), although the Cape Coloureds as a whole followed the general trend (Table 9). The difference between males and females in the percentage

/of C18:1.....

of C18:1 although significant regardless of race (Table 1), was not significant on considering each racial group separately. (Table 11). Thus the differences found between males and females in all the subjects studied, regardless of age and race, do not hold in every single group, but almost all groups follow the general trend.

In order to find the best method of expressing these differences between the sexes it is necessary to consider the interconversions that may take place between those fatty acids which show differences between the sexes.

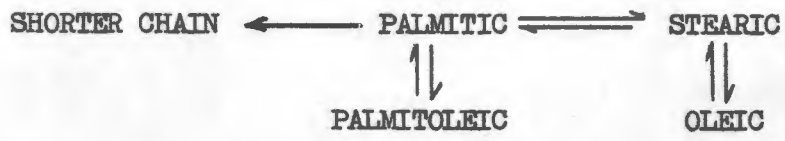
INTERCONVERSION OF SATURATED AND MONOUNSATURATED FATTY ACIDS

It will be recalled from Chapter 2 that the fatty acid composition of mammalian body fat was determined by: (a) Direct deposition of dietary fat. (b) Synthesis from carbohydrate or other sources. (c) Alteration of the fatty acids derived by the other two methods. It is the third factor (c) that may be independent of the diet and therefore the factor that could be responsible for these sex differences.

The interconversions that may take place between the saturated and monounsaturated acids have been extensively studied. Schoenheimer and his collaborators in a series of experiments feeding deuterium labelled fatty acids to rats found: (1) On feeding saturated fatty acids containing deuterium the unsaturated fatty acids also contained deuterium(1). (2) Feeding deuterated unsaturated fatty acids gave rise to deuterium containing saturated fatty acids(2). They concluded, because of the various proportions of deuterium in the tissue components, that at least two-thirds of the deuterium in the saturated fatty acids was the result of the hydrogenation of the fed unsaturated acids. Further experiments by this group showed that deutero-palmitic acid was partially converted to stearic and palmitoleic acid(3). Linoleic acid on the other hand, that was obtained from rats maintained on deuterium water, contained no appreciable amount of deuterium despite the finding that other fatty acids did(4). These findings

/led Schoenheimer.....

led Schoenheimer to suggest that fatty acids were converted according to the scheme:



It can however be argued that the deuterium content of the saturated fatty acids could have been derived from both the breakdown products of the unsaturated acids as well as from the body water itself. The conversion of monounsaturated to saturated acids therefore need not be the result of a direct process. Subsequent experiments, using isotope labelling have elucidated this question.

The administration of C^{14} tripalmitin to rats whether labelled at the first or sixth carbon showed no difference in their elimination as $C^{14}O_2$.(5) From this it was concluded that the oxidation of palmitic acid, once initiated, went to completion with no apparent accumulation of shorter chain-length acids such as myristic, lauric etc. The administration of tristearin labelled in the same manner gave different results; the stearic acid labelled at the sixth carbon was oxidized more slowly than that labelled at the carboxyl group. The subsequent isolation of palmitic and stearic acids from the rats revealed the presence of considerable activity in the palmitic acid fraction of those rats receiving the stearic acid labelled at the sixth carbon(6). The reverse reaction - conversion of palmitic to stearic, has also been shown to occur, both by direct conversion from labelled palmitate(7) as well as from palmitate synthesized from labelled acetate(8). This finding has been confirmed in man by the injection of acetate-1-C^{14} (9) where it was shown that the peak activity occurred first in the palmitic acid and was followed by the peak in stearic acid. The <u>in vitro</u> uptake of acetate-1-C^{14} by human adipose tissue and its incorporation into fatty acids has recently been shown(10). That some synthesis of palmitic acid occurs from elongation of the shorter chain acids was shown by feeding myristic acid-1-C^{14} to rats(11).

/The.....

The distribution of activity in fatty acids synthesized from acetate-1-C^{14} by the mouse showed, from the distribution of radio activity in the fatty acid chain, that palmitic acid is totally synthesized from acetate and that stearic acid is largely synthesized by elongation of palmitate[12]. These experiments[11,12] further indicated that the unsaturated acids corresponded closely in activity to that of the saturated acids of the same chain length, indicating that the monounsaturated acids were formed directly by dehydrogenation of the saturated acids. In the experiments of giving labelled acetate to man[9] when blood was sampled at progressive time intervals the peak activity of oleic acid followed that of stearic acid. These findings, that the monounsaturated acids were derived from the saturated acids were shown by other workers[13] to apply to both palmitoleic and oleic acids. These findings have suggested to Mead[14] that Schoenheimer's original scheme be modified, the fatty acid interconversions now believed to be taking place is as illustrated in Fig. 3. From the results of the <u>in vitro</u> uptake of labelled acetate by human adipose tissue[10], the activity found in the various fatty acid fractions appear to support the sequence suggested by Mead[14].

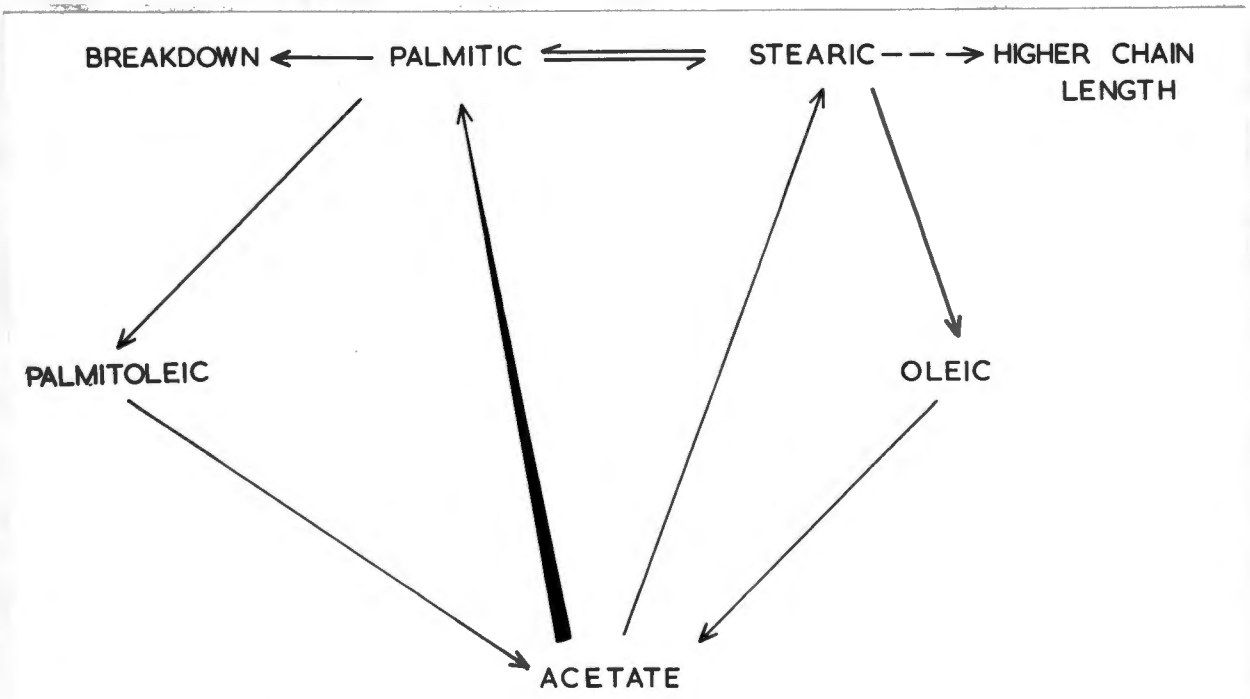


FIG. 3. The interconversions of the saturated and monounsaturated fatty acids.
Taken from Mead.[14]

To sum up, palmitic acid is the key acid. This is largely synthesized and degraded with little formation of intermediate chain-length acids, although this does occur to some extent. Stearic acid is largely formed by elongation of palmitic acid; the reverse reaction also occurring by the removal of 2 carbons. Stearic acid is in addition synthesized directly from acetate to some degree. Palmitoleic and oleic acids are formed from dehydrogenation of palmitic and stearic acids respectively, but the reverse reaction, involving hydrogenation of the monounsaturated acids, appears, not to occur. From the suggested scheme (Fig.3) it appears that the saturated fatty acids can follow two routes in their breakdown to acetate; either by direct degradation of palmitic acid, or by the initial conversion to the monounsaturated, palmitoleic and oleic acids, and subsequent degradation to acetate. The formation of higher acids, beyond C18, is still speculative.

The potent role of the dietary fat in determining the depot fat composition cannot be too heavily stressed. It is therefore so much more remarkable that sex differences have been found in this cross section of people whose diets differ so markedly. It seems therefore that where the organism is able to alter the depot fat composition it appears to do so differently in males and females. It is suggested that what may be the important difference between the sexes is not so much the actual proportion of the C16 and C18 fatty acids but the relationship of C16:0 to C16:1 and C18:0 to C18:1. This concept could be tested by examining the ratios C16:0/C16:1 and C18:0/C18:1 in each sex and comparing the sexes. Table 14 shows the ratios C16:0/C16:1 and C18:1/C18:0 in all the males and females studied.

/TABLE 14.....

FATTY ACID RATIO	MALES Mean	(S.D.)	FEMALES Mean	(S.D.)	diff.	P.
C16:0/C16:1	3.33	(0.9)	2.55	(0.7)	0.78	<0.001
C18:1/C18:0	9.8	(3.5)	14.6	(4.9)	4.8	<0.001

TABLE 14. The fatty acid ratios C16:0/C16:1 and C18:1/C18:0 have been compared between all the males and females studied. The difference between the means is highly significant in each case.

On examining each of these ratios in the males and females in each racial group and at each age level (Tables 15 - 18) the differences between the sexes become more consistent than on comparing the percentage of each of these C16 and C18 fatty acids. It is only in the older age group (Tables 15 and 17) of subjects that the difference between the sexes, although following the general trend (Table 14) in every group, is not always significant. The difference in these ratios between the sexes is striking, particularly because there is considerable variation between the racial groups.

C16:0/16:1

AGE(Yrs)	20 - 29						40 - 49					
SEX	MALES		FEMALES				MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	2.96	(0.49)	2.43	(0.52)	0.53	<0.05	3.08	(0.56)	2.31	(0.60)	0.77	<0.0
C	3.74	(0.92)	2.73	(0.36)	1.01	<0.01	3.83	(1.21)	3.44	(0.71)	0.39	N.S
B	3.50	(0.75)	2.14	(0.37)	1.36	<0.001	2.73	(0.57)	2.27	(0.80)	0.46	N.S

TABLE 15.

/TABLE 16.....

C16:0/16:1

AGE(Yrs) 20 - 29 + 40 - 49

SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	3.01	(0.52)	2.36	(0.55)	0.65	<0.001
C	3.78	(1.04)	3.13	(0.70)	0.65	<0.01
B	3.13	(0.80)	2.19	(0.58)	0.94	<0.001

TABLE 16.

C18:1/18:0

AGE(Yrs)	20 - 29						40 - 49					
SEX	MALES		FEMALES				MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	10.38	(3.38)	14.90	(4.81)	4.52	<0.02	11.30	(1.44)	15.31	(3.62)	4.01	<0.01
C	9.06	(4.34)	12.78	(3.66)	3.72	<0.05	9.55	(3.88)	11.28	(2.18)	1.73	N.S.
B	8.65	(3.29)	17.26	(6.31)	8.61	<0.001	10.16	(2.74)	16.15	(6.16)	5.99	<0.02

TABLE 17.

C18:1/18:0

AGE(Yrs) 20 - 29 + 40 - 49

SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	10.80	(3.26)	15.14	(4.06)	4.34	<0.001
C	9.28	(4.06)	11.97	(2.97)	2.69	<0.01
B	9.37	(3.06)	16.56	(6.02)	7.19	<0.001

TABLE 18.

/The primary.....

The primary difference between the sexes is therefore the relatively higher proportion of the monounsaturated C16 and C18 carbon-chain length fatty acids in the females. This is evident in every group of subjects despite the fact that there are obvious differences between the racial groups.

This difference is not due to a sexual difference in the proportion of the C16 and C18 saturated and monounsaturated fatty acids deposited since they represent the same percentage of the total fatty acids in both sexes (Table 19).

AGE (Yrs.)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
FATTY ACIDS	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
C16:0 + C16:1	30.9	(3.4)	31.4	(3.7)	0.5	N.S.
C18:0 + C18:1	49.6	(4.0)	49.6	(3.7)	0	-

TABLE 19. The mean percentage for the sum of the C16:0 + C16:1 and C18:0 + C18:1 is the same in both males and females.

It would therefore appear that there is a relatively greater rate of conversion of palmitic to palmitoleic and of stearic to oleic acids in the female than there is in the male.

The other pathways the 16 and 18 carbon-chain length fatty acids may take can be seen from Fig. 3 and are as follows: (i) Palmitate is readily broken down to acetate with little accumulation of shorter chain length intermediates. The deposition of C14:0 will therefore depend primarily on dietary factors. It has already been shown that the percentage of C14:0 does not show a difference between the sexes (Table 1) nor is there any difference between the sexes at any age level in any race (Tables 2 and 3). There are however differences between the races which will be discussed in the next chapter.

(ii) Palmitate.....

(ii) Palmitate and stearate are largely interconvertible. On comparing the ratio C16:0/C18:0 between males and females (Tables 20 and 21) it is apparent that there is a trend for the ratio to be higher in the female.

C16:0/C18:0

AGE(Yrs)	20 - 29					40 - 49				
SEX	MALES		FEMALES			MALES		FEMALES		
RACE	Mean	(S.D.)	Mean	(S.D.)	diff. P.	Mean	(S.D.)	Mean	(S.D.)	diff. P.
W	5.44	(1.6)	6.83	(2.4)	1.39 N.S.	6.09	(1.4)	7.29	(1.8)	1.18 N.S.
C	4.87	(2.0)	6.08	(1.9)	1.21 N.S.	5.26	(1.5)	5.79	(1.5)	0.53 N.S.
B	4.19	(1.9)	8.74	(3.1)	4.55 <0.001	4.82	(1.2)	6.87	(2.5)	2.05 <0.05

TABLE 20.

C16:0/C18:0

AGE(Yrs)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	5.74	(1.5)	7.09	(2.0)	1.35	<0.02
C	5.05	(1.7)	5.92	(1.7)	0.87	N.S.
B	4.49	(1.6)	7.96	(3.0)	3.47	<0.001

TABLE 21.

The meaning of these latter findings however is not so specific. It could be due to either a decreased rate of conversion of C16:0 to C18:0, or to an increased rate of conversion of C18:0 to C18:1 in the female. Alternatively it could mean an increased rate of conversion of C18:0 to C16:0 in the females. The question of the possible rate of conversion of C18:0 to higher chain length fatty acids also cannot

/be ignored.....

be ignored. This pathway is however still uncertain and in the depot fat, at least, is not very evident since the fatty acids with a chain length greater than 18 carbon atoms are very scanty. Further the finding of an identical value between males and females for the total percentage of C18:0 + C18:1 (Table 19) serves to support the view that at least the major direction for C18:0 conversions is to C18:1. Similarly the interconversion of C18:0 and C16:0 would appear not to be a distinguishing factor since C16:0 + C16:1 shows no sex difference (Table 19).

Possibly the best manner in which to resolve the problem of the C16:0 and C18:0 interconversions, which may take place in either direction, is to combine the values. Expressing the fatty acid ratios as the total monounsaturated over the saturated C16 and C18 fatty acids (C16:1 + C18:1/C16:0 + C18:0) and comparing this difference between males and females, gives rise to a heightening of the consistency in the sex difference. (Tables 22 and 23).

C16:1 + C18:1/C16:0 + C18:0

AGE(Yrs)	20 - 29						40 - 49					
SEX	MALES		FEMALES				MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	1.88	(0.24)	2.28	(0.25)	0.40	<0.01	1.88	(0.28)	2.27	(0.45)	0.39	<0.05
C	1.73	(0.33)	2.12	(0.22)	0.39	<0.01	1.72	(0.41)	1.95	(0.24)	0.23	N.S.
B	1.93	(0.33)	2.19	(0.28)	0.26	<0.05	2.06	(0.30)	2.45	(0.48)	0.39	<0.05

TABLE 22.

AGE(Yrs)	20 - 29 + 40 - 49					
SEX	MALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W	1.88	(0.25)	2.27	(0.37)	0.39	<0.001
C	1.73	(0.36)	2.02	(0.24)	0.29	<0.01
B	1.99	(0.32)	2.30	(0.39)	0.31	<0.01
W+C+B	1.86	(0.33)	2.21	(0.35)	0.35	<0.001

TABLE 23.

/The.....

The primary difference between the sexes is the relatively higher proportion of the monounsaturated to the saturated C16 and C18 chain-length fatty acids in the females. This is evident in every group of subjects despite the fact that there are obvious differences between the racial groups (Figs. 4 and 5). It is worthy of note that the consistency of the sex difference is greater in the younger than in the older subjects.

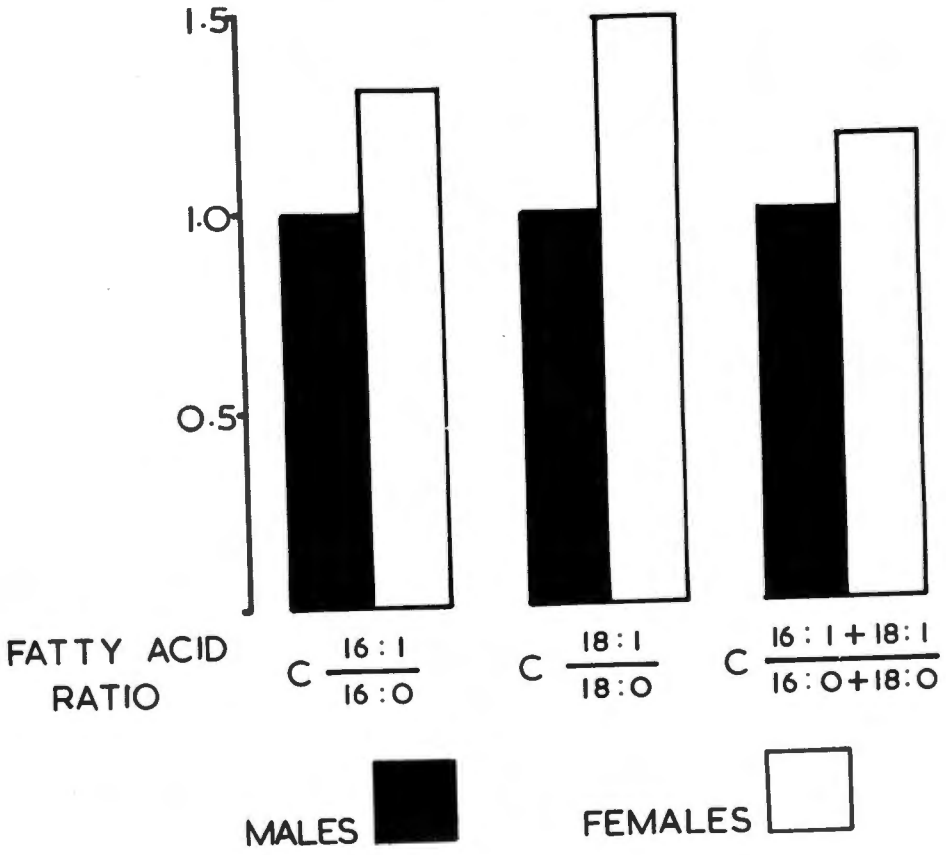


FIG. 4. Comparing the fatty acid ratios of the monounsaturated to the respective saturated acids in males and females. The ratio in the males is taken as 1, and the ratio in the females expressed as an index of this.

/In summary.....

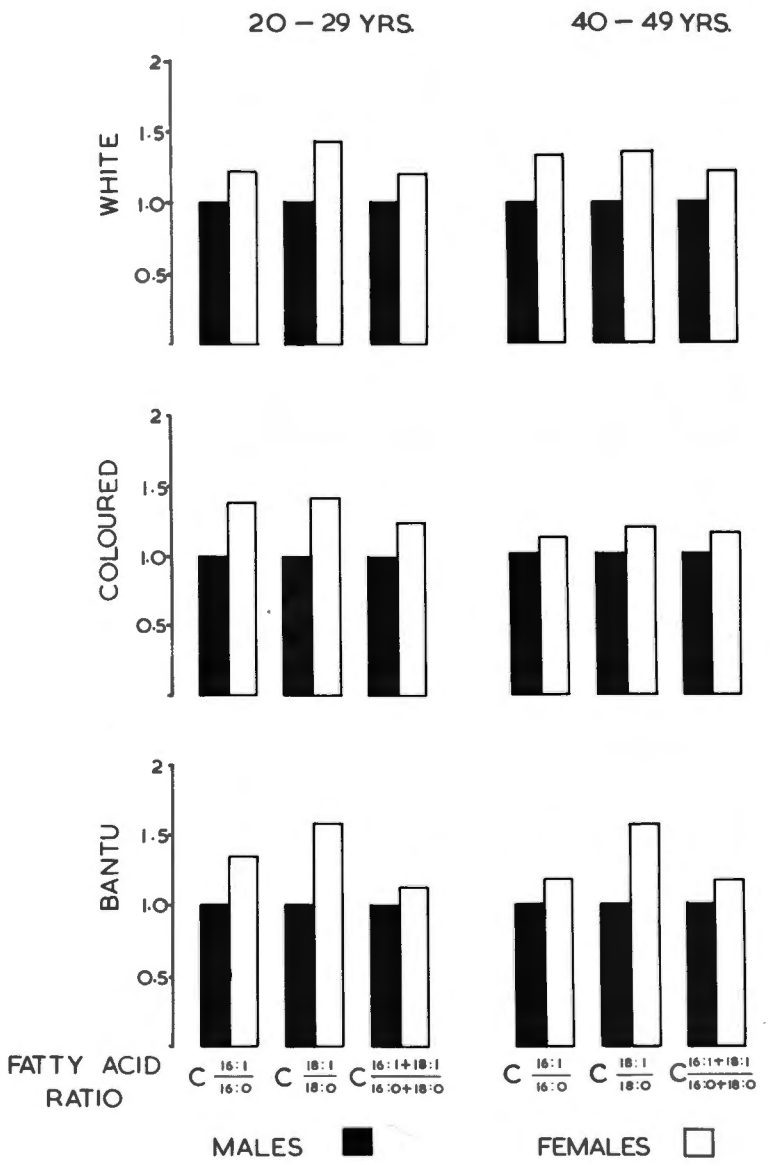


FIG. 5. The ratio of the monounsaturated to the saturated fatty acids comparing males with females. The ratio in the males in each race and at each age level is taken as 1. The ratio in the females of the same age range and same race is expressed as an index of this. Note that at each age level and in each race the value in the females is higher than in the males.

In summary then it would appear that females as a class show a relatively greater proportion of the monounsaturated to the saturated 16 and 18 carbon chain-length fatty acids in their depot fat than do men. This difference is reflected in each racial group and at each age level and is statistically significant in every group with the exception of the older group of Cape Coloured subjects (Table 22). It will be noticed that the Cape Coloured subjects in general tend to have a lower percentage of C16:1 and C18:1 at each age level than do the men and women among the White and Bantu subjects (Tables 6 and 10). This seems to be a feature common to the Cape Coloureds as a group and may account for their discrepancy with regard to the ratio of the C16 and C18 monounsaturated to saturated fatty acids. This point will be discussed in the next chapter.

It is of considerable interest that despite all the multiple factors that may influence the depot fat fatty acid composition and the heterogeneity of the groups from a dietary point of view, studied here, a difference between the sexes has emerged. This difference would appear to be a true metabolic difference, largely, if not entirely, independent of dietary factors.

This observation of a sex difference in the depot fat fatty acid composition appears not to have been made previously in either man or animals. Hirsch et al.[15] have studied the fatty acid composition of human adipose tissue. The only values they have to date reported for normal adults is based on the combined data of 7 males and 5 females. They apparently did not consider a possible difference between the sexes since they make no mention of such a comparison. Gellhorn and Marks[10] also studied human adipose tissue fatty acids. They state that there were no sex differences in fatty acid composition. It is unfortunately not possible to evaluate their findings. They give no separate values for males and females and do not state the number of subjects in each sex. They indicate that the age range of their subjects was from

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about 15 to about 75 years, but it is not known whether the females were pre- or post-menopausal.

The only other study on the body fat composition that appears to have been reported to date is that by Loeb and Burr[16] in 1947. They fed 3 groups of rats from the eighth to the fifteenth week of life on diets with different fat content, both qualitative and quantitative. The iodine number of the carcass fat in the animals differed with the diet in the expected manner, but they reported no sex difference in the degree of unsaturation in any of the groups. Their numbers were however rather small (3 - 7 rats per group) and the determination of the iodine number measures only total unsaturation and not fatty acids.

Possible Mechanisms

The possible mechanisms by which this sex difference in the depot fat fatty acid composition may be brought about is not known. The first requirement would be to determine whether this difference could reasonably be attributed to the effect of oestrogens. This could be done by comparing the depot fat fatty acids in post-menopausal women with the pre-menopausal subjects of this study. Similarly the possible role of androgens might be determined in the same manner, but here the problem is not so easily resolved because the decline in androgen secretion in men is not so sharp as oestrogen is in women. Studies on sexually immature subjects would also be helpful in establishing whether sex hormones determine the differences between men and women. It is probably of some significance that the sex difference in every case was more consistent in the younger subjects. This may be an expression of declining oestrogen activity among the older females.

There is a fair amount of indirect evidence to indicate that oestrogen plays a significant role in the metabolism of the depot fat. The experiments by the Deuel group (Chapter 1) almost three decades ago appear not to have been further elaborated

/to date.....

to date. They found that, on fasting, women excrete ketone bodies 3 - 4 times the level of that in men. This was associated with a profound drop in the CO_2 combining power after 4 days (26.9 vols.%) whereas in men the fall is small (46.6 vols.%). They extended these studies to animals and found that the same sex differences were evident in rats. This was associated in the females with an increase in the neutral fat content of the liver and a decrease in glycogen content when compared with males. They further showed that these sex differences were not present in ovariectomised or in immature or old rats. They found that the fatty liver produced in rats on a choline free diet was greater in females than in males and that the mobilization of this fat, which again was neutral fat, proceeded at a faster rate in the female than in the male. Some of these observations have been confirmed by others, and similar trends were also apparent in guinea pigs and hens. It was concluded by Deuel that fat in the female was more labile than in the male and he thought that this indicated at least a quantitative difference in the metabolism of fat between the sexes. Since these movements of fat concerned the neutral fat the source of this fat in fasting animals must be the adipose tissue.

There is therefore evidence that oestrogens affect the metabolism of the adipose organ.

More recently evidence has appeared establishing a role for oestrogens in fatty acid metabolism. It has been shown that oestrogens stimulate the conversion of acetate to fatty acids <u>in vitro</u>[17,18]. This is associated with an increased rate of oxidation to CO_2 by adipose tissue of glucose 1-C^{14}. The hexose monophosphate shunt (H.M.P.) as determined by the oxidation of glucose-1-C^{14} to CO_2 was significantly higher in the female rat adipose tissue than that from littermate males[18]. This increased H.M.P. shunt in the oxidation of glucose is of importance in fatty acid synthesis since it gives rise to the evolution of reduced triphosphopyridine nucleotide (T.P.N.H.). T.P.N.H. is an important factor in the fatty acid synthesis from acetyl coenzyme A,

/acting as.....

acting as a specific reductant in fatty acyl dehydrogenase reactions[19]. Oestrogens have further been shown to participate in the transhydrogenation of pyridine nucleotides, activating the transfer of hydrogen from T.P.N.H. to D.P.N. (diphosphopyridine nucleotide)[20].

There is thus a fair amount of evidence to indicate a significant role for oestrogens in fat metabolism. The majority of the more recent evidence would appear to indicate that oestrogens have the potential to promote fatty acid synthesis. This obviously cannot be a process moving in a single direction or the adipose organ in the female would continue to enlarge. There must be mechanisms promoting fatty acid breakdown in order to maintain an equilibrium condition. The early work by the Deuel group cited above and the finding of an increased rate of oxidation of glucose-1-C^{14} by female rat adipose tissue[18] support this concept. These reports tempt a speculative suggestion for the findings in this study.

It will be recalled from Fig.3, showing the interconversions between fatty acids, that one of the two pathways in the breakdown of fatty acids to acetate involved an initial dehydrogenation of palmitic and stearic acids to palmitoleic and oleic acids respectively. If the rate of fatty acid turnover is greater in the female one can visualise that this pathway would be accelerated and thus possibly account for the findings in the depot fat fatty acids. The mechanism by which this may be brought about is unknown. In vitro studies on human adipose tissue have been reported as showing no sex difference in the rate of incorporation of acetate-1-C^{14} into mixed lipids[10]. Criticisms of this work by Gellhorn and Marks[10] on general grounds have already been made. It is therefore similarly not possible to evaluate these latter findings. To speculate further on this point would be unprofitable at this stage and one must await the elaboration of a fuller understanding of fatty acid metabolism.

/Relation to.....

Relation to Body Fatness

The possibility that these findings between the sexes may be related to the size of the adipose organ merits consideration. It will be recalled from Chapter 4 that females in general, at each age level and in each racial group, had considerably larger skinfold measurements than did the males. This is a feature common between the sexes in man and animals (Chapter 2). In order to test this possibility the correlation coefficient between skinfold thickness and the fatty acid ratio C16:1 + C18:1/C16:0 + C18:0 was calculated in the females only. To have included the males in this calculation might have given rise to a spurious association since the males in general are leaner than the females (Chapter 4) and have a lower C16:1 + C18:1/C16:0 + C18:0 ratio (Tables 22 and 23 above). No correlation was found ($r = +0.1482, p > 0.1$). The sex difference then cannot be ascribed to relative obesity.

LINOLEIC ACID

The evidence for the view that there is a lesser requirement for linoleic acid among females was discussed in Chapter 1. The hypothesis was there put forward that, given the same diet, females should show a relatively greater proportion of linoleic acid than males in their depot fat. This was based on the reasoning that if the intake of linoleic acid is the same in males and females, the relatively lesser utilization of this fatty acid by the latter would result in the accumulation of this fatty acid in the depots.

The percentage of C18:2 in the depot fat of males and females is compared in Table 1. The mean percentage of C18:2 of the total fatty acids is, in general, the same in males and females. On examining the percentage of C18:2 between the sexes in each race (Table 13) there is still no significant difference. At each age level however a significant difference between the sexes is seen between the 40-49 year old Bantu subjects (Table 12). Here the females show a value significantly lower than the males. This group of Bantu females however have a proportion of C18:2 lower than that in the

/20 - 29 year.....

20 - 29 year old Bantu females (Chapter 7). This trend is not consistent with that seen in any of the other groups. No general conclusions can be drawn from this isolated finding.

It can be seen from Fig. 6 that despite considerable differences between the racial groups in the percentage of linoleic acid, the level in males and females in each racial group, with one exception, show no significant differences. These findings indicate that where the dietary factors determining the deposition of C18:2 is the same in males and females, the proportion of this fatty acid in the depot fat is also the same. It would therefore appear that the requirement for this fatty acid is no greater in males then females.

It may be argued that the females, having a relatively greater store of body fat, have a larger total C18:2 than the males. This, in general, must be the case. The same argument can however be extended to every other fatty acid, the females in general having quantitatively more of each fatty acid. The greater quantitative amount of C18:2 would only indicate a relatively lesser requirement for this fatty acid, among females, if it were shown that the release of C18:2 from the fat depot behaved in a different manner from the other fatty acids; e.g. if C18:2 were drawn on only for specific needs and not as a non-specific energy source, then the greater quantitative amount of C18:2 in the females may have special significance. If this were so it would be expected that the lean females would have a relatively greater proportion of C18:2 in their depots than the more obese females. This is obviously not the case. The younger age group of females show considerably smaller skinfolds than the older females in each race (Chapter 4), yet there is no difference between age groups, except between Bantu females (Chapter 7), the more obese group showing a smaller percentage of C18:2.

The little evidence there is to date on the metabolism of C18:2 in depot fat appears to indicate that C18:2 is mobilised from the depots and is oxidised, at least in part. Hirsch et al.[15] found that feeding linoleic acid at a high level of intake

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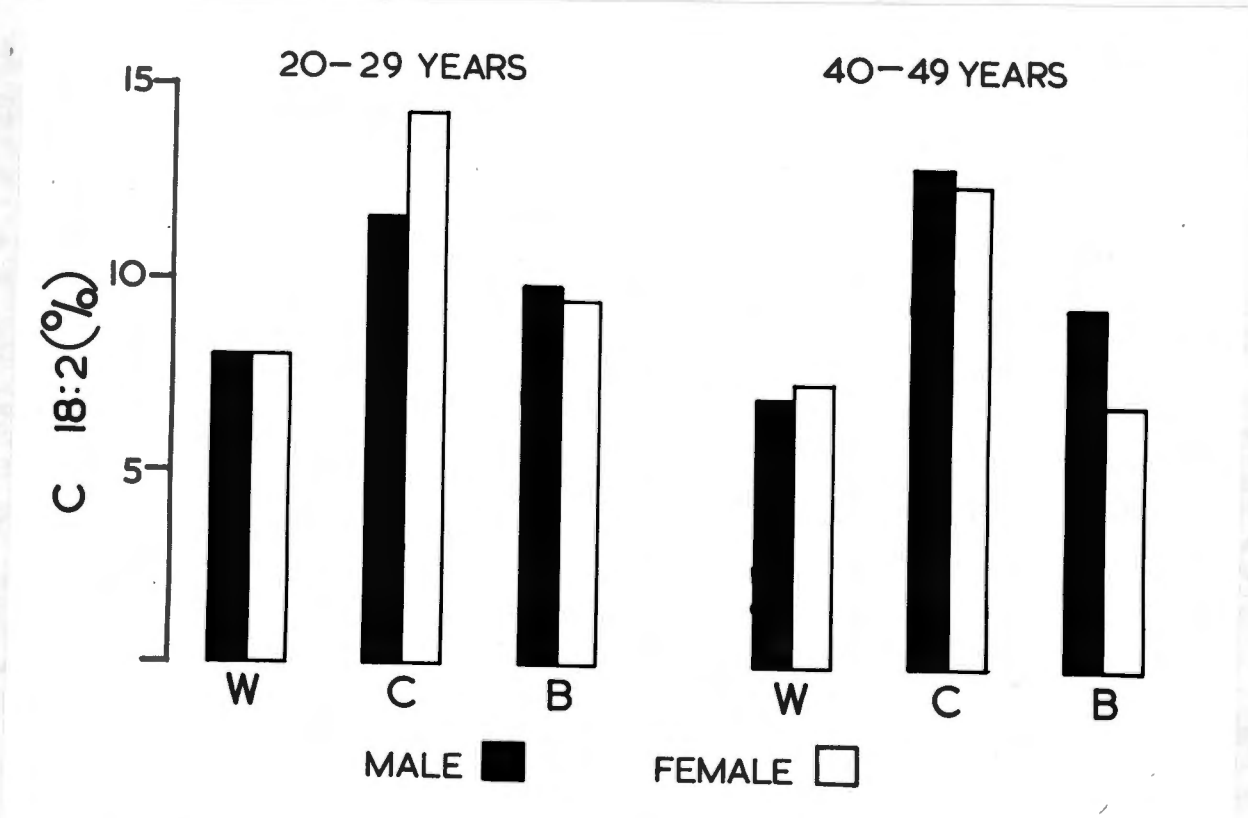

FIG. 6. The percentage of C18:2 shown as a mean, comparing males with females at each age level in White (W), Cape Coloured (C) and Bantu (B) subjects.

produced only a very gradual increase in the proportion of this fatty acid found in the depot fat. During 10 weeks of such a diet the subject ingested more than 3.5 kg. of linoleic acid which is an amount calculated to be about three times that present in his entire adipose tissue; yet there was no rise in the proportion of linoleic acid in his depot fat. The caloric balance during this feeding period was maintained, so presumably his total adipose organ did not increase in size. On the basis of these and other studies Hirsch et al. consider that this is evidence for the non-participation of the whole adipose organ in the metabolism of this tissue. They have postulated the existence of at least two separate metabolic compartments in adipose tissue, the large compartment serving as an inert store for fat, the smaller compartment maintaining a rapid turnover rate. This postulate however assumes that linoleic acid is mobilised and redeposited. If a fatty acid is mobilised it is presumably to provide energy. If it is to provide energy it must be degraded. Since it is believed that linoleic acid cannot be synthesized by the mammalian organism[14] the acetate derived from linoleic acid breakdown will not be resynthesized to linoleic acid. This would lead to the eventual depletion of linoleic acid from the depots. The maintenance of the linoleic acid content of the depots after 38 days on a fat free diet was also shown by Hirsch et al. in an adult male. During this period of time the subject's caloric expenditure was equivalent to his total fat stores. It would therefore appear that linoleic acid was either not mobilised or not totally degraded when mobilised. If linoleic acid is essential to body metabolism it is presumably drawn upon from the depots on a fat free diet. A possible explanation for the findings by Hirsch et al. may be that linoleic acid when mobilised is not totally degraded. Only part of the molecule being used for energy purposes and the tail of the molecule, containing the characteristic double bonds, left intact. Reconversion of this to an 18 carbon chain-length fatty acid by the addition of 2 carbon units will reconstitute linoleic acid. Evidence that this may

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occur in human adipose tissue has recently been published. It was found that incubating adipose tissue with acetate-1-C^{14} gave rise to the recovery of labelled linoleic acid[10]. The authors considered that this finding may be the result of contamination. Their separations however were done by gas-liquid chromatography. It is difficult to see how significant contamination could have occurred, particularly as the fraction preceding C18:2 in their separation (C18:1) had only half of the activity of the C18:2 fraction. The more likely explanation is that suggested above.

Our knowledge of the metabolism of linoleic acid, its mobilization, degradation and resynthesis is very inadequate. The little available evidence there is has been discussed above and the interpretation of this evidence is also subject to controversy. It has been interpreted as indicating that linoleic acid, when mobilized, is not necessarily wholly degraded, as is generally the case with palmitic acid[14]. This may indicate that the metabolism of linoleic acid differs from other fatty acids in adipose tissue.

The findings between the sexes in this study with respect to the percentage of linoleic acid do not resolve the question of the relative requirements for this fatty acid. Until such time as more is known about its metabolism no definite conclusions can be drawn. If it is shown that its deposition and mobilisation in depot fat does not differ greatly from that of the other fatty acids then these findings would indicate that there is no sex difference in the requirements for this fatty acid. If it behaves differently, then the relatively greater size of the female adipose organ may be suggestive of a decreased requirement among females.

It must also be pointed out that although C18:2, as separated by gas-liquid chromatography, and linoleic acid, is spoken of synonymously this may not be the case. C18:2 is not necessarily linoleic acid (see Chapter 3). In these studies however, even if a certain proportion of C18:2 were not linoleic acid, it would not be too great a

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criticism of the findings being the same in both sexes. Geometric isomers of linoleic acid are rare. If they are eaten in any abundance by one or other of the racial groups, the relative intake of these unnatural fatty acids is very likely much the same in both sexes of the same race. It is therefore unlikely that this possibility might explain the results.

/CONCLUSIONS.....

CONCLUSIONS

The depot fat fatty acid composition shows differences between males and females. These differences are confined to the 16 and 18 carbon chain-length saturated and monounsaturated fatty acids. In general the proportion of C16:0 and C18:0 is higher and C16:1 and C18:1 lower in the males than in the females. These sex differences are best expressed as the ratio of the monounsaturated to the saturated fatty acids. These sex differences are present in each racial group, but are more consistent in the younger than in the older age groups.

There is no difference between the sexes in the percentage of C14:0 and C18:2. That there is no sex difference in the proportion of C18:2 between the sexes would appear to indicate that there is no sex difference in the requirement for linoleic acid. This is, however, a complicated problem.

/SUMMARY.....

SUMMARY

The fatty acid composition of depot fat is compared between males and females, both in general and in regard to age and race. Differences between the sexes were found and the possible mechanisms by which these differences may be brought about are discussed.

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CHAPTER 9

THE FATTY ACID COMPOSITION OF DEPOT FAT IN THE THREE RACIAL GROUPS

The marked differences in the dietary habits between the White, Cape Coloured and Bantu subjects sampled have been demonstrated. These differences are primarily a difference in the relative proportion of daily calories derived from carbohydrate and fat as well as in the type of fat. The protein intake does not vary strikingly between the racial groups. The subjects of this study in general follow the established dietary patterns in the 3 races (Chapter 4). It is therefore of interest to determine whether these dietary differences are expressed in the depot fat composition, and if so, in what way.

Each of the common fatty acids in the depot fat have been compared between the racial groups. Because of differences between the sexes shown in Chapter 8 these comparisons will be made between races in each sex.

RESULTS

The percentage of each of the fatty acids, C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2, in each of the racial groups, males and females, is shown as a mean in Table 1 and Figs. 1 and 2.

	MALES			FEMALES		
FATTY ACID	W.	C.	B.	W.	C.	B.
C14:0	4.1	3.7	2.6	3.9	3.2	2.5
C16:0	24.1	23.6	22.3	21.8	21.8	22.5
C16:1	8.2	6.6	7.5	9.6	7.3	10.8
C18:0	4.5	5.2	5.5	3.3	3.9	3.2
C18:1	45.0	42.0	46.9	46.4	44.3	47.4
C18:2	7.5	12.2	9.6	7.6	13.3	8.3

TABLE 1. The mean value for the percentage of each of the common fatty acids in White (W), Cape Coloured (C), and Bantu (B), Males and Females. Note the consistent trends between races in both the males and females for the percentage of C14:0, C16:1, C18:1 and C18:2.

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It can be seen from Table 1 and Figs. 1 and 2 that the percentage of C14:0 falls progressively from White through Cape Coloured to Bantu subjects in both the males and females. The percentage of C16:1 and C18:1 is lowest and the percentage of C18:2 is highest in the Coloured males and females. There are no other consistent differences between races. The percentage of each of these common fatty acids will now be compared between each race and at both age levels, taking account of sex.

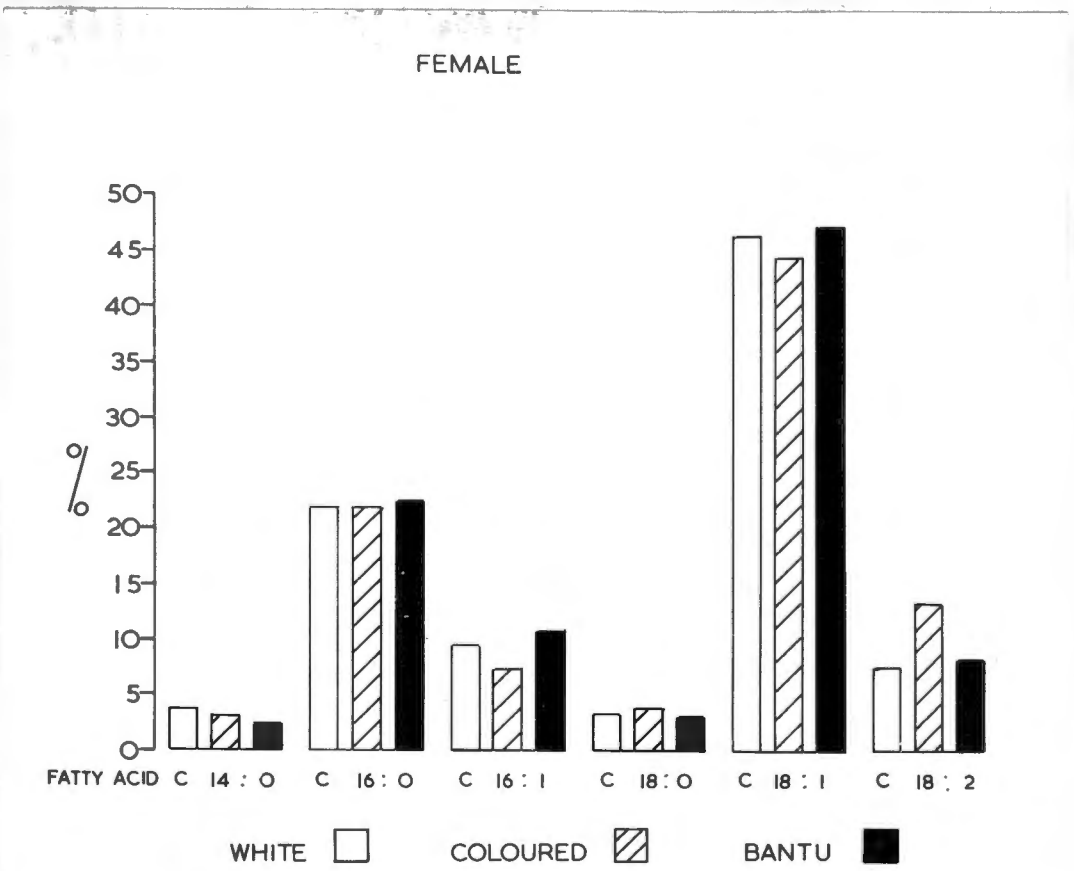

FIG. 1. The percentage of the common fatty acids shown as a mean among the females of each race.

/Tables 2 - 19.....

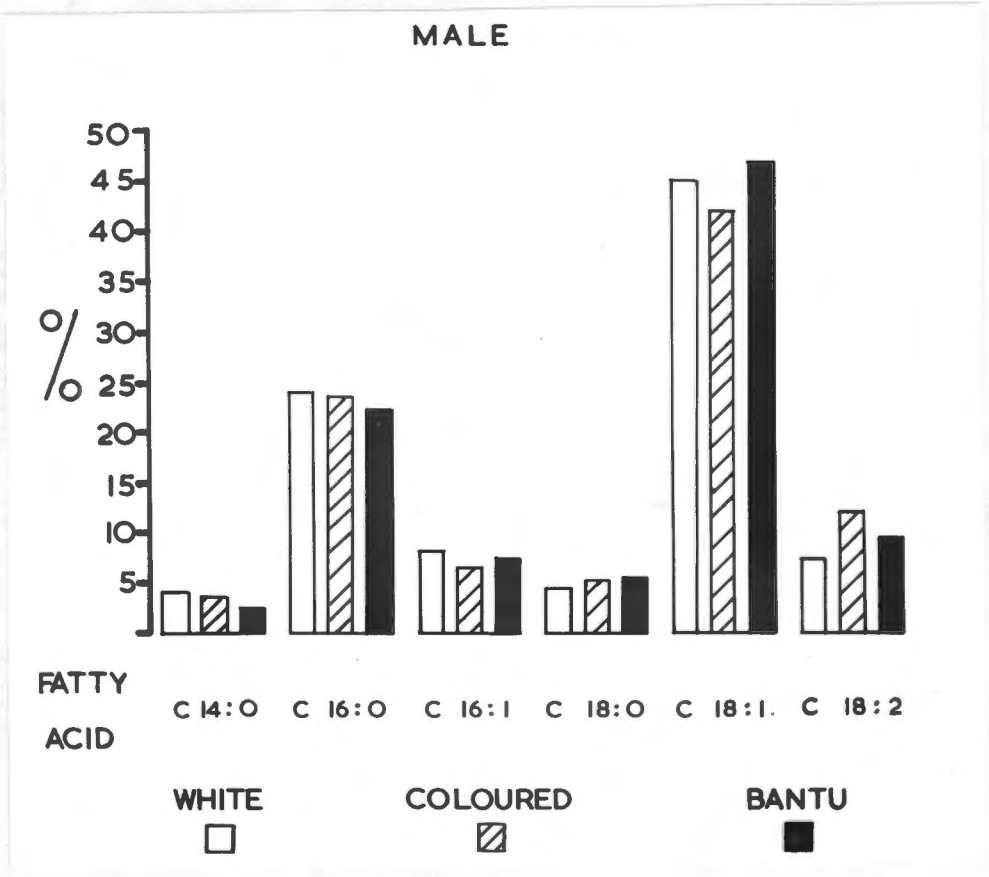

FIG. 2. The percentage of the common fatty acids shown as a mean among the males of each race.

Tables 2 - 19 show the mean, one standard deviation (S.D.), the difference between means (diff.) and the significance of this difference (P) between White and Cape Coloured (W vs.C), White and Bantu (W vs.B) and Bantu and Cape Coloured (B vs.C) in the 20 - 29, 40 - 49 and the combined (20 - 29 + 40 - 49) age groups in both males and females.

C14:0 (%)

AGE(Yrs)	20 - 29					40 - 49				
SEX	MALES		MALES			FEMALES		FEMALES		
RACE	Mean	(S.D.)	Mean	(S.D.)	diff. P.	Mean	(S.D.)	Mean	(S.D.)	diff. P.
W vs.C	4.3	(1.5)	3.8	(1.6)	0.5 N.S.	4.2	(0.9)	3.5	(1.2)	0.7 N.S.
W vs.B	4.3	(1.5)	2.8	(0.3)	1.5 <0.01	4.2	(0.9)	2.6	(0.7)	1.6 <0.00
B vs.C	2.8	(0.3)	3.8	(1.6)	1.0 <0.05	2.6	(0.7)	3.5	(1.2)	0.9 <0.05

TABLE 2.

C14:0 (%)

AGE(Yrs)	40 - 49					40 - 49				
SEX	MALES		MALES			FEMALES		FEMALES		
RACE.	Mean	(S.D.)	Mean	(S.D.)	diff. P.	Mean	(S.D.)	Mean	(S.D.)	diff. P.
W vs.C	3.9	(1.4)	3.5	(0.8)	0.5 N.S.	3.8	(1.2)	3.0	(0.6)	0.8 <0.05
W vs.B	3.9	(1.4)	2.5	(0.5)	1.4 <0.02	3.8	(1.2)	2.3	(0.9)	1.5 <0.01
B vs.C	2.5	(0.5)	3.5	(0.8)	1.0 <0.01	2.3	(0.9)	3.0	(0.6)	0.9 <0.05

TABLE 3.

C14:0 (%)

AGE(Yrs)	20 - 29 + 40 - 49					20 - 29 + 40 - 49				
SEX	MALES		MALES			FEMALES		FEMALES		
RACE	Mean	(S.D.)	Mean	(S.D.)	diff. P.	Mean	(S.D.)	Mean	(S.D.)	diff. P.
W vs.C	4.1	(1.5)	3.7	(1.3)	0.4 N.S.	3.9	(1.1)	3.2	(1.0)	0.7 <0.05
W vs.B	4.1	(1.5)	2.6	(0.4)	1.5 <0.001	3.9	(1.1)	2.5	(0.7)	1.4 <0.00
B vs.C	2.6	(0.4)	3.7	(1.3)	1.1 <0.001	2.5	(0.7)	3.2	(1.0)	0.7 <0.01

TABLE 4.

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From Tables 2, 3 and 4 it can be seen that Bantu males and females have a consistently lower percentage of C14:0 than both the White and Cape Coloured subjects at each age level. The trend for the Coloured subjects to show a decrease compared with the White subjects is of probable significance in the Coloured females only (Table 4). The percentage of C14:0 falls from White, through Cape Coloured to Bantu in each age group and in both sexes.

There is no consistent trend between the races in the percentage of C16:0 (Tables 5, 6 and 7).

C16:0 (%)

AGE(Yrs)	20 - 29					20 - 29			
SEX	MALES	MALES				FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.		Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	23.6 (1.4)	23.3 (2.8)	0.3	N.S.		21.3 (0.9)	20.8 (1.8)	0.5	N.S.
W vs.B	23.6 (1.4)	22.1 (3.5)	1.5	N.S.		21.3 (0.9)	23.0 (2.2)	1.7	<0.02
B vs.C	22.1 (3.5)	23.3 (2.8)	1.2	N.S.		23.0 (2.2)	20.8 (1.8)	2.2	<0.02

TABLE 5.

C16:0 (%)

AGE(Yrs)	40 - 49					40 - 49			
SEX	MALES	MALES				FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.		Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	24.8 (2.3)	23.9 (3.0)	0.9	N.S.		22.1 (2.9)	22.9 (2.6)	1.8	N.S.
W vs.B	24.8 (2.3)	22.4 (2.2)	2.4	<0.05		22.1 (2.9)	21.7 (2.7)	0.4	N.S.
B vs.C	22.4 (2.2)	23.9 (3.0)	1.5	N.S.		21.7 (2.7)	22.9 (2.6)	1.2	N.S.

TABLE 6.

/ TABLE 7.....

C16 : 0 (%)

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	24.1 (1.9)	23.6 (2.8)	0.5	N.S.	21.8 (2.3)	21.9 (2.4)	0.1	N.S.
W vs.B	24.1 (1.9)	22.3 (2.9)	1.8	<0.05	21.8 (2.3)	22.5 (2.5)	0.7	N.S.
B vs.C	22.3 (2.9)	23.6 (2.8)	1.3	N.S.	22.5 (2.5)	21.9 (2.4)	0.6	N.S.

TABLE 7.

The significant differences between White and Bantu and Bantu and Cape Coloured females (Table 5) is not reflected in either the males of the same age group nor in the females of the older age group (Table 6). These differences are also not reflected in the combined age groups (Table 7). The value in the Bantu males tends to be somewhat lower than in the White and Coloured males. The Bantu females tend to be higher; the level in the Bantu males and females being the same, unlike that found between the sexes in the other racial groups (Chapter 8).

The trend for the percentage of C16:1 to be lowest in the Cape Coloured subjects (Table 1, Figs.1 and 2) is seen at each age level, and in the combined age groups, in both males and females (Tables 8, 9 and 10). These differences however are not consistently statistically significant.

C 16 : 1 (%)

AGE(Yrs)	20 - 29				20 - 29			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	8.1 (1.1)	6.5 (1.5)	1.6	<0.01	9.2 (2.4)	7.7 (1.1)	1.5	N.S.
W vs.B	8.1 (1.1)	6.7 (1.7)	1.4	<0.05	9.2 (2.4)	11.0 (1.7)	1.8	<0.05
B vs.C	6.7 (1.7)	6.5 (1.5)	0.2	N.S.	11.0 (1.7)	7.7 (1.1)	3.3	<0.001

TABLE 8.

/TABLE 9.....

C16 : 1 (%)

AGE(Yrs)	40 - 49				40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	8.2 (1.4)	6.7 (1.8)	1.5	<0.05	9.9 (1.5)	7.0 (1.5)	2.9	<0.00
W vs.B	8.2 (1.4)	8.5 (1.7)	0.3	N.S.	9.9 (1.5)	10.5 (2.9)	0.6	N.S.
B vs.C	8.5 (1.7)	6.7 (1.8)	1.8	<0.05	10.5 (2.9)	7.0 (1.5)	3.5	<0.01

TABLE 9.

C16 : 1 (%)

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	8.2 (1.2)	6.6 (1.6)	1.6	<0.001	9.6 (1.9)	7.3 (1.4)	2.3	<0.00
W vs.B	8.2 (1.2)	7.5 (1.9)	0.7	N.S.	9.6 (1.9)	10.8 (2.2)	1.2	N.S.
B vs.C	7.5 (1.9)	6.6 (1.6)	0.9	N.S.	10.8 (2.2)	7.3 (1.4)	3.5	<0.00

TABLE 10.

It would appear that the Cape Coloured subjects, males and females, tend to have a lower percentage of C16:1 than the White subjects (Table 10), but only the Coloured females have a significantly lower percentage of C16:1 than the Bantu females (Tables 8, 9, 10). The difference between the Coloured and Bantu males is not so striking (Tables 8, 9, 10).

The percentage of C18:0 does not show any striking or consistent difference between the races (Tables 11, 12, 13).

C18 : 0 (%)

AGE(Yrs)	20 - 29				20 - 29			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	4.7 (1.3)	5.5 (2.0)	0.8	N.S.	3.4 (1.0)	3.7 (1.0)	0.3	N.S.
W vs.B	4.7 (1.3)	6.1 (2.1)	1.4	N.S.	3.4 (1.0)	3.0 (1.1)	0.4	N.S.
B vs.C	6.1 (2.1)	5.5 (2.0)	0.6	N.S.	3.0 (1.1)	3.7 (1.0)	0.7	N.S.

TABLE 11.

/TABLE 12.....

C18 : 0 (%)

AGE(Yrs)	40 - 49						40 - 49					
SEX	MALES		MALES				FEMALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W vs.C	4.3	(1.2)	4.9	(1.6)	0.6	N.S.	3.2	(0.9)	4.1	(0.8)	0.9	<0.01
W vs.B	4.3	(1.2)	4.9	(1.4)	0.6	N.S.	3.2	(0.9)	3.5	(1.2)	0.3	N.S.
B vs.C	4.9	(1.4)	4.9	(1.6)	0	N.S.	3.5	(1.2)	4.1	(0.8)	0.6	N.S.

TABLE 12.

C18 : 0 (%)

AGE(Yrs)	20 - 29 + 40 - 49						20 - 29 + 40 - 49					
SEX	MALES		MALES				FEMALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W vs.C	4.5	(1.2)	5.2	(1.8)	0.7	N.S.	3.3	(0.9)	3.9	(0.9)	0.6	N.S.
W vs.B	4.5	(1.2)	5.5	(1.2)	1.0	<0.05	3.3	(0.9)	3.2	(1.2)	0.1	N.S.
B vs.C	5.5	(1.8)	5.2	(1.8)	0.3	N.S.	3.2	(1.2)	3.9	(0.9)	0.7	<0.05

TABLE 13.

The percentage of C18:1 which appeared to be lower in the Coloured subjects (Table 1, Figs. 1 and 2) is not statistically significant in every case (Tables 14, 15), but does in general follow that trend.

C18 : 1 (%)

AGE(Yrs)	20 - 29						20 - 29					
SEX	MALES		MALES				FEMALES		FEMALES			
RACE	Mean	(S.D.)	Mean	(S.D.)	diff.	P.	Mean	(S.D.)	Mean	(S.D.)	diff.	P.
W vs.C	44.5	(2.7)	42.3	(3.9)	1.2	N.S.	46.6	(4.9)	43.9	(2.5)	2.7	N.S.
W vs.B	44.5	(2.7)	46.8	(3.5)	2.3	N.S.	46.6	(4.9)	45.5	(3.1)	1.1	N.S.
B vs.C	46.8	(3.5)	42.3	(3.9)	4.5	<0.01	45.5	(3.1)	43.9	(2.5)	1.6	N.S.

TABLE 14.

/TABLE 15.....

C18 : 1 (%)

AGE(Yrs)	40 - 49				40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	45.7 (3.2)	41.7 (4.3)	4.0	<0.05	46.2 (4.1)	44.9 (2.2)	1.3	N.S.
W vs.B	45.7 (3.2)	47.1 (2.8)	1.4	N.S.	46.2 (4.1)	50.1 (2.0)	3.9	<0.02
B vs.C	47.1 (2.8)	41.7 (4.3)	5.4	<0.01	50.1 (2.0)	44.9 (2.2)	5.2	<0.001

TABLE 15.

C18 : 1 (%)

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	45.0 (2.9)	42.0 (4.0)	3.0	<0.01	46.4 (4.3)	44.3 (3.8)	2.1	N.S.
W vs.B	45.0 (2.9)	46.9 (3.1)	1.9	<0.05	46.4 (4.3)	47.4 (3.6)	1.0	N.S.
B vs.C	46.9 (3.1)	42.0 (4.0)	4.9	<0.001	47.4 (3.6)	44.3 (3.8)	3.1	<0.01

TABLE 16.

The Coloured males and females have a lower percentage of C18:1 than the other groups. This difference between the racial groups, although not always significant, is consistent.

The percentage of C18:2 is consistently higher among the Coloured subjects, both males and females at each age level. The Bantu subjects tend to be intermediate between White and Coloured.

C18 : 2 (%)

AGE(Yrs)	20 - 29				20 - 29			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	8.0 (2.0)	11.6 (4.4)	3.6	<0.02	8.0 (2.2)	14.3 (3.3)	6.3	<0.001
W vs.B	8.0 (2.0)	9.8 (2.2)	1.8	N.S.	8.0 (2.2)	9.4 (2.5)	1.4	N.S.
B vs.C	9.8 (2.2)	11.6 (4.4)	1.8	N.S.	9.4 (2.5)	14.3 (3.3)	4.9	<0.001

TABLE 17.

/TABLE 18.....

C18 : 2 (%)

AGE(Yrs)	40 - 49				40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	6.9 (1.6)	12.9 (3.8)	6.0	<0.001	7.3 (2.0)	12.4 (4.0)	5.1	<0.001
W vs.B	6.9 (1.6)	9.3 (2.0)	2.4	<0.01	7.3 (2.0)	6.8 (1.6)	0.5	N.S.
B vs.C	9.3 (2.0)	12.9 (3.8)	3.6	<0.02	6.8 (1.6)	12.4 (4.0)	5.6	<0.001

TABLE 18.

C18 : 2 (%)

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	7.5 (1.9)	12.2 (4.1)	4.7	<0.001	7.6 (2.0)	13.3 (3.7)	5.7	<0.001
W vs.B	7.5 (1.9)	9.6 (2.0)	2.1	<0.01	7.6 (2.0)	8.3 (2.5)	0.7	N.S.
B vs.C	9.6 (2.0)	12.2 (4.1)	2.7	<0.02	8.3 (2.5)	13.3 (3.7)	5.0	<0.001

TABLE 19.

It can be seen from Tables 17, 18 and 19 that in every case the percentage of C18:2 among the Coloured subjects, at each age level and in the combined age groups, is significantly higher than among the White subjects. The trend for the percentage of C18:2 to be higher in the Coloured than in the Bantu is also statistically significant in each comparison except in the young male subjects (Table 17). There also appears to be a trend for the percentage of C18:2 to be higher in the Bantu males than in the White males, but this is primarily due to a difference between the older age group of male subjects.

/DISCUSSION.....

DISCUSSION

In looking for trends in the depot fat fatty acid composition between the races, features that are consistent between males and females of a particular race will give the best guide.

MYRISTIC ACID

The percentage of C14:0 decreases from White, through Coloured, to Bantu in the males and females; both in general (Table 1, Figs.1 and 2) and at each age level (Tables 2 and 3). The difference between the Bantu, and both the Coloured and White subjects was significant at each age level in the males and females (Tables 2, 3 and 4). The Cape Coloured subjects show an intermediate percentage which was not impressively lower than that in the White subjects.

This pattern between the racial groups in the percentage of C14:0 is reminiscent of their respective fat intakes (Chapter 4). If the total fat intake is compared with the percentage of C14:0 in both males and females (Fig.3) a striking correlation is evident.

It will be recalled from Chapter 8 that the oxidative degradation of palmitic acid gives rise to little accumulation of shorter chain-length intermediates. The source of this fatty acid would therefore be almost exclusively dietary, since the ability of the organism to synthesize C14:0 is minimal. It is of interest that myristic acid constitutes, on the average, approximately 3% of the total fatty acids in the natural fats and oils derived from both animal and vegetable sources[1]. The percentage of C14:0 in the depot fat fatty acids would therefore appear simply to reflect the total fat intake.

/LINOLEIC ACID.....

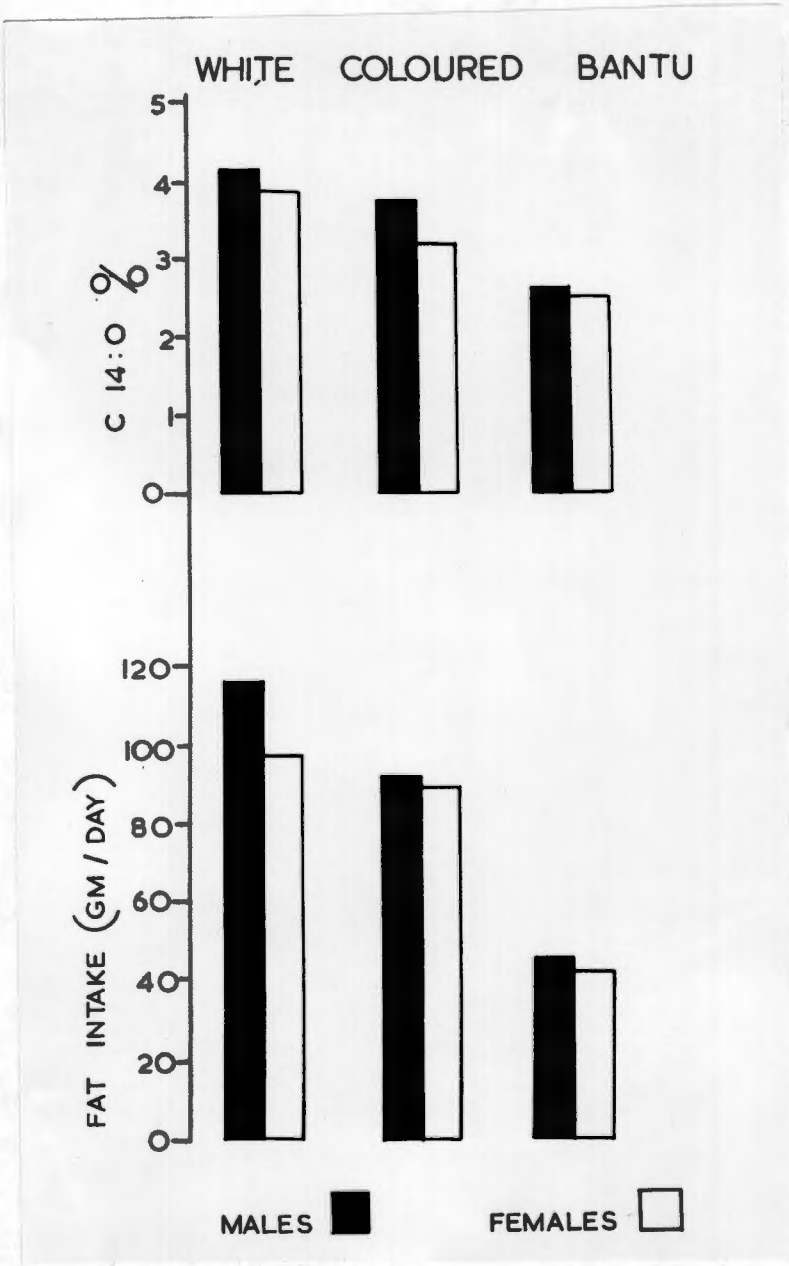

FIG. 3. Showing the mean value for the percentage of C14:0 and the mean total daily fat intake in gms., in each race and sex. Note that the fall in C14:0 is paralleled by the fall in fat intake.

LINOLEIC ACID

Relation To Diet

The trend for the Coloured subjects to have a higher percentage of this fatty acid (Tables 1, 17, 18, 19) (Figs. 1 and 2) is a most striking feature related to race. The percentage of this fatty acid is in general intermediate in the Bantu and lowest in the White subjects (Table 1, Figs. 1 and 2).

The deposition of this fatty acid, as has been indicated in Chapter 8, is believed to be dependent upon its availability in the diet. One might therefore expect that the relative proportion of linoleic acid found in the depot fat among the racial groups would be related to their relative intake of this fatty acid. No such simple relationship is however evident whether the linoleic acid intake is expressed as total intake or as a percentage of total calories (Table 20).

	MALES			FEMALES		
RACE	W	C	B	W	C	B
gm./day	7.6	7.3	6.9	6.8	6.6	5.0
% Total Calories	3.2	3.1	2.9	3.0	3.4	2.9

TABLE 20. The mean linoleic acid intake expressed in gm./day and as a percentage of the total daily calories in each race and sex. There is no correlation between intake and the percentage of depot fat linoleic acid among the racial groups. The intake of linoleic acid is remarkably similar between the racial groups.

This finding serves to indicate a complex relationship between diet and essential fatty acid requirements.

The findings in the depotfat of the local racial groups suggest that the intake of linoleic acid among the Cape Coloured subjects is in relatively greater excess of their requirements than among the Bantu and White subjects. Factors said to increase the demand for essential fatty acids are dietary fat and cholesterol[2]. Deuel et al.[3] reported that increasing the dietary fat level increases the demand for essential fatty

/acids.....

acids. The relationship between the total fat intake, or the animal fat intake, in the local racial groups (Chapter 4) and the percentage of C18:2 in their depot fat also does not correlate. It has since been claimed that not total fat, but saturated fat, increases the demand for essential fatty acids[4]. The saturated fat intake in the groups studied, whether expressed as a percentage of total fat or as a percentage of total calories still does not correlate with the percentage of C18:2 found in the racial groups (Table 21).

	MALES			FEMALES		
RACE	W	C	B	W	C	B
% Total Calories	23.3	18.4	8.5	21.3	20.0	9.7
% Total Fat	49.0	47.6	40.7	47.6	47.2	40.1

TABLE 21. The mean intake of saturated fat expressed as a percentage of total calories and as a percentage of total fat in the groups studied.

By subtraction therefore the unsaturated fat intake as such also would not correlate with the depot fat percentage of C18:2 found in the racial groups.

It has recently been shown that a lipid rich yeast (Torulopsis utilis) is able to convert oleic acid-1-C^{14} to what is assumed to be linoleic acid[5]. It is therefore conceivable that such a transformation, previously thought not to occur in nature, may take place in man. The oleic acid intake has not been calculated in the subjects studied, but the vegetable fat intake, relatively rich in oleic acid, may be used as an index. This however also does not correlate with the percentage of C18:2 in the racial groups. The total vegetable fat intake (Chapter 4) does not show the same trends between the races as does the percentage of C18:2 in the depot fat, nor is there a similar trend when the vegetable fat intake is expressed as a percentage of the total calories (Table 22).

/TABLE 22.....

RACE	MALES			FEMALES		
	W	C	B	W	C	B
% Total Calories	6.3	8.0	9.5	11.2	11.8	10.5

TABLE 22. The mean vegetable fat intake expressed as a percentage of the total calories.

The dietary cholesterol content has also been shown to increase the demand for linoleic acid[6,7]. Only the serum cholesterol concentration is available in the subjects studied. Serum cholesterol concentration does not simply depend upon the dietary cholesterol content, but is largely determined by the amount and type of dietary fat ingested[8, 9]. The serum cholesterol concentration therefore is not an indicator of dietary cholesterol content. In general terms, however, serum cholesterol concentration in man may be regarded as reflecting the proportion of unsaturated to saturated fat in the diet[8]. The serum cholesterol concentration might therefore be used as an index of the interplay between the dietary factors in a mixed diet that determine the demand for linoleic acid. The serum cholesterol concentration however also does not correlate with the percentage of C18:2 in the depot fat. This is evident both when considering the trends in serum cholesterol concentration in the racial groups (Chapter 4) and the trends shown in this chapter in the percentage of C18:2 (Figs. 1 and 2) in the depot fat. The correlation coefficient between serum cholesterol concentration and the percentage of C18:2 in the depot fat is not significant ($r = -0.053$, p 0.1) (Fig. 4).

A dietary factor having a direct and potent effect on the proportion of C18:2 in the depot fat has recently been demonstrated in rats. Kaunitz et al.[10] fed rats, depleted of depot fat linoleic acid, a variety of diets. Feeding these rats specially prepared triglycerides made up of predominantly medium chain-length and predominantly long-chain saturated fatty acids did not give rise to the deposition of C18:2 in the depot fat. On adding 2% of linoleic acid to the diet, C18:2 appeared in the depot fat.

/When the.....

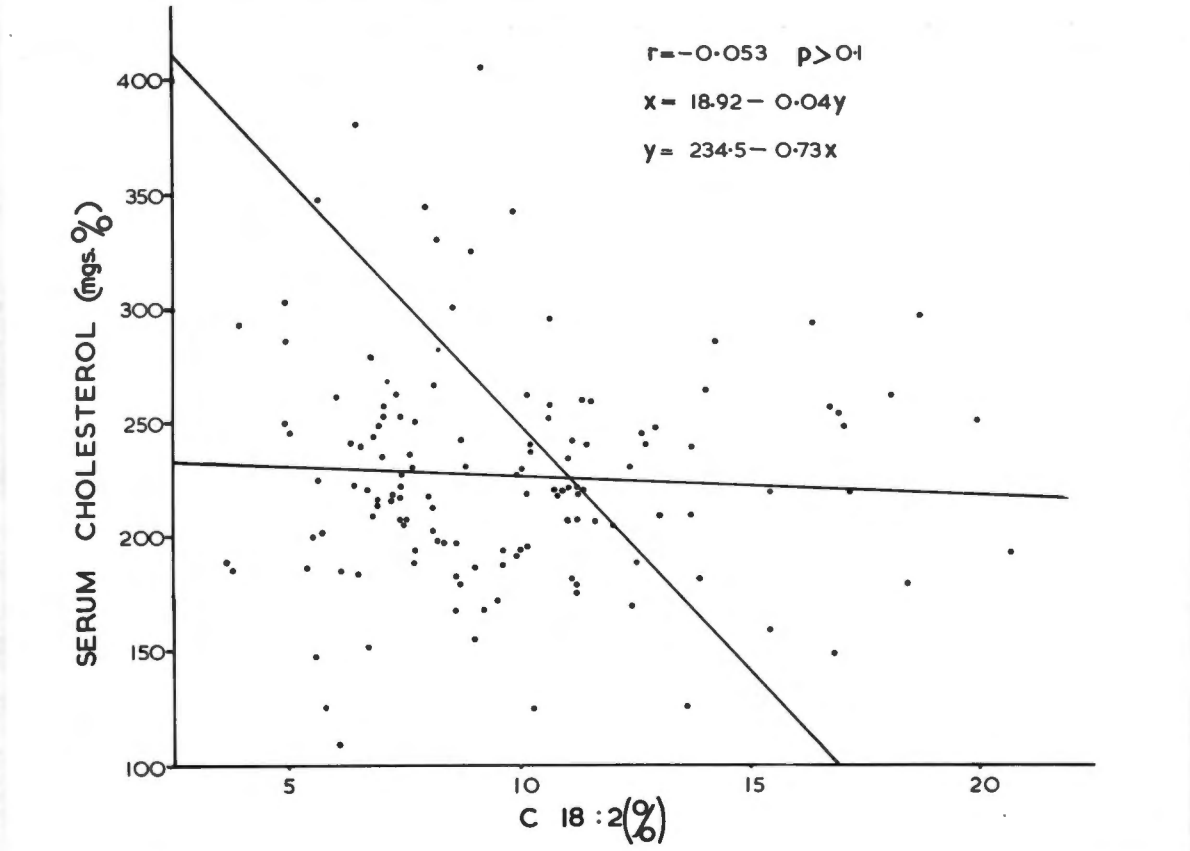

FIG. 4. Showing the relationship between serum cholesterol concentration (mg.%) and C18:2 (%).
There is no significant correlation.

When the linoleic acid was fed with a fat-free diet the proportion of C18:2 in the depot fat fatty acids was 21.1%. When fed together with a diet containing 20% medium chain-length triglycerides, C18:2 represented 11.1% of the depot fat fatty acids and when fed with 20% of long-chain triglycerides the depot C18:2 was 6.0% of the total. Thus a relatively small change in the fatty acid composition of the saturated dietary fat may halve the proportion of depot fat C18:2. What is also of interest is that the rats fed no fat had the largest proportion of depot fat C18:2. These findings may be interpreted as indicating an increased demand for linoleic acid by the feeding of saturated fat, particularly if of long-chain fatty acid.

It is not possible to determine from the dietary data whether this may be the dietary factor accounting for the findings among the local racial groups.

It is apparent that the proportion of C18:2 in the depot fat is not simply a reflection of the net dietary intake of this fatty acid. A number of factors contribute in determining its proportion in body tissues, as has been shown in animal studies. There appears to be little hope of determining the factors, dietary or otherwise, that may account for the findings in this study until more is known about the metabolism of this fatty acid. It is of course possible that dietary details obtained by recall may not be accurate enough to assess details of linoleic acid intake. Another factor of probable importance is that the linoleic acid content of the various foodstuffs was calculated from tables published in America. These tables might well be incorrect for local foods. No tables are however available for the linoleic acid content of local foods.

Hirsch et al.[20] have shown in man that the percentage of linoleic acid in the depot fat will ultimately approach that found in a specific dietary fat, fed at a very high level of intake (more than 20% of daily calories). There does not however seem to be the same direct relationship in subjects on natural diets with a linoleic acid content of about 3% of the total calories.

/RELATIONSHIP.....

RELATIONSHIP TO OTHER FATTY ACIDS

It can be seen from Table 1 and Figs. 1 and 2 that while the percentage of C18:2 is highest among the Coloured subjects, C16:1 and C18:1 are lowest in this group. This relationship is evident between the races at each age level (Tables 8 - 10, and 14 - 16; Fig. 5). Calculating the correlation coefficient for the percentage of C18:2 and C16:1, C18:2 and C18:1 and C18:2 and C16:1 + C18:1 showed that there was a significant negative correlation in each case (Table 22, Figs. 6, 7, 8). These correlations were significant among the males, females and all subjects.

	MALES		FEMALES		ALL SUBJECTS	
FATTY ACIDS	++ r	p	r	p	r	p
18:2/16:1	-0.465	< 0.001	-0.476	< 0.001	-0.439	< 0.001
18:2/18:1	-0.340	< 0.01	-0.376	< 0.01	-0.356	< 0.001
18:2/16:1 + 18:1	-0.441	< 0.001	-0.555	< 0.001	-0.476	< 0.001

TABLE 23. The monounsaturated fatty acids, both singly and together show a significant negative correlation with C18:2 when each is expressed as a percentage of the total fatty acids.
++r = correlation coefficient.

A similar relationship has been noted by Kaunitz et al.[10]. They noted in the feeding experiment on rats, cited above, that as the percentage of C18:2 in the depot fat rose there was a fall in the percentage of C18:1. From their tables however it is apparent that there was also a fall in the percentage of C16:1, the other major fatty acids showing relatively little proportional change.

The fact that there is, statistically, a highly significant negative correlation between C18:2 and the monoenoic fatty acids does not necessarily mean that they have a direct functional relationship. There is however evidence in the literature to connect these two groups of fatty acids. As early as 1938 Nunn and Smedley-MacLean[11] demonstrated an increase in the trienoic acid content of tissues from essential fatty acid (E.F.A.) deficient rats. This observation has been confirmed by others[12, 13, 14].

/The major amount.....

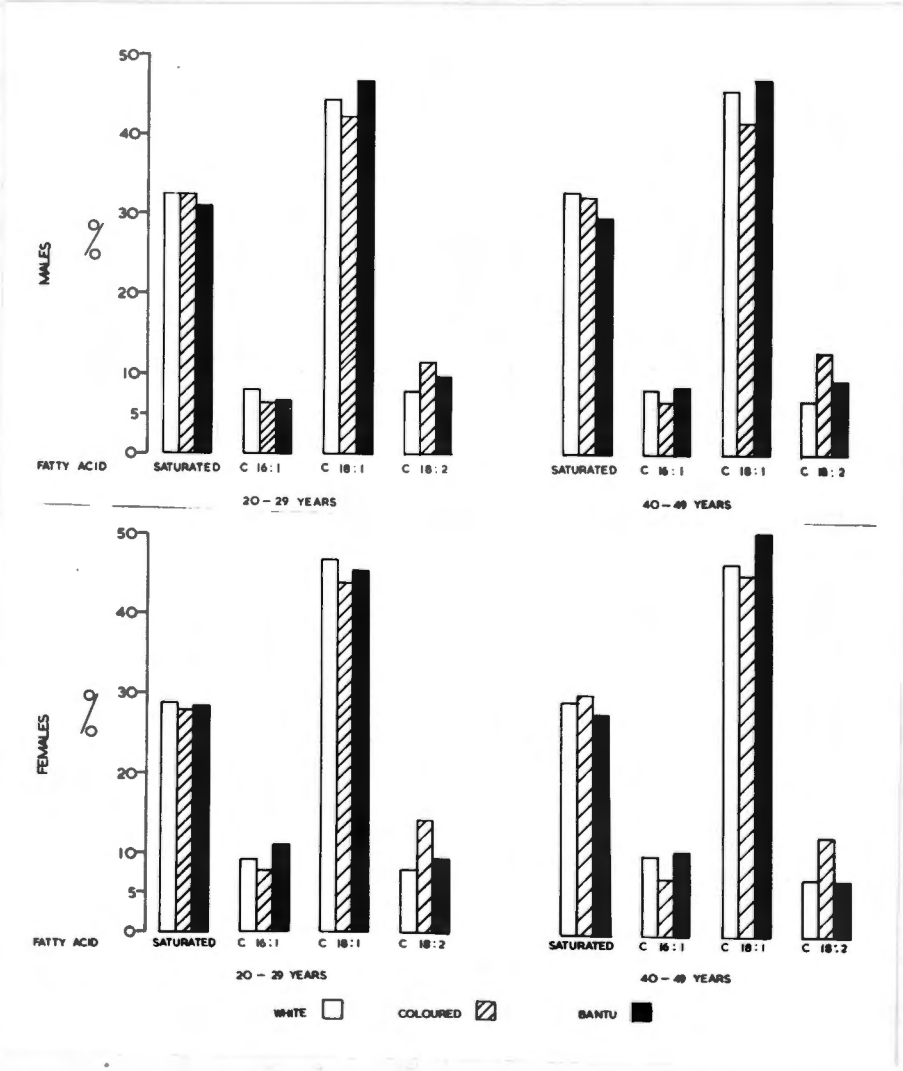

FIG. 5. The percentage of the saturated fatty acids and of C16:1, C18:1 and C18:2 is shown as a mean in each race, in both males and females at each age group.

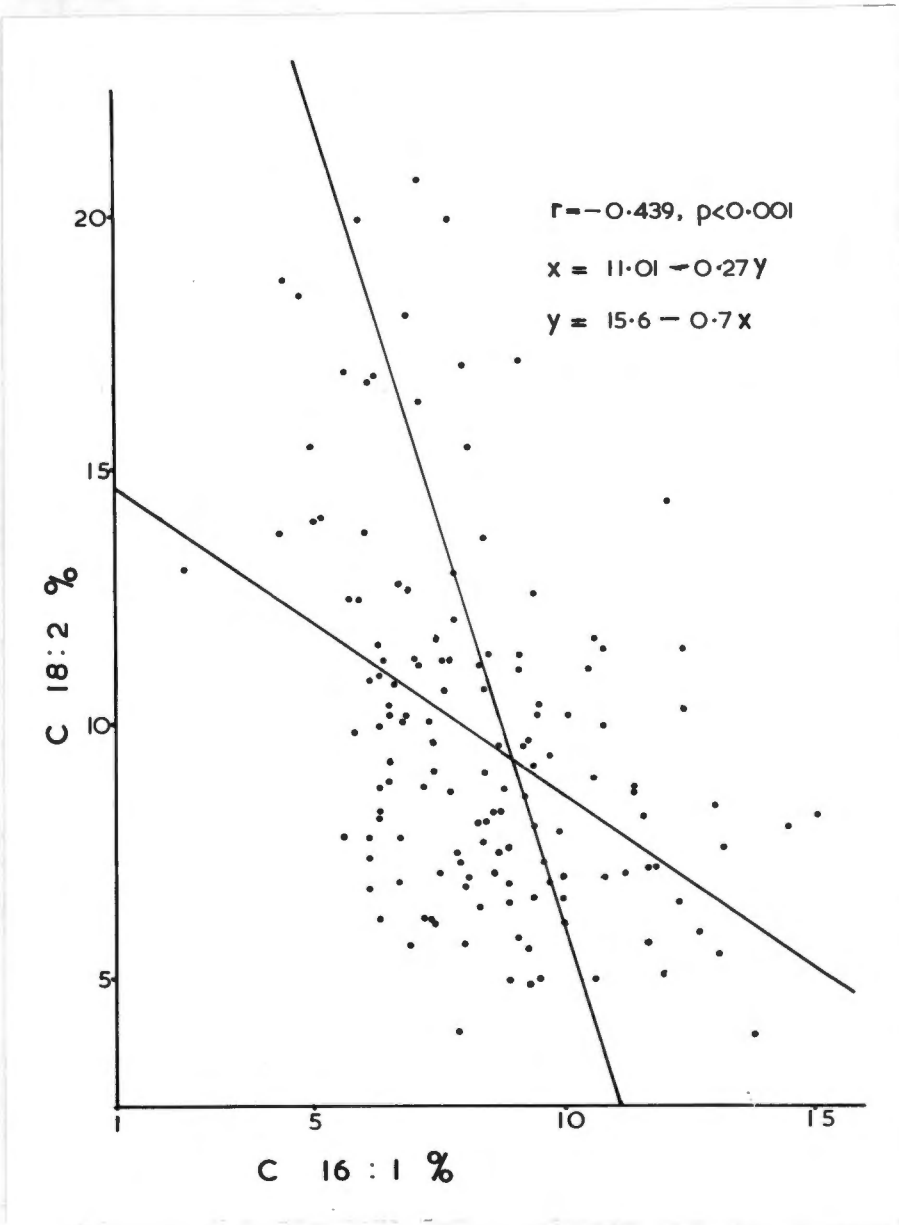

FIG. 6. The relationship between the percentage of C18:2 and C16:1 for all subjects. There is a highly significant negative correlation.

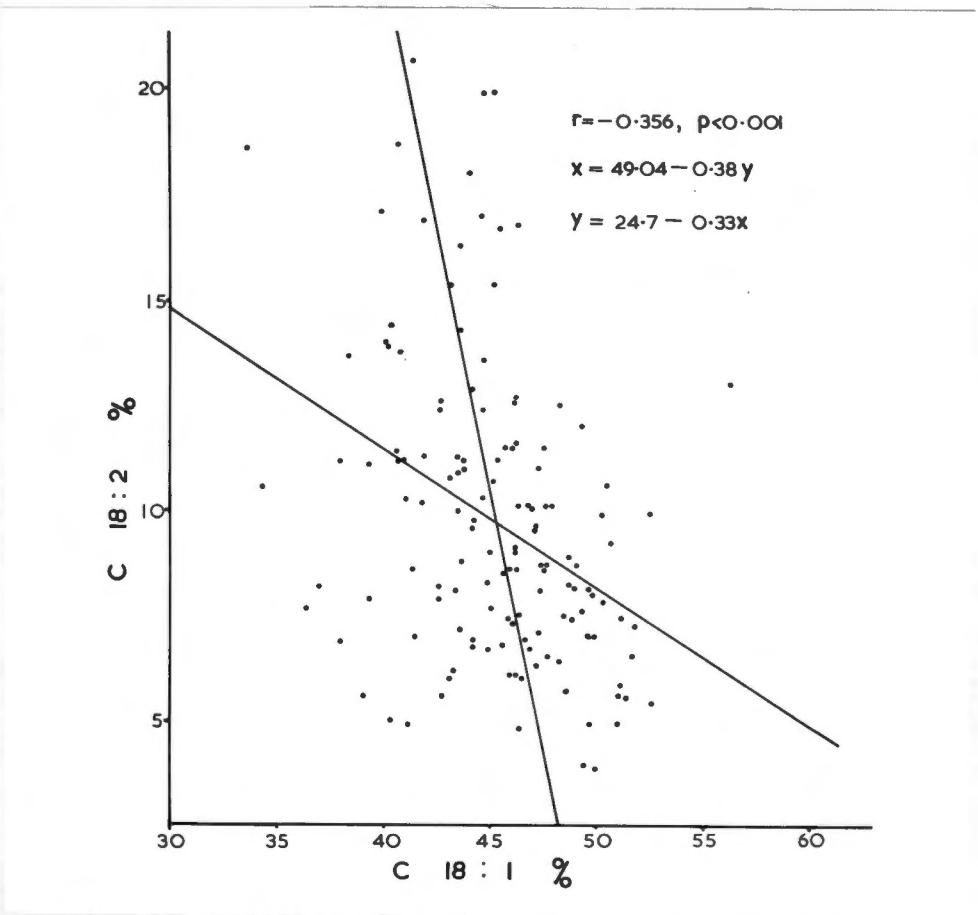

FIG. 7. The relationship between the percentage of C18:2 and C18:1 for all subjects. There is a highly significant negative correlation.

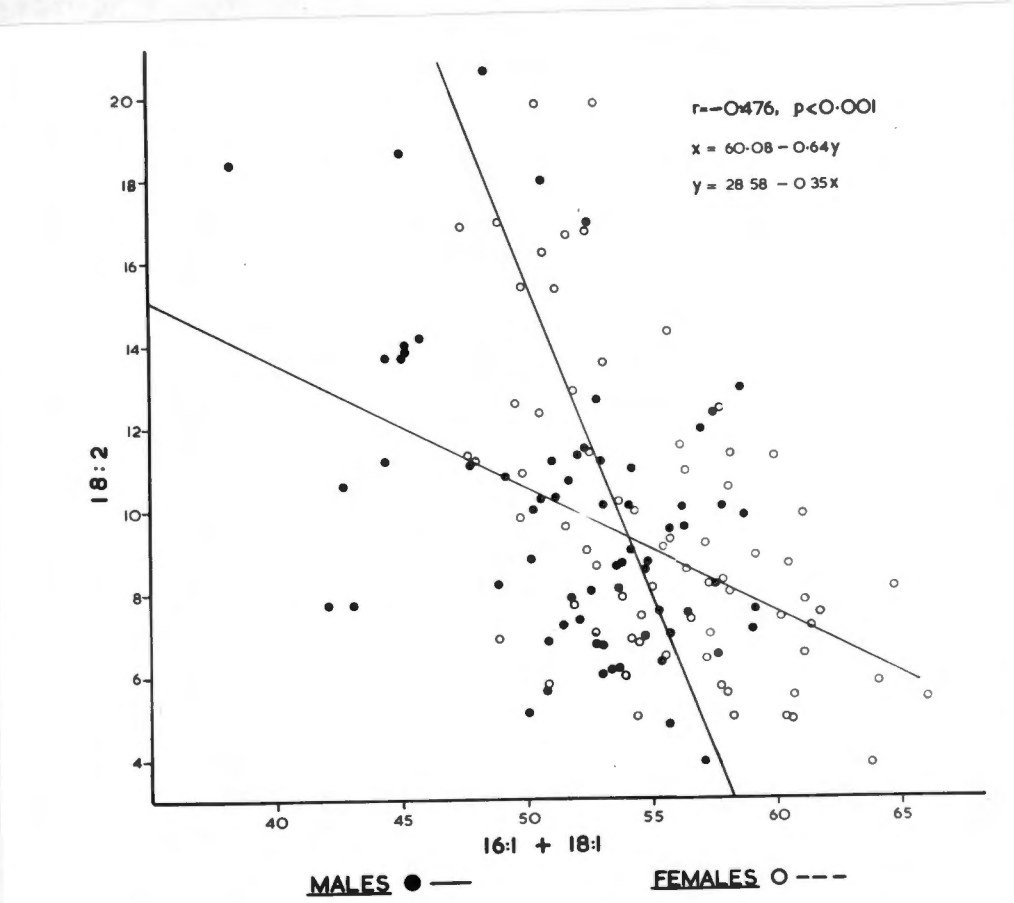

FIG. 8. The relationship between the percentage of C18:2 and C16:1 + C18:1. There is a highly significant negative correlation.

The major amount of these trienoic acids is 5, 8, 11- eicosatrienoic acid[15]. This fatty acid is considered to be related to oleic acid[16], the latter having been shown to be the *in vivo* precursor of eicosatrienoic acid[17]. Another source for eicosatrienoic acid is palmitoleic acid (C16:1)[18], the tissue content of which increases enormously in E.F.A. deficiency[19]. There is therefore evidence to indicate that with a fall in linoleic acid there is a rise in the monounsaturated 16 and 18 carbon chain-length fatty acids.

From the data obtained here it is not possible to assess whether the recently reported observation that oleic acid is converted to linoleic acid[5] may be valid. The fatty acids in the depot fat are measured in proportion to each other. One would therefore have to postulate that a relatively greater proportion of oleic acid has been converted into linoleic acid in the Coloured subjects. There is no evidence to suggest why this may be the case. This observation has thus far been made only in yeast and has still to be demonstrated in mammals.

PALMITIC AND STEARIC ACIDS

These fatty acids show no consistent trends among the males and females of any race . This finding is noteworthy because these two fatty acids are the primary fatty acids to be synthesized from acetate (Chapter 8). One might therefore have expected that the Bantu subjects with their high carbohydrate intake, would show a relatively greater proportion of these fatty acids. This not being the case attests to the ability of the organism to convert a wide variety of foodstuffs degraded to acetate into fatty acids.

THE FATTY ACID RATIOS RELATED TO SEX

It will be recalled from Chapter 8 that the fatty acid ratios C16:0/C16:1, C18:1/C18:0 and C16:1 + C18:1/C16:0 + C18:0 which showed consistent differences, statistically significant, among the White and Bantu subjects was less consistent among

/the Coloured.....

the Coloured subjects. It was there suggested that the possible reason might be due to the fact that the general level of C16:1 and C18:1 among the Coloured subjects tended to be lower than in other groups. On comparing these fatty acid ratios between the racial groups in each sex it can be seen that while the White and Bantu groups show no significant differences, the Coloured groups differ from both White and Bantu (Tables 24,25,26).

C16:0/C16:1

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	3.01 (0.52)	3.78 (1.04)	0.77	<0.01	2.36 (0.55)	3.13 (0.70)	0.77	<0.001
W vs.B	3.01 (0.52)	3.13 (0.80)	0.12	N.S.	2.36 (0.55)	2.19 (0.58)	0.17	N.S.
B vs.C	3.13 (0.80)	3.78 (1.04)	0.65	<0.05	2.19 (0.58)	3.13 (0.70)	0.94	<0.001

TABLE 24.

C18:1/C18:0

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	10.80 (3.26)	9.28 (4.06)	1.52	N.S.	15.14 (4.06)	11.97 (2.97)	3.17	<0.01
W vs.B	10.80 (3.26)	9.37 (3.06)	1.43	N.S.	15.14 (4.06)	16.56 (6.02)	1.42	N.S.
B vs.C	9.37 (3.06)	9.28 (4.06)	0.18	N.S.	16.56 (6.02)	11.97 (2.97)	4.59	<0.01

TABLE 25.

C16:1 +C18:1/C16:0 +C18:0

AGE(Yrs)	20 - 29 + 40 - 49				20 - 29 + 40 - 49			
SEX	MALES	MALES			FEMALES	FEMALES		
RACE	Mean (S.D.)	Mean (S.D.)	diff.	P.	Mean (S.D.)	Mean (S.D.)	diff.	P.
W vs.C	1.88 (0.25)	1.73 (0.36)	0.15	N.S.	2.27 (0.37)	2.02 (0.24)	0.25	<0.02
W vs.B	1.88 (0.25)	1.99 (0.32)	0.11	N.S.	2.27 (0.37)	2.30 (0.39)	0.03	N.S.
B vs.C	1.99 (0.32)	1.73 (0.36)	0.26	<0.02	2.30 (0.39)	2.02 (0.24)	0.28	<0.01

TABLE 26.

/The slight.....

The slight deviation of the Coloured subjects in the sex difference seen among the White and Bantu groups would therefore appear to be the result of their relatively lower monounsaturated fatty acids. This seems to be related to their higher percentage of C18:2.

It will also be recalled from Chapter 1 that the sex difference in serum cholesterol concentration was expressed as a difference in the total cholesterol concentration as well as in the ratio of the beta- to alph-lipoprotein fractions.

Work done on the serum cholesterol levels on the local racial groups was also quoted there. It was found that the differences in the total serum cholesterol concentration between White, Cape Coloured and Bantu men was due, almost entirely, to a difference in the amount of beta-lipoprotein cholesterol[9]. The work by Bronte-Stewart et al.[8] subsequent to this, demonstrated that qualitative alterations in the dietary fat, producing alterations in the serum total cholesterol, was again due to the changes in the beta fraction. The percentage of beta- to alpha-lipoprotein cholesterol rose from Bantu, through Coloured to White groups. It did therefore appear that dietary factors could give rise, in men, to an alteration in the serum cholesterol lipoprotein fractions, resembling that found in women. In general it appeared that in the Bantu male, the serum cholesterol lipoprotein pattern approached that found among British and American females. It is therefore of interest to determine whether the differences between the sexes in the C16 and C18 saturated and monounsaturated fatty acid ratios could similarly be related to diet. From Tables 24, 25 and 26 it can be seen that this does not appear to be the case. The White and Bantu subjects on no occasion show a significant difference among either the males or the females for any of the fatty acid ratios. The Coloured subjects, both males and females, show differences from the other racial groups. The possible reason for this deviation among the Coloured subjects was discussed above. The sex differences found in the fatty acid ratios (Chapter 8)

/therefore.....

therefore do not follow the trends between the racial groups with respect to serum cholesterol concentration and lipoprotein pattern. It would therefore appear that dietary factors are unlikely to account for the differences between the sexes in these fatty acid ratios.

LOCAL BANTU COMPARED WITH RURAL BANTU

Of the three racial groups, the Bantu subjects sampled from Groote Schuur hospital would be the group that has been exposed to environmental factors most different for their normal living conditions. This change in their environment is not only the result of their admission to hospital, but includes the new influences to which they may be subjected in urban areas. Their coming into contact with different diets may give rise to an alteration in their depot fat composition.

It was possible to obtain samples of depot fat from 3 Basuto resident in Basuto-land aged between 40 and 50 years who were consistently on a typical Basuto diet. This diet probably does not differ greatly from the local Bantu diet. The Basuto generally have a relatively lesser intake of animal protein and fat and probably a relatively greater intake of carbohydrate[21]. The mean values for the common fatty acids in the 3 Basuto subjects are compared with those in the local Bantu males (Table 27).

FATTY ACID	BANTU	BASUTU
C14 : 0	2.6	2.3
C16 : 0	22.3	27.0
C16 : 1	7.5	5.7
C18 : 0	5.5	5.9
C18 : 1	46.9	41.0
C18 : 2	9.6	11.1

TABLE 27. The mean percentage for the common fatty acids in the local Bantu males compared with 3 Basuto males.

As can be seen from Table 27 there are some apparent differences between local Bantu and Basuto. It is however not possible to assess the significance of these

/differences.....

differences because of the small number of Basuto subjects. The Basuto samples do not show any deviation from the range in fatty composition found among local subjects in general (Chapter 5).

CONCLUSIONS

Differences between the racial groups in the proportions of the fatty acids were found for myristic acid (C14:0) and linoleic acid (C18:2). The monounsaturated fatty acids, C16:1 and C18:1, show a negative correlation with C18:2.

The percentage of C14:0 in the depot fat appears to correlate directly with the total dietary fat intake.

It was not possible to define the dietary factors responsible for determining the racial differences in the proportion of C18:2 in the depot fat. The multitude of factors which may be interrelated in affecting this proportion were discussed.

The negative correlation found between C18:2 and the monounsaturated C16 and C18 chain-length fatty acids appears not to be the result of a direct relationship. A relative decrease in C18:2 gives rise to a secondary increase in C16:1 and C18:1. There is no satisfactory evidence to date to support the possible view that these fatty acids are interconvertible, the one group being synthesized to form the other.

The differences found between the sexes in certain of the fatty acid ratios (Chapter 8) do not show the same trends between the races as does serum cholesterol. These trends between the sexes would therefore appear to be related to sex rather than diet.

SUMMARY

The common fatty acids are compared between the racial groups in the males and females. Trends consistent within the racial groups were found for the percentage of C14:0 and C18:2. The possible relationship of dietary factors in determining the

/relative.....

relative proportion of these fatty acids was discussed. A significant negative correlation between the proportions of C18:2 and the monounsaturated fatty acids C16:1 and C18:1 was found. The possible reasons for this relationship were discussed.

The common fatty acids found among the local Bantu males are compared with Basuto males.

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CHAPTER 10

THE DEPOT FAT FATTY ACID COMPOSITION IN MEN WITH ISCHAEMIC HEART DISEASE

The depot fat fatty acid composition, serum cholesterol concentration, and dietary details of 12 White men with ischaemic heart disease have been determined. The results of these determinations are presented below.

RESULTS

Depot Fat Composition

The mean percentage and the range of the common fatty acids are presented in Table 1.

FATTY ACID	MEAN	RANGE
C14:0	3.5	2.3 - 6.1
C16:0	23.8	19.0 - 30.0
C16:1	8.2	6.1 - 10.5
C18:0	3.6	2.7 - 5.2
C18:1	45.3	40.3 - 50.3
C18:2	10.1	5.2 - 15.8

TABLE 1. The mean percentage and the range for the common fatty acids in subjects with ischaemic heart disease.

Dietary Details

The proportion of calories derived from the basic foodstuffs is shown in Table 2.

CARBOHYDRATE	FAT	PROTEIN
53.2	32.4	14.4

TABLE 2. The percentage of calories derived from carbohydrate, fat and protein shown as a mean.

/The.....

The average total daily fat intake was 71.3 gm., consisting of 50.2 gm. of animal fat and 21.0 gm. of vegetable fat. Their mean linoleic acid intake was 6.1gm./day, representing 2.0% of their total calories.

Serum Cholesterol

The mean serum cholesterol concentration for the group was 256.3 mg./100 ml. with a standard deviation of 42.6 mg./100 ml.

DISCUSSION

The depot fat composition in this group of subjects is not in any way different from that found in apparently healthy subjects (Chapter 5). The most suitable control group with which to compare the fatty acid compositions would be the White men of the same age range. The percentage of the common fatty acids in White subjects with ischaemic heart disease is compared with the 40 - 49 year old apparently healthy White males in Table 3.

	I.H.D.	"HEALTHY"		
FATTY ACID	Mean (S.D.)	Mean (S.D.)	diff.	P.
C14:0	3.5 (0.9)	3.9 (1.4)	0.4	N.S.
C16:0	23.8 (3.1)	24.8 (2.3)	1.0	N.S.
C16:1	8.2 (1.7)	8.2 (1.4)	0	-
C18:0	3.6 (0.7)	4.3 (1.2)	0.7	N.S.
C18:1	45.3 (3.1)	45.7 (3.2)	0.4	N.S.
C18:2	10.1 (3.4)	6.9 (1.6)	3.2	<0.02

TABLE 3. The mean, one standard deviation, the difference between the means and the significance of this difference for the common fatty acids in White subjects with ischaemic heart disease (I.H.D.) and apparently healthy White males aged 40 - 49 years.

/It can be seen.....

It can be seen from Table 3 that the only fatty acid showing a significant difference between apparently healthy White subjects and men with ischaemic heart disease is C18:2. This fatty acid has been implicated as the dietary factor, a lack of which is responsible for the development of ischaemic heart disease (I.H.D.)[1,2]. Studies on blood lipid components have on the whole shown no evidence for a lack of this fatty acid in subjects with ischaemic heart disease [3,4,5]. The absence of any striking difference in the proportion of this fatty acid in the plasma of White men with this disease and normal White and Bantu men, matched according to age, has also been noted[6]. The point was raised in Chapter 1 that the absence of a difference in the proportion of this fatty acid in the circulating lipids between subjects with and without ischaemic heart disease may possibly be accounted for by its differential drainage from the depot fat. The proportion of this fatty acid in the depot fat of the respective groups would appear not to support such an hypothesis (Table 4)

	I.H.D.	W	C	B
C18:2	10.1	6.9	12.9	9.3

TABLE 4. The mean percentage of C18:2 in White (W), Coloured (C), and Bantu (B) men and White men with ischaemic heart disease (I.H.D.) aged 40 - 49 years.

The difference in the mean percentage of C18:2 between the apparently healthy White subjects and the subjects with ischaemic heart disease is significant (Table 3) but does not differ significantly from the mean values found in the Coloured or Bantu men ($P < 0.1$ and < 0.6 respectively).

It will have been noted from the dietary details of the group with ischaemic heart disease that their fat intake both quantitative and qualitative, resembled that of the Coloured men more closely than that of the White men (Chapter 4). It will also have been noted that the mean serum cholesterol concentration in the subjects with

/ischaemic heart.....

ischaemic heart disease was also lower, although not significantly so ($P < 0.2$) than in the White men aged 40 - 49 years (Chapter 4). These data suggest that this group of subjects had changed their diets either before or since the manifestations of their disease. It may therefore be argued that they do not in general represent subjects with this disease. There is no real defence against such a criticism. White men in this area, particularly those with a family history of ischaemic heart disease, have become very conscious of the view that fat in excess quantity, and animal fat in particular, may be detrimental to their health. It would therefore have been advisable to sample subjects with a recent onset of ischaemic heart disease. It would then have been possible to assess with more assurance whether linoleic acid protects against this disease. It may however be concluded that a diet in White men with ischaemic heart disease resembling that found among Coloured men, gives rise to the deposition in the former group of a proportion of C18:2 approximating that in the latter. It would therefore appear that I.H.D. does not produce an excess drainage of C18:2 from the body fat.

The dietary intake of linoleic acid in these subjects with I.H.D. (see above) appears to be lower than that found among the apparently healthy men of all races (Chapter 9). This anomaly serves further to illustrate the complexity of the relationship between the intake of linoleic acid and the proportion of C18:2 found in the depot fat (Chapter 9).

Hirsch et al.[7] have also compared the fatty acid composition between normal subjects (5 women and 7 men) with an age range of 20 - 35 years and 5 men aged 32 to 65 years dying within a few days of acute myocardial infarctions. They did not find any striking difference in the percentage of linoleic acid. Björntorp[8] analysed the polyunsaturated fatty acids in the subcutaneous tissue of normal subjects and subjects with essential hypercholesterolaemia by the alkali isomerization technique and found identical values (6.5%) for the dienes when the mean was expressed as a percentage of

/the total.....

the total fatty acids. The dienes in depot fat would be almost exclusively C18:2. Subjects with essential hypercholesterolaemia are known to have an increased susceptibility to ischaemic heart disease. Although the disorder in lipid metabolism is not identical in the two groups of conditions, they have features in common.

CONCLUSIONS

The depot fat fatty acid composition in the group of subjects with ischaemic heart disease does not show any unusual features when compared with apparently healthy subjects. They did however show a significant difference in the percentage of C18:2 when compared with apparently healthy White men of the same age range. Their dietary data suggest that these patients might have altered their diets. The findings however of a similar diet to that of the Coloured subjects with a similar percentage of C18:2 suggest that ischaemic heart disease as such is not associated with an increased demand for linoleic acid.

SUMMARY

The common fatty acids found in the depot fat of subjects with ischaemic heart disease are presented. The findings are compared with apparently healthy White men of the same age range. The dietary details and serum cholesterol concentration is also presented.

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GENERAL SUMMARY

CHAPTER 1.

The background to this study was outlined and the desirability for establishing the depot fat fatty acid composition in relation to various other variables elaborated.

CHAPTER 2.

The literature on the anatomy and physiology of depot fat was reviewed. The large body of evidence accumulated has established that the adipose organ has the potential and does participate to a very high degree in the energy metabolism of the mammalian organism. The former contention, still prevalent, that depot fat is simply a semi-static energy store is completely refuted. This tissue, or organ, is probably responsible for supplying the greater part of the energy requirements of the mammalian organism, and with its balanced regulatory mechanisms it is able to respond to the fluctuating needs of the organism. A considerable advance has been made into the understanding of the mechanisms by which the uptake and release of fat by this tissue are brought about.

CHAPTER 3.

The subjects, the means by which they were selected, and the clinical and chemical methods used are elaborated. The reproducibility of the method is shown. The representivity of the sample obtained by the aspiration technique is demonstrated. The statistical formulae employed are given.

CHAPTER 4.

The dietary details, serum cholesterol concentration and relative obesity have been compared according to age, sex and race. The diets eaten by the respective racial groups were found to follow the usual patterns in the races. The serum cholesterol trends with age also reflected the established racial differences among the males. The females in each race showed similar trends to the males of the same race.

/CHAPTER 5.....

CHAPTER 5.

The fatty acids found in all the subjects arepresented both qualitatively and in their relative proportion. These findings are compared with similar reported studies and in general show good agreement between means. The common, or more abundant, fatty acids found agree with the findings in other studies and were here found to consistently constitute more than 90% of the total fatty acids.

CHAPTER 6.

Fat sampled by the áspiration technique from the buttock is compared with that at other superficial and deep sites. There was good agreement in fatty acid composition between fat from subcutaneous sites. The deep sites showed a little variation which is probably not of significance.

CHAPTER 7.

There was little variation in fatty acid composition between age groups in either the males or females. The single exception to this is discussed. Age as such, at the age levels studied, appears not to be associated with an alteration in fatty acid composition.

CHAPTER 8.

The fatty acid composition between males and females is compared both in general, in each race and at each age level. A difference in the fatty acid composition was found between the sexes which appears to be best expressed as the ratio of the monounsaturated to the saturated C16 and C18 fatty acids. The possible reasons for this finding are discussed. There was no sex difference in the percentage of C18:2.

CHAPTER 9.

The fatty acid composition is compared between the racial groups. Myristic acid was found to fall progressively from White, through Cape Coloured to Bantu.

/The percentage.....

The percentage of C14:0 correlates with the total fat intake in each race and sex.

The percentage of C18:2 was found to be consistently higher among the Cape Coloured subjects at each age level and in males and females. This was associated with a lower proportion of C16:1 and C18:1 in the Coloured subjects. A significant negative correlation between C18:2 and the monounsaturated fatty acids was found. This finding is discussed in relation to other studies. It was not possible to correlate the intake of linoleic acid with the percentage of C18:2 found in the depot fat.

CHAPTER 10.

The depot fat composition in White men with ischaemic heart disease is presented and compared with the other groups of the same age range. The only fatty acid among the men with ischaemic heart disease which showed a significant difference from the apparently healthy White men was C18:2, being higher in the former group. The possible reasons for this finding are discussed.

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/Mrs.M.J.Perrin.....

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Mr. Todd took the photographs.

Miss W.M.Greenshields was faced with the task of deciphering an illegible scrawl and typing the manuscript. This involved her in many long nights and additional strain. She will not allow me to express my gratitude fully, and since she has the ultimate say about what is here documented, one must surrender to her command. The result, however, voices its own eulogy.

The candidate encloses herewith reprints of other published work, namely:-

1. Krut, L.H., Hansen, J.D.L., Truswell, A.S., Schendel, H.E. and Brock, J.F.

   Controlled Field Trial of a Bread Diet Supplemented with Lysine for Children in an Institution.

   S.A. J. Lab. clin. Med., 7: 1, 1961.

2. Krut, L.H., Perrin, M.J. and Bronte-Stewart, B.

   Taste Perception in Smokers and Non-Smokers.

   Brit. med. J., i: 384, 1961.

3. Perrin, M.J., Krut, L.H. and Bronte-Stewart, B.

   Smoking and Food Preferences.

   Brit. med. J., i: 387, 1961.

Signed

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L.H. KRUT.

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CONTROLLED FIELD TRIAL OF A BREAD DIET SUPPLEMENTED WITH LYSINE FOR CHILDREN IN AN INSTITUTION

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INTRODUCTION

Osborne and Mendel in 1914[33] showed, in rat-growth experiments, that the protein of wheat is deficient in lysine. Much subsequent work on the amino-acid composition of proteins prompted Mitchell and Block[30] to state, with some reservations, that the nutritive value of a protein for any biological function is limited by the relative proportions of the essential amino acids contained in it. The nutritive value of wheat protein can be improved for animals by supplementation with lysine. This has been repeatedly shown by many workers including Hutchinson *et al.*,[23] Rosenberg and Rohdenberg,[35] Sure,[38] Bender,[5] Flodin,[12] Deshpande *et al.*,[11] and Harris and Burress.[19]

As far as man is concerned, Hoffman and McNeil,[20] Albanese *et al.*,[1] Bressani *et al.*,[7] and Rice *et al.*,[34] have shown that the nutritive value of wheat can be improved by the addition of lysine.

The investigation here reported was designed to determine whether lysine supplementation of bread improved its nutritive value for children.

Problems of Amino-acid Supplementation

Harper[16] reviewed the possible changes in feeding the indispensable amino acids at levels exceeding the requirement. He elaborated the dangers of coming to general conclusions until such time as the specific imbalances, antagonisms and toxicities have been studied. There is, however, ample good evidence that lysine supplementation of wheat protein at ideal levels is beneficial.[5, 11, 12, 17, 23, 35, 38]

Much has been written on lysine supplementation. It had until recently been concluded by most workers, from rat-growth experiments, that the ideal level of lysine supplementation of wheat protein is 0·25%. Jahnke and Schuck[24] indicated that supple-

mentation in excess of 0·5% may depress growth by producing secondary deficiencies of other amino acids. They also found that at this higher level of lysine supplementation the mean liver fat of the rats increased. Deshpande *et al.*[11] found that on feeding rats a diet consisting of 78% white flour with a protein content of 9·6% (N × 6·25), there was no fatty infiltration of the liver and that lysine was limiting for growth. When they fed white flour with a protein level of 5·4% there was fatty infiltration, but this was prevented by lysine. Similarly, Harper *et al.*[17] could prevent fatty infiltration of the liver in rats on a diet of polished rice by feeding lysine at the 0·4% level.

From the available evidence it seems that the level of protein feeding is also of great importance in the dietary aetiology of fatty infiltration of the liver. Lysine supplementation can prevent this complication from developing on a low-protein diet.

The actual lysine content of wheat is subject to variation. Lawrence *et al.*[27] have shown that it is possible to cultivate wheat with a higher lysine content, although thus far the levels obtained are not adequate to cause a substantial improvement in the nutritive value of the wheat. They have also shown that, as the protein content of wheat falls below 13·5%, the decrease in lysine content is proportionately greater. Thus not only is the wheat lower in protein, but the available protein is less efficient nutritively. This may be an added factor in the fatty infiltration of rat livers found on low-protein diets.

Hutchinson *et al.*[23] and Harris and Burress[19] have shown, in rats, that at each level of protein feeding there is an ideal level of lysine supplementation. The level of supplementation is directly related to the protein content of the wheat, i.e., as the level of protein increases, the lysine requirement also rises.

Sackler and Sophian[36] report that the administration of lysine with a test meal produces a substantial rise in the gastric secretion of pepsin and a moderate rise in HCl secretion. This phenomenon, possibly resulting in stimulation of appetite, may be held by some to be responsible for the nutritional benefit of lysine. This effect, however, was in response to a 5 g. dose, a level far in excess of the amount usually given, and therefore probably producing an abnormal or greatly exaggerated response. Cannon *et al.*[8] report that all nutrients must be given at the same time for supplementation to be effective. There are thus several conditions to be fulfilled for lysine supplementation of wheat to be beneficial.

General Plan of the Trial

The trial was conducted at an institution which serves as a temporary home for destitute non-White children. The children entering the institution had probably all been on deficient diets before admission. It was feasible to plan only a short-term investigation since the children had to be placed with foster parents or returned to their own rehabilitated homes as soon as possible. The trial was divided into 2 parts. Part I ran for 6 weeks and Part II for 5 weeks. The children were divided into 2 balanced groups receiving the same basic diet. One group was given a lysine supplement (lysine group) and the other an isonitrogenous glycine supplement (glycine group) for Part I of the trial. Part II commenced immediately on conclusion of Part I when the amino-acid supplements to the groups were reversed. Thus the former lysine group became the glycine group, each group serving in turn as control for the other.

MATERIALS AND METHODS

The Children

Fifty-eight children, ranging in age from 2 to 8½ years (mean 4½ years), took part in the trial. They were considered to be suitable subjects for the investigation on the basis of a full clinical examination and assessment of nutritional status. All were ambulant and able to feed themselves. The 2 groups (29 children in each) were balanced

according to age, weight, height, skinfold thickness, haemoglobin and haematocrit. Twenty-two children in the lysine group and 28 in the glycine group completed Part I of the experiment. Table I shows the matching of these groups at the start of the trial according to the above criteria and includes serum-albumin and globulin concentrations.

TABLE I. MATCHING OF GROUPS, COMPARING THE MEAN VALUES IN EACH GROUP FOR THOSE CHILDREN COMPLETING PART I.

Factor	*Lysine group (n= 22) mean*	*Glycine group (n=28) mean*	*Difference between means*	*S.E. of difference*
Ages (years)	4·54	4·57	0·03	0·5
Weight (kg.)	14·22	14·35	0·13	1·04
Height (cm.)	96·0	96·7	0·7	3·7
Skinfolds (mm.)	28·7	27·5	1·2	2·4
Albumin (g.%)	4·01	4·04	0·03	0·09
Globulin (g.%)	3·43	3·33	0·10	0·42
Haemoglobin (g.%)	11·4	11·5	0·1	0·31
Haematocrit (%)	38·4	38·3	0·1	0·80

S.E. = Standard error.

Growth Measurements

Weight. The children were weighed unclothed, after having urinated, on a platform balance able to weigh to the nearest ¼ lb. (0·11 kg.). They were always weighed by the same person (L.H.K.) and at the same time of day. The mean group weights recorded at the start of the trial and at the end of each part were determined from the mean of 4 consecutive daily weighings. This technique, employed by Truswell *et al.*[39] was shown to reduce the standard error of the mean group weight.

Height. Height was measured by means of a suitable rule permanently fixed to a rigid support. This procedure was carried out with an assistant who ensured that the children's heels rested firmly on the floor, and with the back of the heels, buttock, thoracic spine and occiput in contact with a vertical support. The recording taken was the highest reading with the head held level.

Skinfold thickness was measured with appropriate calipers at 5 sites [subclavicular, abdominal (para-umbilical in mid-clavicular line), mid-anterior thigh, posterior arm, (midway between acromion and olecranon) and sub-scapular]. The total of these sites was used in the assessment of subcutaneous fat change.

Blood Analysis

Blood was taken by venepuncture at the start and at the end of each part of the trial.

Total serum protein was determined by a biuret method,[41] serum albumin was then similarly determined after precipitation of the globulins with 27% sodium sulphate, and serum globulin was obtained by difference. The factor for the biuret solution was calculated by micro-Kjeldahl estimations of serum nitrogen.

Haemoglobin was determined from oxalated blood by the cyanmethaemoglobin method and read in a Klett colorimeter, calibrated against a standard haemoglobin solution.

Haematocrit readings were measured by centrifugation of oxalated blood in Wintrobe tubes.

Diet

Both groups were offered the same basic diet, under identical conditions, prepared and served by the institution staff under supervision.

EXPERIMENTAL MENU

7 a.m.	Bread sandwiches *ad lib.* Amino-acid drink, black coffee.
10 a.m.	Bread sandwich. Occasionally fruit.
12 noon	Vegetables—variously prepared. Bread sandwiches *ad lib.* Amino-acid drink.
2.30 p.m.	Bread sandwich. Occasionally fruit.
5 p.m.	Vegetable soup fortified with flour used in baking the bread. Bread sandwiches *ad lib.* Amino-acid drink, black coffee. Multivitamin tablet. Iron tablet.

Bread was locally baked and distinctively labelled. It was prepared from a single batch of 87% extraction flour which was the product of 4 local wheat varieties (75%) and Manitoba wheat (25%). To the flour was added 1% yeast, 2% salt (NaCl), 1% vegetable fat and 2% sugar, as part of the normal preparation for baking. This was supplemented with calcium carbonate (0·5%) to provide additional calcium. During the course of the trial the protein content of the fresh bread was determined and found to be 6·4% (N × 5·8)*. This level was much lower than the 8·5% we had expected from reference to South African food tables.[13] The calcium content was increased to 144 mg. per 100 g. Bread was machine-sliced daily to a fairly constant thickness. Three samples of 10 slices were randomly selected, and the average weight of a slice determined. The bread was offered *ad libitum*, individual consumption for every meal and snack recorded, and intake in grams thus determined.

Butter, margarine, and jam or syrup in known amounts were mixed and spread on the bread slices. The intake of these foods by each child was expressed in terms of the number of slices eaten.

Vegetables and fruit were selected, by reference to food tables,[13, 29] on the basis of a protein content not exceeding 1·1%. They were weighed after being prepared for cooking. The children were offered approximately equal helpings, varying with age. Since the age range in the groups was matched, and since the institution staff were not informed of the aims of the trial, it was felt that there would not be selection in the mean helpings offered to each group. The mean intake was determined by dividing the total consumption by the number of children on the diet each day.

* There is some confusion about the factor that is used in converting nitrogen to protein for wheat. The factor 5·7 is in general use for flour. This factor, however, varies, depending upon the relative proportions of the amino acids, and is, in the case of wheat, dependent upon the level of extraction. Taking normal proportions of bran, endosperm and embryo in the grain, these factors give for the whole wheat a conversion factor of 5·83.[25]

Any food refused was recorded and allowances made accordingly.

Other nutrients were a multivitamin* and an iron** tablet given daily, to provide an adequate intake of these substances.

Amino-acid solutions were prepared twice weekly in our laboratories. The required concentration was made up in distilled water. The concentration was such that 45 ml. per day, given in 3 divided doses, delivered the required amount of amino acid. 15 ml. (0·6 ml.) was mixed with about 150 ml. of a sweetened synthetic drink ('oro-crush'), just before serving with the 3 main meals of the day. Samples of the prepared amino-acid solutions were kept refrigerated at our laboratories for 8 days. Kjeldahl determinations of the total nitrogen and Van Slyke gasometric analysis for -amino nitrogen on specimens, made up as administered to the children, were 95% of the expected value. On the basis of anticipated bread consumption it was calculated that the required lysine supplement (as *l*-lysine HCl) was 1 g. per day and the calculated isonitrogenous amount of glycine given to the control group was found to contain 0·822 g. per day.

Statistical Analysis

The significance of the change in the mean values of the parameters in each group was tested by the delta 't' test. The significance of the difference of the change for each parameter, between the groups, was tested by Student's 't' test. Chi-square analysis was employed in comparing the incidence of various developments in the groups.

RESULTS

Part I

Six children, all from the lysine group, were discharged from the institution for administrative reasons in the first 2 weeks of this part of the trial. In addition, 1 child from each group was excluded on clinical grounds. There were thus 22 children in the lysine and 28 in the glycine group who completed Part I. The results are based on the findings in these children. The children initially responded to the *ad libitum* bread offer with great appetite. There was a rapid weight gain by both groups in the first 4 weeks (Fig. 1). Their enthusiasm for this new diet was, however, short-lived and their bread consumption began to fall in the second week (Fig. 2). Their appetite for the other foods remained unchanged.

Growth Measurements (*Table II*)

Weight. There was a significant gain in both groups. Although the gain by the lysine group was the greater, it was not significantly more than in the glycine group.

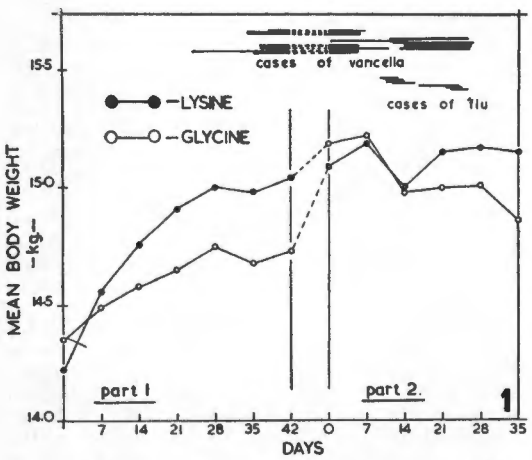

Fig. 1. Mean weekly weight in the groups. The early upward trend was greater in the lysine group. Note that the fall in weight trends coincides with the development of illness. The time of onset and duration of illness in each child affected is shown.

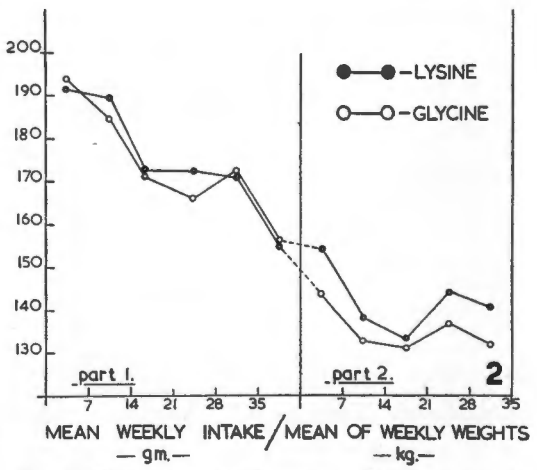

Fig. 2. The mean bread consumption in grams in relation to mean weekly body weight showing a parallel fall in both groups.

Height. A significant increase occurred in both groups by the same amount.

Skinfolds. A remarkable increase occurred in both groups, 39% in the lysine and 38% in the glycine group.

* Composition of multivitamin tablet as given by manufacturers: Vitamin A, 5000 i.u.; B 1, 1 mg.; riboflavin, 0·5 mg.; pyridoxine HCl, 0·25 mg.; ascorbic acid, 25 mg.; nicotinamide, 10 mg.; vitamin D, 400 i.u.; vitamin B_{12}, 0·5 μg.; and brewer's yeast, 50 mg.

** Iron Tablets ('fersamal'): Ferrous fumarate, said to contain about 65 mg. of ferrous iron per tablet.

TABLE II. COMPARISON OF THE MEAN CHANGES IN THE GROUPS FOR PART I (DURATION 6 WEEKS)

Factor	*Lysine supplemented group (n = 22)*				*Glycine supplemented group (n = 28)*				*Significance of difference between (a) & (b)*
	Start	*Finish*	*Change (a)*	*Significance p*	*Start*	*Finish*	*Change (b)*	*Significance p*	*p*
Weight (kg.)	14·2	15·04	0·82	< 0·001	14·35	14·73	0·38	< 0·05	N.S.
Height (cm.)	96·0	96·4	0·4	< 0·02	96·7	97·2	0·5	< 0·01	N.S.
Skinfolds (mm.)	28·7	40·0	11·3	< 0·001	27·5	37·9	10·4	< 0·001	N.S.
Albumin (g.%)	4·01	3·31	−0·70	< 0·001	4·04	3·15	−0·89	< 0·001	N.S.
Globulin (g.%)	3·43	3·21	−0·22	N.S.	3·33	3·29	−0·04	N.S.	N.S.
Haemoglobin (g.%)	11·4	11·8	+0·4	< 0·02	11·5	12·0	+0·5	< 0·02	N.S.
Haematocrit (%)	38·4	36·6	−1·8	< 0·01	38·3	38·2	−0·1	N.S.	< 0·05

N.S. = Not significant at the 5% level.

Blood Analysis (Table II)

Serum albumin. There was a highly significant fall in both groups ($p<0.001$). Although the fall in the lysine group was less than in the glycine group, this difference was not significant.

Serum globulin did not alter significantly in either group.

Haemoglobin levels rose significantly in both groups ($p<0.02$) by much the same amount.

Haematocrit readings fell significantly in the lysine group ($p<0.01$) but not in the glycine group.

Food Intake

The mean intake of protein and calories was not significantly different between the groups. The mean intake of protein in the lysine group was 26·2 g. per day which represented 1·8 g. per kg. per day and in the glycine group this was 25·8 g. per day (1·8 g. per kg. per day). The daily calorie intakes were 1,654 and 1,636 in the lysine and glycine groups respectively. This represented 111 calories per kg. in both groups. Bread provided 90% of the protein and 56% of the calories (Table III).

Part II

Twenty-four children in the lysine and 21 in the glycine group completed this part of the trial. The results (Table IV) are based on these children. Bread consumption continued to fall in both groups (Fig. 2).

Growth Measurements (Table IV)

Body weight. There was no significant change of the mean level in the lysine group. In the glycine group the mean level fell significantly ($p<0.01$). The difference between the changes in the mean weight of the groups was significant ($p>0.01$).

Height. No significant increase was recorded in either group.

Skinfold thickness. A significant decrease occurred in the glycine group only ($p<0.05$). This was, however, not significantly greater than the decrease in the lysine group.

Blood Analysis (Table IV)

Serum albumin. The mean level in the lysine group was maintained. The glycine group showed a highly significant fall ($p<0.001$). The difference between the changes in the means was significant ($p<0.02$).

TABLE III. MEAN INTAKE OF PROTEIN AND CALORIES BY BOTH GROUPS IN PARTS I AND II. NOTE THERE IS NO DIFFERENCE IN INTAKE

Trial	*Lysine supplemented groups*				*Glycine supplemented groups*			
	Protein		*Calories*		*Protein*		*Calories*	
	g./day	*g./kg./day*	*per day*	*per kg./day*	*g./day*	*g./kg./day*	*per day*	*per kg./day*
Part I	26·2	1·8	1654	111	25·8	1·8	1636	111
Part II	22·7	1·5	1587	105	21·9	1·5	1556	103
Parts I and II	24·6	1·6	1623	109	24·0	1·6	1600	108

TABLE IV. COMPARISON OF THE MEAN CHANGES IN THE GROUPS FOR PART II (DURATION 5 WEEKS)

Factor	*Lysine supplemented group (n = 24)*				*Glycine supplemented group (n = 21)*				*Significance of difference between (c) & (d)*
	Start	*Finish*	*Change (c)*	*Significance p*	*Start*	*Finish*	*Change (d)*	*Significance p*	*p*
Weight (kg.)	15·09	15·15	0·06	N.S.	15·19	14·85	0·34	< 0.01	< 0.01
Height (cm.)	98·1	98·2	0·1	N.S.	97·0	97·2	0·2	N.S.	N.S.
Skinfolds (mm.)	37·2	36·9	−0·3	N.S.	39·5	36·4	−3·1	< 0.05	N.S.
Albumin (g.%)	3·25	3·21	−0·04	N.S.	3·34	3·12	−0·22	< 0.001	< 0.02
Globulin (g.%)	3·43	2·80	−0·63	< 0.001	3·27	3·00	−0·27	< 0.01	< 0.02
Haemoglobin (g.%)	12·1	11·5	−0·6	< 0.01	11·8	11·6	−0·2	N.S.	N.S.
Haematocrit (%)	37·9	37·8	−0·1	N.S.	36·7	38·3	1·6	< 0.01	< 0.01

N.S. = Not significant at the 5% level.

Serum globulin. Mean levels fell significantly in the lysine group ($p<0.001$) and in the glycine group ($p<0.01$). The mean fall in the lysine group was significantly greater ($p<0.01$).

Haemoglobin fell in both groups, but only in the lysine group was this significant ($p<0.01$). The difference between the mean falls was, however, not significant.

Haematocrit. There was no significant change of the mean value in the lysine group. A significant rise was found in the glycine group ($p<0.01$). The difference between these mean changes was also significant ($p<0.01$).

Food Intake

The intake of protein and calories again did not differ between the 2 groups (Table III). The mean protein intake in the lysine group was 22·7 g. per day (1·5 g. per kg. per day) and in the glycine group 21·9 g. per day (1·5 g. per kg. per day). The mean daily calorie intake was 1,587 (105 calories per kg.) in the lysine and 1,556 (103 calories per kg.) in the glycine groups respectively. Bread provided 87·4% of the protein and 48% of the calories.

Combined Findings of Part I and Part II

The mean change in each of the parameters in the lysine group, over both parts of the trial, was compared with those in the glycine group. A total of 46 children (22+24) in the lysine group and 49 (28+21) in the glycine group completed both parts of the trial.

Food Intake

The mean protein and calorie intake was the same in both groups (Table III). This represented a mean protein intake per child of 24·6 g. per day (1·6 g. per kg. per day) in the lysine group and 24·0 g. per day (1·6 g. per kg. per day) in the glycine group. The mean daily calorie intakes were 1,623 (109 calories per kg.) and 1,600 (108 calories per kg.) in the lysine and glycine groups respectively. Bread provided 88·8% of the protein and 52·4% of the calories.

Growth Measurements (Table V)

Body weight. The mean gain in the lysine group of 0·42 kg. was significantly greater than the 0·07 kg. in the glycine group ($p<0.02$).

Blood Analysis (Table V)

Serum albumin. The mean level in the lysine group fell by 0·36 g. per 100 ml. This was significantly less than the fall of 0·60 g. per 100 ml. in the glycine group ($p<0.01$).

Haematocrit showed a fall of 0·93% in the lysine group and a rise of 0·65% in the glycine group. This difference of 1·59% was highly significant ($p<0.001$).

No significant difference between the groups was found comparing the mean changes in height, skinfold thickness, serum globulin and haemoglobin (Table V).

The protein efficiency ratio (P.E.R.)* in the lysine group was 0·22 (5·46/24·6) and 0·04 (0·91/24·0) in the glycine group.

*P.E.R. = Weight gain (g. per day)/protein intake (g. per day).

TABLE V. COMPARISON OF THE OVERALL MEAN CHANGES FOR PARTS I AND II BETWEEN THE GROUPS (DURATION 11 WEEKS)

Factors	*Lysine supplemented group*			*Glycine supplemented group*				
	Part I ($n = 22$)	*Part II* ($n = 24$)	*Parts I and II* ($n = 46$)	*Part I* ($n = 28$)	*Part II* ($n = 21$)	*Parts I and II* ($n = 49$)	*Difference between* (*e*) *and* (*f*)	*Significance p*
	change (*a*)	*change* (*c*)	*mean change* (*e*)	*change* (*b*)	*change* (*d*)	*mean change* (*f*)		
Weight (kg.)	0·82	0·06	0·42	0·38	−0·34	0·07	0·35	< 0·02
Height (cm.)	0·4	0·1	0·24	0·5	0·2	0·36	0·12	N.S.
Skinfolds (mm.)	11·3	−0·3	5·2	10·4	−3·1	4·6	0·6	N.S.
Albumin (g.%)	−0·70	−0·04	−0·36	−0·89	−0·22	−0·60	0·24	< 0·01
Globulin (g.%)	−0·22	−0·63	−0·43	−0·04	−0·27	−0·14	0·29	N.S.
Haemoglobin (g.%)	0·4	−0·6	−0·12	0·5	−0·2	0·24	0·36	N.S.
Haematocrit (%)	−1·8	−0·1	−0·76	−0·1	1·6	0·65	1·59	< 0·001
MCHC (%)	2·59	−1·45	0·49	1·66	−1·94	0·12	0·37	N.S.

N.S. = Not significant at the 5% level.

COMPLICATIONS OF THE TRIAL

Clinical Effects

In Part I, 1 child from each group was excluded. The child in the glycine group developed detectable oedema with skin changes in the fourth week of the trial. The child in the lysine group had no specific lesion but was not thriving and had a poor appetite. At the end of Part I, 2 more children were excluded, both from the glycine group. One had developed skin changes and the other had lost weight and was eating poorly.

In Part II, 3 children were excluded. One was a child in the lysine group who became anorexic with the onset of chicken pox. The other 2 children, 1 from each group, developed oedema with no skin changes, in the fifth week.

There were thus 7 children whose nutritional state was adversely affected by the conditions of the trial; 4 in the glycine and 3 in the lysine group. The mean age of these children was 2·9 years (range 2 - 4 years). It was thus the younger children who were affected. The incidence of children affected was not significantly different between the groups.

Intercurrent Illness

Chicken pox. One of the children developed chicken pox on the 25th day of the investigation. The infection spread to affect another 6 children during Part I (4 from the glycine and 3 from the lysine group), and 6 more in Part II (4 from the glycine and 2 from the lysine group). There were in all 5 children from the lysine and 8 from the glycine groups thus affected (Fig. 1).

Influenza. An outbreak occurred in the second week of Part II. Four children in the glycine and 3 in the lysine group were affected.

Intercurrent illness thus affected 8 children in the lysine group and 12 in the glycine group. This difference in incidence was not significant ($x^2 = 0.725$; $p > 0.3$). There were, however, different numbers affected in the groups, and as can be seen from Fig. 1, illness seemed to influence the group mean weights. Since more children in the glycine group became ill, the mean changes in this group may have been more markedly affected. Also, children who had lost weight while ill would tend to gain weight more rapidly on recovery and in this way results might be artificially influenced. In view of these variables, the mean changes in body weight and serum albumin were recalculated, excluding those children affected by chicken pox in Part I from both parts, and those affected by chicken pox or influenza in Part II from this part only. There were thus a total of 35 children in each group who completed the full trial period. The mean weight gain was then found to be 1·16 kg.

in the lysine group and 0·29 kg. in the glycine group. This difference was significant ($t=2·149$; $p>0.05$). Similarly, the mean fall in the serum albumin in the lysine group was 0·29 g. per 100 ml. and 0·78 g. per 100 ml. in the glycine group. This difference was also significant ($t=3·737$; $p<0.001$). The significantly smaller relative mean fall in serum globulin in the glycine group in Part II (Table V) was found to be not significant when the above children were excluded and the data recalculated.

DISCUSSION

It has been shown that the lysine group gained significantly more weight than did the glycine group. Also the fall in serum albumin in the lysine group was significantly less than in the glycine group (Table V).

Body Weight

It is probable that the greater gain in the lysine group was due to a true body-tissue increase. The increase in subcutaneous fat was not significantly different between the groups (Table V). This then is unlikely to account for all of the difference in body weight. Bressani *et al.*[7] and Rice *et al.*[34] have shown that lysine supplementation of wheat can significantly improve nitrogen retention. It thus appears that the difference in mean body weight can, to a considerable degree, be best accounted for by greater nitrogen retention in the lysine group.

Serum Albumin

The fall in serum-albumin concentration in both groups, including the lysine group, can be ascribed to the low-protein content of the bread (6·4%). A protein level of 8·5%, expected from food tables, could have potentially increased the dietary protein by about 30%, but whether even this protein level would have been adequate to provide minimal protein requirements is by no means certain. Bosshardt *et al.*[6] showed that proteins of poor quality must be fed at relatively higher levels than those of good quality in order to obtain their maximal efficiency ratios. Wheat in particular has been shown to approach its maximal efficiency when the protein content nears 20%.[3, 19]

The calorie intake was high (108 calories per kg. per day) and corresponded to the 75 percentile level for 'upper middle class' Americans of the same age, whereas the mean protein intake (1·6 g. per kg. per day) was in the range of their lowest levels.[4] This high-calorie, low-protein diet was reflected in the gain in body weight in both groups, due largely to the laying down of fat, and a falling serum albumin. Another manifestation of this diet was the progressive fall in bread consumption (Fig. 2). A similar observation was made by Allison[2] in puppies on a wheat diet. He noted an increase in body weight with laying down of fat, despite a falling food intake.

These factors of a high-calorie, bulky and rather monotonous diet, combined to limit the protein intake. The younger children tired of this diet sooner than the older. Also, the low-protein bread was less able to meet their relatively greater protein requirement. It is noteworthy in this connection that the mean age of those children who had to be withdrawn from the trial was 2·9 years (range 2 - 4 years). It is apparent that bread as a sole source of protein is inadequate in this age group unless perhaps wheat of sufficiently high-protein content is used. The data do however demonstrate that lysine supplementation led to better utilization of available protein.

The intake of protein and calories was the same in both groups (Table III). The differences in body weight and serum albumin cannot thus be accounted for by a difference in food intake. Lysine, as used in this investigation, did not result in any appetite stimulation as has been suggested by Sackler and Sophian.[36] The favourable effect of lysine supplementation can thus be ascribed to an improvement of the amino-

acid pattern of the wheat protein. This is in accord with the work of many investigators.[1, 5, 7, 11, 12, 19, 20, 23, 24, 34, 35, 38]

Level of Lysine Supplementation

The level of supplementation in relation to bread consumption was 0·27% in Part I and 0·33% in Part II of the trial. This was near the recommended level of 0·25%. More recent work suggests, however, that the level of supplementation is best related to the protein content of the diet.[19, 23] It has been shown by Hutchinson *et al.*[23] that 0·1% lysine added to a 7·1% wheat-protein diet produced the maximal effect in rats. The question of a possible 'imbalance' produced by amounts exceeding this level must also be considered.[16, 18] Harris and Burress,[19] however, have found that lysine levels 5 - 8 times the ideal level, in relation to the dietary protein content, produced no 'toxicity', the excess amount, in their view, not being metabolized. In this trial the level of lysine supplementation may have been in excess of the best level for the low-protein wheat used, but it is well within the non-toxic limits found by Harris and Burress and it did not have any deleterious effect that could be detected.

Haematology

The mean haemoglobin levels rose and fell equally in both groups. The mean haematocrit in the lysine group, however, showed a fall and in the glycine group there was a rise. The difference between these changes was significant ($p<0.001$) (Table V). It would thus appear that the mean corpuscular haemoglobin concentration (MCHC)* was higher in the lysine group. Analysis of the changes in MCHC, however, showed no significant difference between the groups when these changes were compared for both parts of the trial (Table V).

The initial rise in haemoglobin concentration in both groups was probably in response to the iron supplement since iron deficiency is common among Cape Coloured children.[26] The subsequent fall could best be ascribed to the suboptimal protein diet.[40, 44] The role of lysine in haemopoiesis has not yet been fully established, but it may play some part. Sebrell and McDaniel[37] and Hogan *et al.*[21] suggest that it may be beneficial, whereas Orten and Orten[32] are more doubtful. Gillespie *et al.*[14] found that lysine deficiency produced a mild anaemia with a slightly greater decrease in haemoglobin than red cells, i.e. the MCHC would fall. While it was expected that our data would confirm this point, there was no statistically significant difference in the changes in MCHC between the groups (Table V). We therefore cannot make any claims for lysine in haemopoiesis.

General Comments

The use of glycine to provide the control group with the same potential nitrogen intake in a non-essential form is not without possible deleterious effects. Christensen *et al.*[9] found that high glycine feeding affected the distribution ratios of cellular and extracellular glycine, as well as other amino acids. Derrick and Hanley[10] showed that a glycine load of 0·62 g. per kg. results in a urinary loss of some non-essential and essential amino acids, notably leucine, lysine, threonine and valine. Glycine cannot therefore be considered to be entirely devoid of metabolic effects. The above findings, however, were in response to glycine loads about 10 times that used in this trial. It does not follow from the above reports that small doses of glycine will produce similar effects. There is no evidence, to our knowledge, to indicate that the amount of glycine administered to the control group in this investigation may have been deleterious. We therefore consider that glycine, used as reported here, is a valid method for providing a control group in this experimental design.

The rapidity with which differences

*MCHC% = Haemoglobin in g. per 100 ml./haematocrit as percentage × 100.

between the 2 groups was shown is noteworthy. Widdowson and McCance[42] failed to demonstrate differences on different bread diets in orphanage children over long periods. It has since been pointed out that in the particular diets offered those children, lysine was no longer limiting.[22, 31] In an investigation with a similar design conducted at the same institution by some of us[39] differences between groups were also shown over a similar period of time.

The children taking part in the trial, although well nourished as judged clinically, were in fact underdeveloped, since they had probably all been on low and poor diets before admission. The mean weights and heights of those from 2 to 6 years were 6% and 3% less respectively than those reported for 'normal' Coloured children.[43] The children of 6 - 8½ years were 7% and 5% less in weight and height respectively than the normal values for these ages in Coloured children.[28] It can be seen from Fig. 3 that the weights and to some extent the heights of the children in the trial were considerably below the mean values for their age as found in 'normal' Coloured children. Hand and wrist radiographs of all the children showed a mean age of 3·9 years (± 1·8 years) when estimated against the development for age given by Greulich and Pyle[15] in American children. The mean chronological age in our group of children was 4·5 years (± 1·76).

This disparity between the mean bone age and mean chronological age in this group of children therefore adds further evidence of their retarded physical development and undernutrition. This is probably the most likely factor to explain the rapidity with which differences were shown. The significant fall in serum albumin over such a short period, despite gain in weight, suggests a small protein reserve even though their initial serum-albumin concentrations were normal (Table I).

Intercurrent Illness

The chicken-pox outbreak was mild. The affected children were isolated until free from skin lesions. Other than the development of impetigo in one child there were no complications and all made normal recoveries. The influenza outbreak was widespread and not limited to the children on the trial. This was a more severe illness than the chicken pox and was characterized by

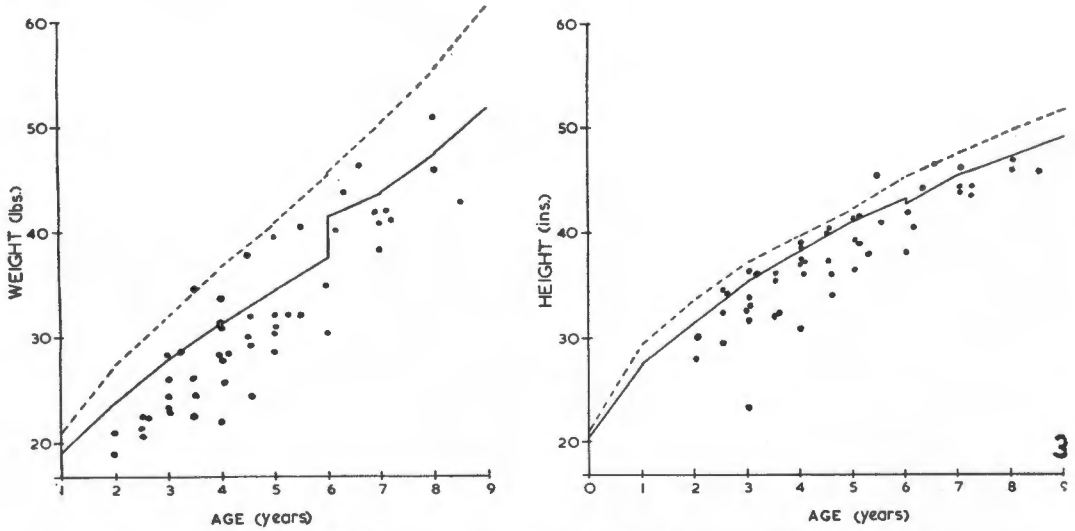

Fig. 3. The weight and height of the individual children at the commencement of the trial in relation to the mean values for normal Cape Coloured children (solid line). The dotted line represents these mean values for White children in Cape Town.[28, 43]

headache, fever and malaise for 1 - 2 days, but with a rapid recovery in 3 - 4 days. The incidence of the illnesses in the lysine and glycine group was not significantly different ($p>0.3$).

Complications of the Trial

The single case of oedema in the fourth week of Part I was at the time not considered to be indicative of any general protein deficiency since the other children were all happy, eating well and gaining weight (Fig. 1). With the appearance of skin lesions in a second child at the end of 6 weeks we still felt that these signs of protein deficiency were referable to single cases since both children were very young (2 years). In the fifth week of Part II another 2 children developed oedema and, since the serum-albumin levels for the whole group were showing a fall, it was decided to conclude the trial at this point. The number of children excluded from the trial because of the manifestations of deficiency symptoms was 4 in the glycine and 3 in the lysine group. All affected children recovered rapidly on a high-protein diet.

The fall in serum albumin in both groups, despite the beneficial effect of lysine supplementation, has demonstrated the unsuitability of bread (6·4% protein) as the principal source of dietary protein for young children. The outbreaks of chicken pox and influenza were additional stress factors and no doubt contributed to the general failure to maintain nutritional status. The rapidity with which apparently normally nourished children, as judged clinically and by serum-albumin concentration, can become obviously protein-depleted on a type of diet which is commonly given to many such children, was most alarming. While serum-albumin levels showed the commencement of protein deficiency sooner than it was suspected clinically, they did not indicate the initially limited protein reserve in these children. An effective method of assessing protein reserve would be of great value in areas where protein malnutrition occurs.

CONCLUSIONS

Lysine supplementation of a diet in which bread provided 88·8% of the protein and 52·4% of the calories has been shown to be beneficial for young children as assessed by serum-albumin concentration and body weight, when compared with a control group receiving an isonitrogenous glycine supplement. The effect of lysine cannot be ascribed to a greater protein intake, nor did it show any appetite stimulation in the amount and manner given. The effect of lysine was presumably to improve the biological value, and therefore the utilization, of the wheat protein.

Lysine did not appear to have any specific rôle in haemopoiesis.

Bread of low-protein content (6·4%) was found to be unsuitable as the principal protein source in the diet of young children. The bulk of food that must be consumed and the satisfaction of appetite by starch calories would seem to be the factors that limit the protein intake. Monotony and palatability of the diet are also factors that influence food intake.

SUMMARY

The effect of lysine supplementation of bread was studied in children at an institution. The children had probably all been on low and poor diets before admission and were underdeveloped compared with normal controls.

Fifty-eight children with a mean age of 4½ years (range 2 - 8½ years) were divided into 2 groups, matched according to age, body weight, height, skinfold thickness, serum albumin and globulin, haemoglobin and haematocrit.

The trial was run in 2 parts — each was intended to be for 6 weeks. Part I ran for the full 6 weeks; Part II was concluded after 5 weeks. In Part I, one group received the lysine supplement and the control group an

isonitrogenous amount of glycine. In Part II the supplements were changed over so that the former glycine-supplemented group received the lysine supplement, each group serving in turn as control for the other.

Bread (6·4% protein) was offered *ad libitum* and provided 88·8% of the protein and 52·4% of the calories in the diet. Other foods included butter, margarine, jams, low-protein vegetables and fruit, multivitamin and iron tablets. The amino acids were given in the form of a drink at the end of each meal at recommended levels. The protein and calorie intake was the same in both groups.

Forty-five children completed the full period of the trial. The trial was concluded sooner than planned because of the development of nutritional oedema in 3 children and the general fall in serum-albumin concentration.

The lysine-supplemented group gained significantly more weight than the glycine group. The serum-albumin concentration fell in both groups, but significantly less in the lysine group.

The trial was complicated by outbreaks of chicken pox and influenza.

Lysine supplementation of this diet was of benefit when compared with glycine. In view of the fall in serum-albumin concentration, however, such a bread diet is not considered to be suitable for young children.

Permission to hold this trial at Bonnytoun Place of Safety, Wynberg, Cape, was kindly given by the Commissioner for Coloured Affairs.

We are particularly grateful to Major A. J. de Beer, Principal at Bonnytoun, and his wife and staff for their willing help and interest in the trial; and to Dr. L. Sive, Medical Officer to the Institution for his cooperation.

Financial support was received from the South African Council for Scientific and Industrial Research, E. I. du Pont de Nemours, and the Williams Waterman Fund for the Combat of Dietary Diseases, Research Corporation, New York. The work was aided in part by a grant from the Food and Nutrition Board, National Research Council of the USA.

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Technical assistance was given by Misses C. Liadsky, N. Posthumus, E. Webb, C. Freesemann and J. Sutherland.

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SMOKING AND FOOD PREFERENCES

BY

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It was of interest to determine whether the differences in taste perception that exist between smokers and non-smokers (Krut, Perrin and Bronte-Stewart, 1961) had any bearing on their food preferences. There is a belief that smokers do prefer more savoury foods, and that, with abstinence from smoking, sweeter foods are preferred, and this possibly accounts for the subsequent increase in weight that has been reported (Brožek and Keys, 1957). As these impressions are without any factual foundation, this study was planned to obtain data in this regard.

Material and Methods

The subjects of this study were sampled from two sources—a group of 80 second-year medical students and a group of 76 males and females from an insurance firm whose ages ranged from 17 years to 50 years.

Firstly, in a printed questionary the subjects, who consisted of 79 smokers and 77 non-smokers, were asked to indicate their preferences for salty, spiced, sour, or bland foods, examples of each kind being given.

Secondly, in conjunction with the questionary, a diet history was taken by the system of recall, paying particular attention to the fat content of the diet derived from common foods: 63 smokers and 62 non-smokers were questioned.

Finally, the male smokers (all smoking more than 20 cigarettes/day) and non-smokers, whose ages matched, were selected from the insurance personnel tested for taste sensitivity. Thus sex, age, and socio-economic background were matched so that the variable factors were limited so far as possible. From these men, two small groups, A and B, consisting of heavy smokers and non-smokers, were selected with the use of random number tables (Fisher and Yates, 1957) for a more detailed dietary investigation.

Nine smokers and eight non-smokers formed group A and eleven smokers and nine non-smokers group B. A detailed description of the daily diet was obtained, paying attention not only to the main meals but also to snacks, the variations during the week and week-ends, and the methods used in cooking. The amounts eaten were gauged on average portions, but if there were obvious deviations these were taken into account. The fat content of various foods was obtained from standard food tables (Fox and Golberg, 1944; McCance and Widdowson, 1946).

Results

Subjective Taste Preferences.—Of the 79 smokers who completed the questionary on their preferences for salty, spiced, sour, and bland foods, 36 said they preferred salty foods, 50 spiced food, 24 showed preference for sour food, while 40 preferred bland food. Of the 77 non-smokers, 29 preferred salty food, 39 spiced, 30 sour, and 61 bland food. The differences were significant for bland food ($P<0.001$), but for salty and spiced food combined ($0.1>P>0.05$) the result was not quite significant.

Semi-quantitative Dietary Survey.—The total fat assessed from common fatty foods came to a mean of 577 g./week for smokers and 543 g./week for non-smokers—that is, smokers ate a slightly more fatty diet than non-smokers. However, this brief history did not take into account all the fat eaten in the diet, and the results were not significant.

Detailed Dietary Surveys

In group A, smokers consumed 1,215 g. fat/week as compared with 1,094 g. fat/week for non-smokers, but this was not statistically significant ($0.5>P>0.4$). On more detailed examination, however, marked differences existed in the types of foods that constituted the overall

fat intake in these two groups. Heavy smokers consumed significantly more meat and more eggs than did non-smokers, but non-smokers consumed somewhat more fat in the form of cakes, sweets, and chocolate.

Almost the same results were shown in group B—that is, the total fat was 1,155 g./week for smokers and 931 g./week for non-smokers. Similarly, heavy smokers consumed significantly more meat and more eggs than did non-smokers, but the intake of fat from other foods was again not very different (Fig. 1).

For the purpose of statistical analysis, group A and group B were combined and the results shown as a

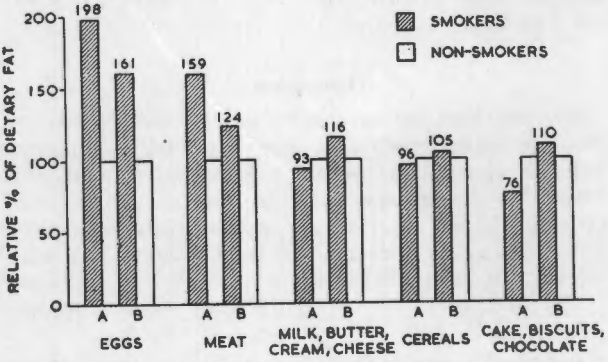

FIG. 1.—Relative fat intake expressed as a percentage (taking non-smokers as 100% in each case), showing comparative results from group A and group B in the different food groups.

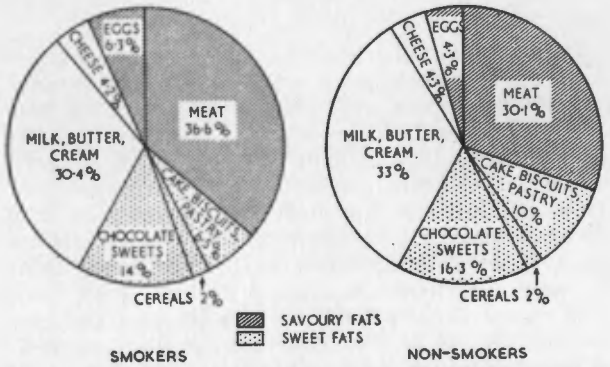

FIG. 2.—Percentage distribution of weekly dietary fat. A greater percentage of fat in the food of smokers compared with non-smokers is in the form of savoury fats. Note the segment taken up by dairy fats is similar in the two groups, but that from sweet food fats is greater in the non-smokers.

mean. The differences between smokers and non-smokers for meat and eggs were significant for these samples ($0.02>P>0.01$ and $0.05>P>0.02$ respectively). No statistically significant difference was seen in the other food groups, nor in the difference between total fats—that is, 1,176 g. fat/week for smokers compared with 1;029 g. fat/week for non-smokers ($0.1>P>0.05$).

The percentage of fat derived from cheese, butter, milk, and cream combined remains almost constant for smokers and non-smokers. Cereals are also eaten in aimost the same quantities by these two groups. Meat and eggs, which are savoury foods and are often cooked with additional fat, are preferred by the smoker, whereas sweet foods are preferred by the non-smoker (Fig. 2).

Discussion

By their very nature, dietary surveys cannot provide absolute values, and with regard to food preferences one has to rely on subjective information. For this reason, so far as was possible, the information was collected blindly in that the individual recording the dietary data was unaware whether a smoker or a non-smoker was being questioned.

The problem was tackled using three different approaches. Firstly, the subjects indicated their preference for certain foods with distinctive tastes. Next an assessment of the fat intake from the more common foods in their daily diet was made, Finally, two separate random samples, drawn on two separate occasions, were subjected to a detailed dietary analysis.

Each approach directs one's attention to differences in food preferences between smokers and non-smokers. The results indicate that smokers on the average seek gustatory satisfaction in consuming foods that have had their flavours and palatability enhanced by spices, herbs, vinegar, condiments, and fat. The differences in total fat intake, although not great, were consistent and of the same order in each instance. This was also seen in the 1955 survey in the same area (Bronte-Stewart, 1956). However, the differences in food preferences, particularly with regard to fatty foods between smokers and non-smokers, is seen to best advantage not when one takes food consumption as a whole but when one pays attention to individual food items. It was because of this that the analysis of fat intake from the different food items was repeated in a second random sample (group

B), the results of which confirmed the findings in group A—namely, that a significant difference exists between smokers and non-smokers with regard to consumption of meat and eggs.

In controlled feeding studies (Bronte-Stewart *et al.*, 1956) it was shown that meat and eggs elevate the serum cholesterol levels more rapidly and to a greater extent than most other fats.

Possibly this could account for the differences in serum cholesterol levels that have been reported in surveys on smokers and non-smokers (Gofman *et al.*, 1955; Thomas, 1958; Karvonen *et al.*, 1959; Bronte-Stewart, 1961).

Summary

The 156 individuals who were subjected to the tasting tests were questioned about their food preferences.

1. Whether they preferred salty, spiced, sour, or bland food. It was shown that non-smokers preferred bland food, and a fairly high proportion of smokers preferred salty and spiced food.

2. In addition, the fat content of their diet was analysed, and it was shown that smokers consumed more fat than non-smokers, but the differences were small.

3. On more detailed examination marked differences existed in the types of foods that constituted the overall fat intake in two independent groups. Heavy smokers consumed significantly more meat and eggs than did non-smokers. Non-smokers consumed more fat in the form of cakes, sweets, and chocolate.

It was concluded that in these samples cigarette smoking affected food preferences.

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TASTE PERCEPTION IN SMOKERS AND NON-SMOKERS

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Taste is a complex sense. In addition to sensations arising in taste receptors, it is influenced by chemical, tactile, warm, and cold receptors in the mouth, and in particular by olfactory sensations (Houssay, 1955). It is presumably the complexity of the taste sense that has limited its objective study. The subject is, however, simplified by the generally held view that there are but four primary taste modalities: sweet, sour, salt, and bitter (Houssay, 1955; Best and Taylor, 1955; Fulton, 1955; Bell, Davidson, and Scarborough, 1959), and that odour plays no part in the recognition of these pure tastes.

Smoking is popularly held to decrease taste perception. This is attested by almost everyone subject to the habit, and particularly those who have mastered their addiction to it. There are, however, few objective studies on the effects of smoking on taste.

Bronte-Stewart (1956) discussed the relationship between smoking and ischaemic heart disease, and put forward the hypothesis that smoking could affect food preferences via the taste mechanism. To test this hypothesis, taste thresholds for sweet, sour, salt, and bitter in groups of smokers and non-smokers were determined

and compared. Taste thresholds were similarly determined immediately before and immediately after the smoking of a cigarette in a sample of the above groups. In view of the findings, the relationship between the ability to taste phenylthiocarbamide (P.T.C.) and smoking was also examined.

Material and Methods

Two groups of subjects from different walks of life were tested in an identical manner a few weeks apart: (1) 80 medical students, 40 of whom were smokers—the mean age of this group was 20.5 ($\pm$3.1) years; and (2) 76 employees of a local insurance company, 39 of whom smoked, having a mean age of 27.6 ($\pm$9.3 years). Thus a total of 79 smokers and 77 non-smokers were tested. Tests were carried out over several days between 9.30 and 11 a.m. on each of these groups.

Test Procedure.—The subjects were asked to complete a questionary giving details of their age, sex, and smoking habits. They were informed of the aim of the experiment and of the taste modalities to be tested. They were told that there would be no fixed order in the substances to be tasted and that tap-water would be used from time to time. The observer recording the thresholds was kept ignorant of the smoking habits of the subjects. These tests were thus done in a "blind" manner. It was arranged that there were approximately equal numbers of smokers and non-smokers having threshold determinations on each day. Stepwise dilutions of sucrose (20–0.15%), citric acid (5–0.15%), sodium chloride (5–0.15%), and quinine hydrochloride (0.1–0.006%) were made up into solutions with tap-water. Each step in the titre series was a multiple of 0.5 of the preceding step. A single drop of a solution was deposited on to the centre of the protruded tongue by means of a pipette. The subject then withdrew his tongue, tasted the solution at leisure, and swallowed the drop. The threshold for each substance was determined in turn by depositing increasing concentrations of each solution, beginning with the most dilute. When two consecutive correct answers were given, the lower concentration was recorded as the threshold in each case. Mouths were rinsed with tap-water after each taste was identified. In this manner it was possible to determine the mean threshold value for each substance in each group of subjects.

Immediate Effects of Smoking.—Sixty of the above individuals, including 29 smokers, were subjected to a second series of tests. Initial threshold determinations were made as above, and the subject then went out for 10 minutes. The smokers all smoked a cigarette, while the non-smokers waited for a similar period until recalled. Each subject was then retested and thresholds were again determined.

Phenylthiocarbamide (P.T.C.). — Titres (0.13–0.00025%) were made up into solution in tap-water. Each step in the titre series was again a multiple of 0.5 of the preceding step. These threshold values were determined in the same manner as those above in 75 subjects, of whom 32 were smokers, drawn from both the student and the insurance groups.

Results

Thresholds for the Four Primary Taste Modalities.—The student group showed no significant differences between smokers and non-smokers in the mean taste thresholds for sweet, sour, or salt. The mean threshold for bitter, however, was significantly higher ($P<0.001$) in the smokers than in the non-smokers. These findings were exactly reproduced in the insurance group of subjects. Neither age nor sex variables between the groups account for this finding (Table I). Thus in two

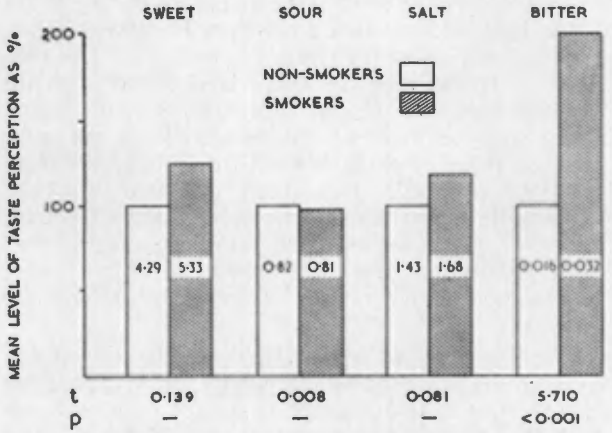

FIG. 1.—Combined student and insurance groups' mean threshold values for each of the taste primaries, comparing 79 smokers with 77 non-smokers. Note the significantly higher threshold among smokers for bitter only.

TABLE I.—*Mean Thresholds for Quinine Solutions in Relation to Age and Sex in Smokers and Non-smokers. Note that Allowing for Age and Sex Variables Does Not Affect the Finding that Non-smokers Have a Significantly Better Taste Acuity for Bitter than Smokers*

Class	Smokers		Non-smokers		t	P
	No.		No.			
All subjects (Mean age)	79	0·032 (25·6±9·5)	77	0·016 (22·0±4·9)	5·710	<0·001
Males only (Mean age)	66	0·032 (23·6±8·2)	54	0·017 (21·3±5·4)	5·017	<0·001
Males < 40 yrs. (Mean age)	61	0·031 (22·5±1·6)	54	0·017 (21·3±5·4)	4·684	<0·001
Females only (Mean age)	13	0·035 (33·6±13·4)	23	0·013 (22·5±5·6)	3·019	<0·01

separate groups it was shown that smokers taste bitter less well than non-smokers (Fig. 1).

Age in Relation to Thresholds for Bitter.—The mean threshold values in the non-smokers showed no significant change in considering the age-groups: under 20 years (0.016%), 20–30 years (0.014%), over 30 years (0.021%). On analysis of variance $P>0.2$. In the smokers, however, there was a progressive deterioration with age: under 20 years (0.026%), 20–30 years (0.034%), over 30 years (0.044%). Analysis of variance of the mean quinine thresholds in these age groups showed that this deterioration was significant ($0.05>P>0.01$) (Fig. 2).

Amount Smoked in Relation to Thresholds for Bitter.—The light smokers (not more than 10 cigarettes/day) had a mean threshold level of 0.027%. This was significantly less than the mean level (0.040%) in the moderate smokers (11–20 cigarettes/day) ($P<0.001$). There was, however, no further change in the mean level of those smoking more than 20 cigarettes/day (0.033%) ($P<0.4$). This mean threshold was also significantly higher than in the light smokers ($P<0.02$) (Fig. 3). Light smokers are thus less affected than moderate smokers, but there appears to be no further deterioration if more than 20 cigarettes a day are smoked.

Immediate Effects of Smoking.—In the 60 subjects tested it was found that there was on the whole a slight improvement in taste perception for the taste primaries in both smokers and non-smokers. None of these changes were, however, statistically significant, nor was the improvement in the smokers significantly different from that in the non-smokers (Table II). The smoking

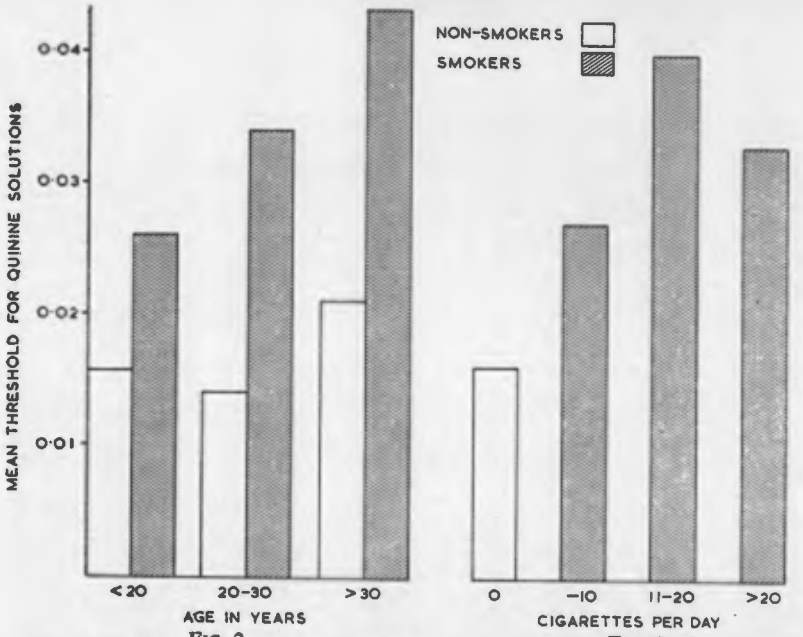

FIG. 2

FIG. 3

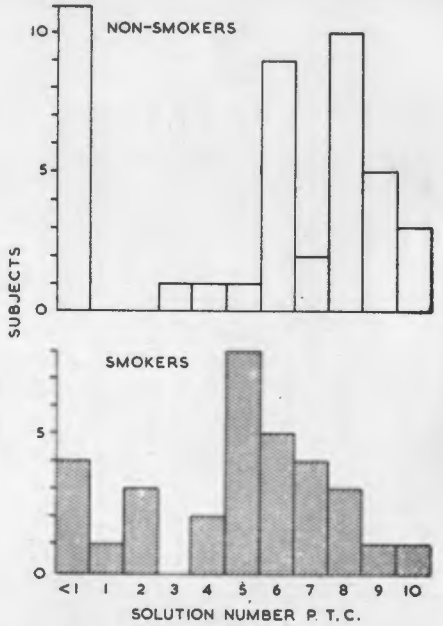

FIG. 4

FIG. 2.—Mean threshold for quinine solutions in relation to age level in smokers and non-smokers, showing a significant and progressive deterioration with age among the smokers only. This effect is thus attributed to the duration of smoking. FIG. 3.—Mean threshold for quinine solutions in relation to amount smoked. The moderate smokers showed a significantly higher threshold ($P<0.001$) than the light smokers. The threshold in the heavy smokers was not significantly different from that in the moderate smokers ($P<0.4$), but was significantly greater than in the light smokers ($P<0.02$). FIG. 4.—Number of subjects identifying the taste of P.T.C. as bitter at each concentration from 0.13 to 0.00025%, corresponding to solution numbers 1–10. Those subjects not identifying this taste from one drop are grouped under solution number <1 The antimode of these distributions is taken as being between solution numbers 2 and 3. The distribution between "tasters" and "non tasters" was the same in smokers and non-smokers on chi-square analysis ($P>0.9$).

of a cigarette, then, had no immediate effect on taste perception.

Phenylthiocarbamide (P.T.C.)—Thresholds for this substance showed the classical bimodal distribution into "tasters" and "non-tasters" in both smokers and non-smokers (Fig. 4). The antimode of these distributions is taken as being between solution numbers 2 and 3. Chi-square analysis showed that the distribution of smokers between "tasters" and "non-tasters" is the same as for non-smokers ($P>0.9$). Analysis of the P.T.C. tasters showed that in smokers the mean threshold was a titre of 0.0053%, whereas in the non-smokers it was lower (0.0034%). The difference, however, was not significant ($0.2>P>0.1$).

Discussion

It has been shown that the taste acuity of smokers for sweet, sour, and salt is not significantly different from that of non-smokers. The perception of bitter is, however, significantly worse in smokers (Fig. 1).

There are few reported studies with which to compare our results. As in our study, Richter and Campbell (1940) report that excessive smoking did not affect taste sensitivity for sucrose solutions. Cooper, Bilash, and Zubek (1959), in a study of the effects of age on taste, mention that in a sample of 58 smokers and 42 non-smokers from their subjects there were no significant differences in taste sensitivity for any of the taste primaries. Their technique, using a sipping method, did not ensure that a fixed amount of each solution was offered for tasting on each occasion.

Age has been shown to influence taste acuity, but not over age range (Harris and Kalmus, 1950–1 ; Kalmus, 1958 ; Cooper *et al.*, 1959). Sex has also been shown to influence taste in that females, as a group, are more sensitive (Falconer, 1946–7 ; Harris and Kalmus, 1950–1 ; Kalmus, 1958 ; Pangborn, 1959). This variable also cannot account for the difference in sensitivity to bitter between smokers and non-smokers, since, although there were more females among the non-smokers, their exclusion did not affect the results (Table I).

Unfortunately no record of the age of onset of smoking is available. Since, however, most persons start smoking in their late teens, the finding of a progressive deterioration in sensitivity for bitter among the

smokers, but not the non-smokers, with increasing age (Fig. 2) is probably best explained by the effect of duration of smoking. The slight but non-significant changes in taste acuity on repeat testing in smokers and non-smokers (Table II) is a phenomenon reported on by others (Harris and Kalmus, 1950–1 ; Pangborn, 1959) and is attributed to learning. Certainly the immediate effects of smoking a cigarette are not responsible for our findings. It would thus appear that continued smoking progressively affects taste acuity for bitter.

The same finding in two separate unselected samples of subjects and the fact that the association was relative to the age of the smokers, and thus presumably the duration of smoking, as well as the amount smoked (Fig. 3), strengthens our conviction that this defective taste for bitter among smokers does exist.

The possibility that individuals with an inherently defective taste for bitter were more likely to become smokers was entertained. Fox (1932) discovered that all people, regardless of race, age, or sex, are divided into "tasters" and "non-tasters" of P.T.C., and that this character is genetically determined. This was shortly thereafter shown by Blakeslee (1932) to be not absolute, but to follow a bimodal distribution. Salmon and Blakeslee (1935) and Falconer (1946–7) found no correlation between smoking and taste for P.T.C. Our results, in showing the same bimodal distribution for both smokers and non-smokers (Fig. 4), concur with their findings. Falconer (1946–7) and Kalmus (1958) found that there was some correlation between thresholds for P.T.C. and quinine. We found that, while the non-smokers among the P.T.C. tasters were more sensitive that the smokers, the difference was not significant ($P<0.2$). It is therefore unlikely that the decreased acuity for bitter among smokers, as tested by quinine, arises on a genetic basis and we attribute it to the effects of smoking.

The possible explanation for the selective inhibition for bitter, and not sour, salt, or sweet, by smoking is speculative. El-Baradi and Bourne (1951) have shown by histological techniques that the alkaloid quinine strongly inhibits an esterase in the taste buds, suggesting that enzyme inhibition is the mechanism in taste perception. Nicotine and other alkaloids are abundant in tobacco smoke (Henry, 1949 ; Salter, 1952 ; Goodman and Gilman, 1955 ; Sollmann, 1957). It is conceivable

TABLE II.—*Comparison of Taste Thresholds for Each of the Taste Primaries immediately Before and After Smoking a Cigarette. The Non-smokers were Similarly Tested, Allowing the Same Time Interval Between Testing as in the Case of Smokers. No Significant Changes were found. The Immediate Effects of Smoking a Cigarette thus do not Influence Taste Acuity for any of the Taste Primaries.*

	Smokers (29)					Non-smokers (31)					(A)–(B)
	Before	After	Diff. (A)	t	P	Before	After	Diff. (B)	t	P	P
Sweet ..	6·21	5·06	−1·15	1·272	—	4·38	4·12	−0·26	0·659	—	—
Sour ..	0·954	0·866	−0·088	0·595	—	0·719	0·739	+0·020	0·049	—	—
Salt ..	1·94	1·78	−0·16	0·495	—	1·23	1·11	−0·12	0·003	—	—
Bitter ..	0·035	0·027	−0·008	1·526	—	0·016	0·015	−0·001	1·561	—	—

that the alkaloids of tobacco smoke may act in a manner similar to quinine, and in this way possibly fatigue the mechanisms for perception of bitter.

Conclusions and Summary

In two completely separate population groups, a comparison of taste perception among samples of smokers and non-smokers revealed that the taste thresholds for bitter (using quinine hydrochloride) was significantly higher in smokers than in non-smokers. There was no significant difference in the taste thresholds for sweet, sour, or salt between smokers and non-smokers. Bitter is thus specifically affected. The age of the smoker, and thus presumably the duration of smoking, as well as the amount smoked, both adversely affected sensitivity to quinine solutions.

The smoking of a cigarette has no immediate effect on taste, for any of the taste primaries in smokers. Smoking, in decreasing sensitivity to bitter, appears to be the result of prolonged addiction to the habit.

As determined by P.T.C. testing, there does not appear to be a genetic inability among smokers to taste bitter.

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