FAT INTAKE, SERUM CHOLESTEROL CONCENTRATION, AND ATHEROSCLEROSIS IN THE SOUTH AFRICAN BANTU. PART I. LOW FAT INTAKE AND THE AGE TREND OF SERUM CHOLESTEROL CONCENTRATION IN THE SOUTH AFRICAN BANTU[1]

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During the past few decades, interest in the effects of overnutrition and undernutrition on blood cholesterol values, and their bearing on the pathogenesis of atherosclerosis, has led to the carrying out of numerous experimental studies on both animals and man (1, 2). Several studies have shown that diets characterized by reduced fat intake, are associated with, or give rise to reductions in serum cholesterol levels (3, 4). Dealing specifically with changes in fat intake, Keys, Mickelsen, Miller, and Chapman (5), using iso-caloric diets, found that when fat intake is substantially altered, well-marked changes appear in serum cholesterol concentration, which, moreover, are independent of cholesterol intake.

Confirmation of the correlation between fat intake and cholesterol values is afforded by two types of evidence. Firstly, populations subjected to dietary restrictions, which invariably include reduced fat intake, have been shown to have lower cholesterol levels than when the relevant conditions returned to normal. Thus, in the last war, very low cholesterol values were observed among the inmates of various European concentration camps (6–8). Conversely, a significant rise in mean blood cholesterol level occurred in groups of German subjects from 1947 to 1949, this change being attributed to dietary improvements, including increased fat intake (9).

The second type of evidence is provided by populations habituated to a high fat intake, *e.g.*, the inhabitants of the United States (10) and Denmark (11), who have higher mean serum cholesterol values than peoples accustomed to a lower fat intake, *e.g.*, population groups in Italy (12), Spain (13), China (14), India (15), Belgian Congo (16), Southern Rhodesia (17), and Bechuanaland (18).

In addition to the observation that serum cholesterol level varies directly with fat intake, the evidence indicates that the extent of the rise in serum cholesterol which occurs with age is influenced by the amount of fat customarily consumed. Thus, Keys and his co-workers (10) examined a series of American adult males habituated to a high fat intake (that of the nation as a whole supplies a mean of 38 per cent of calories), and observed a sharp regular rise in cholesterol levels from youth to late middle age (51 to 60 years), followed by a recession of values. On the other hand, with other workers, Keys (13) investigated the serum cholesterol age trend of a comparable series of Italians, who are used, *inter alia,* to a much lower fat intake (the mean national intake supplies about 20 per cent of calories); they found that the mean serum cholesterol level began on approximately the same plane, rose until the middle thirties when a plateau was reached (12).

This last investigation stimulated us to determine the age trend of serum cholesterol concentration in groups of the South African Bantu, whose average intake of fat is less than a half of that usual in the United States, and also less than that consumed by the Italian groups cited.

MATERIAL

Fat Intake

Among the Bantu of Southern Africa, maize, wheat, and, in certain areas, "kaffir corn" (*sorghum*), together with small amounts of legumes such as "cow peas" (*Vigna unguiculata*), "sugar beans" (*Phaseolus vulgaris*), "Jugo beans" (*Voandzeia subterrannea*) are the staple sources of calories: the fat intake is low (19–23). In Johannesburg in 1941, Fox and Janisch (20) carried out a study of African income and expenditure in 987 families,

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and reported an average daily consumption of 35 g. fat, which supplied 17 per cent of the mean estimated calorie intake. In a study carried out in 1951, du Toit (23) investigated the food intake of 100 families in an urban Bantu township in Springs, near Johannesburg. Families were selected to constitute a random sample, and observations extended over three months. The mean fat intake per man unit *per diem* was 45 g., which supplied 14 per cent of mean estimated calorie intake. (Daily diet provided a mean of 2,950 calories, and 84 g. gross protein.) There is additional information on the food habits of these urban and peri-urban Bantu population groups, which, although based on less factual data, confirms the low proportion of calories, certainly less than 20 per cent, which are provided by dietary fat.

Subjects

Bantu subjects used for determining the age trend of serum cholesterol. We found it impossible to collect blood from persons pursuing their every-day life in the Bantu townships around Johannesburg, the chief reason being the reluctance of non-hospitalized natives to give blood. Our subjects were chosen from patients attending Baragwanath Non-European Hospital (1,350 beds), which serves Johannesburg and the surrounding townships. The 218 persons examined included 167 men and 51 women, whose ages ranged from 15 to 93 years. Subjects were mainly ambulant out-patients awaiting treatment for minor surgical lesions, *e.g.*, hydrocele, wounds, otorhinolaryngal complaints, skin diseases, etc.; in the light of present knowledge these conditions are not considered likely to seriously affect general nutritional state or serum cholesterol values. The number of elderly persons attending out-patients departments, unfortunately, is small; the majority of such subjects were patients with cataract just admitted to hospital for operative treatment. All subjects were in fair state of nutrition; there were no cases of undernourishment or emaciation.

Bantu subjects from other South African territories (*a*) *Bechuanaland.* The diet of the Tswana of South Bechuanaland resembles that consumed by urban Bantu. Reports by Squires (24, 25), together with our own impressions, suggest that the consumption of "kaffir corn" is probably higher, and possibly that larger amounts of meat and vegetables are consumed. The average amount of fat eaten is considered to be of the same order as in urban areas. The above reports indicate that, speaking generally, the state of nutrition is reasonably good. At Lobatsi and Kenya, blood samples were obtained from 38 subjects, *i.e.*, 23 male and 15 female subjects, their ages ranging from 21 to 38 years, mean age being 30 years. Of the male subjects, 10 were prison laborers, and 10 were agricultural workers; the remaining three males and all the females were attending out-patients departments (the female subjects mainly on account of their children) in the two centers mentioned. All subjects were in outward good health.

(*b*) *Basutoland Bantu.* The indigenous Basuto consume a diet of similar pattern to that of the urban Bantu, except that their consumption of maize is higher and of wheat lower compared with town dwellers (26). The intake of fat is not believed to be different. In Johannesburg, the Witwatersrand Native Labour Association Headquarters is the clearing station through which pass annually about a quarter of a million Bantu mineworkers before being directed to the different mines, where they work for recurrent periods of about nine months. Blood samples were obtained from 31 subjects immediately after their arrival from Basutoland. Ages ranged from 21 to 40 years, mean age being 29 years. All subjects were passed as medically fit.

Bantu consuming a partially Europeanized diet with higher fat content. Serum cholesterol values were determined on 69 Bantu adult males from two local centers of employment which provided rations for their workers. The diets, although still containing a large amount of cereal products, differed from those usually consumed mainly on account of their higher energy and higher fat contents, the latter being about 65 to 80 g. *per diem* and providing 20 to 25 per cent of estimated calorie intake. Ages ranged from 21 to 40 years, mean age being 31 years. Subjects had been used to the diet for a minimum of one month, though usually for a longer period. All subjects were medically fit.

European control subjects. The European subjects examined were either on the staff of this Institute, or were blood donors to the South African Blood Transfusion Service. All persons were in a good state of health. Two age groups were selected for study. The first, 21 to 30 years, numbered 55 subjects, with mean age 28 years. The second group 41 to 50 years, with mean age 46 years, was composed of 46 subjects. Unfortunately, no recent dietary surveys have been undertaken on local Europeans. In the family Budget Inquiry (27) carried out in 1936, the middle income groups dwelling in Johannesburg consumed a mean daily fat intake of 108 g. which supplied roughly 36 per cent of the estimated calorie intake. In le Riche's study in Pretoria (28), the lower class European group consumed a mean of 91 g. fat *per diem*, which provided about 24 per cent of estimated calorie intake. Our impressions are that the fat consumption of our European subjects differed little from that of the corresponding strata of population found in the United States, *i.e.*, fat intake supplied at least 30 to 35 per cent of calorie intake.

Time of Sampling

Blood was collected approximately mid-way between breakfast and lunch time, except that about half of the bloods of the Bantu consuming the Europeanized diet were collected between noon and 4 p.m.

METHODS OF ANALYSIS

Serum cholesterol. Serum was shaken with the usual Bloor mixture, and after evaporation, the extracted cholesterol was determined by the Liebermann-Burchard reaction, the final color being stabilized as described by

TABLE I

Serum cholesterol data on Johannesburg Bantu of different age groups *

Age groups (years)	Average age (years)	No. of subjects	Range of serum cholesterol	Mean and S.D. values	Minnesota data	
					Average age	Mean and S.D. values
20	18	25	99–224	155±32	18	168±31
21–30	26	45	88–236	166±28	25	184±34
31–40	38	40	92–265	169±40	35	200±43
41–50	45	54	85–240	179±37†	45	236±37†
51–60	54	28	73–252	184±39†	55	256±46†
60+	68	26	103–283	183±42†	70	225±42†

* Cholesterol values given in mg. cholesterol per 100 ml. serum.
† Means differ significantly ($P < 0.01$).

Sheftel (29). To obviate any uncertainty, the method employed will be described in detail.

All glassware is thoroughly dried beforehand. Into a 15 ml. stoppered centrifuge tube are put 8 ml. absolute alcohol and 3 ml. ether; 0.2 ml. serum is added slowly. After shaking for one min., the tube is allowed to lie horizontally for one hour, and then centrifuged for 10 min. at 3,000 r.p.m. The supernatant liquor is poured into a small flat-bottomed flask, and the alcohol-ether mixture evaporated off at low temperature on a sand bath. When cool, 5 ml. dry chloroform are added, the precipitate dissolved, and the solution poured into a small glass stoppered reagent bottle. The standard solution, 5 ml. chloroform containing 0.4 mg. cholesterol (prepared from stock chloroform solution containing 100 mg. per cent cholesterol), is poured into a similar bottle. To each bottle is added 1 ml. glacial acetic acid and then 2 ml. acetic anhydride containing 10 per cent sulphuric acid (this mixture is freshly prepared on each occasion and cooled before use). The green colors produced are allowed to develop in the dark for 30 min., and measured in a Hilger Spekker Spectrophotometer using Spectrum Red filter 608.

The method followed records all color as cholesterol. Hence, all values are very slightly too high because the color intensity of cholesterol esters is greater than that of free cholesterol (29).

Using this method, average difference between 100 duplicates was 5.8 mg. cholesterol, per 100 ml. serum. Fifty determinations were carried out in parallel with Dr. W. M. Politzer of the Routine Biochemistry Department of this Institute, using the same method. The average difference between results was 6.2 mg. cholesterol per 100 ml. serum. Finally, on taking five sera of cholesterol value of about 150 to 170 mg. per cent, and adding pure cholesterol sufficient to double this value, the mean recovery rate was 98 per cent (range 95 to 101 per cent). With five sera having values about 300 to 320 mg. per cent, and treated correspondingly, mean recovery of added cholesterol was 97 per cent(range 94 to 102 per cent).

Liver function tests. Determinations were made of thymol turbidity (30), cephalin-cholesterol flocculation (31), Takata Ara (32), and gamma globulin (33).

TABLE II

Serum cholesterol data on different Bantu and South African European groups

Race	Age group (years)	Average age (years)	No. of subjects	Cholesterol values
Bechuanas	21–38	30	38	149±36* (86–210)
Basutos	21–40	29	31	153±37* (94–221)
Johannesburg Bantu group	21–40	31	85	167±33* (88–265)
Europeanized Bantu group	21–40	31	69	178±36* (100–249)
South African Europeans	21–30	28	55	206±35 (145–322)
	41–50	46	46	238±38 (186–328)

* The means of Bechuanas and Basutos are significantly lower than that of the Johannesburg Europeanized Bantu ($P < 0.05$).

RESULTS

The results are summarized in Tables I to III.

COMMENT [2]

The American data cited refer to values obtained when subjects were at basal condition. According to the findings of Keys and his co-workers (10) the values we obtained are likely to be approximately 4 mg. per cent higher than at basal state.

The age trend of serum cholesterol concentration in the Bantu. Below 40 years, there is no statistical difference between the mean cholesterol values of the Bantu and American age groups. After 40

[2] Statistical analysis of our results was undertaken by Mr. Walter Lutz B.A. of the Department of Mathematics of the Witwatersrand University.

TABLE III

Liver function data on different Bantu groups

	Bechuanas	Basutos	Europeanized urban Bantu group*
No. of subjects	38	31	57
Per cent showing liver dysfunction	68	71	65
Mean serum cholesterol in dysfunction group	151±37	151±39	180±37
Mean serum cholesterol in group not showing dysfunction	145±34	155±36	175±35

* The liver function tests on the Johannesburg Bantu consuming the Europeanized diet were undertaken by Dr. I. Bersohn and Miss C. D. Sussman, B.Sc. of the Diagnostic Department of this Institute.

The liver function data were obtained in an endeavor to learn whether there was any correlation between presence of liver dysfunction and level of cholesterol concentration. Initially, a subject was judged to show liver dysfunction when the thymol turbidity value exceeded 2 units, the cephalin cholesterol (24 hour reading) and Takata Ara tests were positive, and the gamma globulin concentration exceeded 1.25 g. per 100 ml. serum. Since this classification revealed dysfunction present in 80 to 90 per cent of subjects, a re-grading was carried out in which the thymol turbidity ceiling level was raised to 3 units, and the gamma globulin to 1.40 g. per 100 ml. serum. Subjects with liver function data equal to or below the stated values were considered to show no or mild liver dysfunction; the remainder, for the purposes of argument, were regarded as having marked dysfunction.

years, the means of the Bantu groups are significantly lower than the American means ($P < 0.01$).

The mean annual increments were computed as the slopes of the least squares regression lines fitted to the data over age groups showing no significant curvilinearity, *i.e.*, 17 to 45 years. The slopes differ significantly ($P < 0.01$). The rises in serum cholesterol per year are 0.378 ± 0.89, and 2.29 ± 0.180 mg. per 100 ml. serum, respectively, for the Bantu and Minnesota groups. There is no significant difference between the corresponding origins, 141.7 and 127.7 mg. per cent respectively.

Cholesterol values of other Bantu groups. There is no significant difference between mean cholesterol values of the 21 to 40 age group of the Johannesburg Bantu, the Bechuanas, and the Basutos.

The mean cholesterol value of the 21 to 40 year group of the urban Bantu consuming the Europeanized diet is significantly higher than the values for the Bechuanas and Basutos ($P < 0.05$), although not for the Johannesburg Bantu.

Cholesterol values of the European groups. The serum cholesterol value of the 21 to 30 year group of South African Europeans, although slightly higher, does not differ significantly from the corresponding American value. Likewise, our 41 to 50 year group does not differ from the American group significantly in mean serum cholesterol value.

Liver function tests. Among the three Bantu groups examined (Table III) there is no significant difference between the mean cholesterol values of the sections showing little or no dysfunction, and those showing marked dysfunction.

DISCUSSION

It will be apparent that the low fat intake of the South African Bantu correlates with their low serum cholesterol values. The factors that may share in the responsibility for the correlation will now be considered.

Race

Populations, both White and Non-White, who are habituated to a low fat diet are marked by low serum cholesterol levels. Furthermore, Snapper (14) noted low values in poor Chinese in Peiping, yet high values among commercial Chinese dwelling in Indonesia. In addition, Verhoef (34) reported low values among indigenous Javanese, compared with normal values occurring in Javanese who had been resident in Holland for some time. Our Europeanized Bantu group on their higher fat intake, had a higher mean cholesterol concentration in the serum compared with the two rural Bantu groups consuming their usual low fat diet. Bearing these points in mind, we agree with van Oye and Charles (16) in doubting whether the racial factor has any relevance in the present issue.

State of health

Subjects in the main Bantu group examined either were attending or were recently admitted to hospital. Hence, understandably, their cholesterol data may be treated with some reserve. Nevertheless, our results reveal no significant difference between the serum cholesterol concentrations of the 21 to 40 year age groups of the hospital Bantu on the one hand, and the Bechuanas, and Basutos on the other. Since all three groups were believed to be accustomed to much the same pattern of diet, and since the two rural groups were

known to be in a good state of outward health, it would seem that the complaints for which the Johannesburg Bantu were attending hospital were not markedly affecting their serum cholesterol figures. It is therefore probable that the age trend of serum cholesterol values observed in the hospital Bantu group is likely to be equally valid for Non-hospitalized Bantu pursuing their usual avocations.

It is apparent that every-day good health as experienced by the Bechuana and Basuto subjects is compatible with low serum cholesterol concentrations.

Dietary factors

Fat intake. At first sight, it would seem reasonable to consider that the low fat intake of these people is likely to be primarily responsible for the low cholesterol values observed. There are, however, a number of observations which preclude the unreserved acceptance of this view: (*a*) The serum cholesterol value of the Johannesburg Bantu on the higher fat diet was less affected by the increase in fat intake than was anticipated: actually, there was no significant difference between the mean cholesterol value and that of the Johannesburg Bantu consuming their usual low fat diet; (*b*) In a comprehensive Dutch study (35), it was found that a diet of almost exclusively vegetable products had the effect of reducing the mean cholesterol value of 20 subjects, mean age 38 ± 8 years, from 260 to 200 mg. per cent in three months, although the diet was rich in vegetable fat—a mean of 108 g. *per diem,* providing 36 per cent of calories; (*c*) In a further recent Dutch study (36), the mean serum cholesterol value of 55 strict vegetarians ("vegans"), of mean age 39 years, was found to be 166 mg. per cent, although, here again, the fat content of the diet provided no less than 34 per cent of calorie consumption. In studies (*b*) and (*c*), it is clear that a factor or factors other than reduced or low fat intake were in operation. While there is little doubt that the low fat intake of the Bantu is implicated in the low cholesterol values recorded, present knowledge allows no preciseness of incrimination.

Cholesterol intake. Certainly the cholesterol content of the habitual diet of the Bantu is low in comparison to the levels of intake that are common overseas. In America, the comprehensive studies of Keys, Mickelsen, Miller, and Chapman (5, 13) have demonstrated that alterations in cholesterol intake have virtually no influence on serum cholesterol value. Whether such results would be equally demonstrated in the Bantu pattern of diet, we are not in a position to say.

Caloric deficiency. Keys and his associates (4) have critically examined reported data on blood lipids in acute and semi-starvation. They consider that the latter produces little if any change in blood lipid levels except for a decrease in cholesterol, which contrasts with the suggestive slight increase in this component in acute starvation. Unfortunately, there is little information available on cholesterol levels accompanying mild degrees of caloric insufficiency such as occur occasionally at certain seasons with the Bantu of Southern Africa. As noted earlier, upon altering fat intake *only* in diets of iso-caloric content, Keys, Mickelsen, Miller, and Chapman (5) obtained highly significant changes in serum cholesterol concentration. But the influence of alterations in caloric intake *only* in diets of iso-fat content has been less thoroughly investigated. Walker (37) has recently studied changes in various blood lipid components of subjects during weight reduction on diets already low in fat but of reduced calorie content; he observed significant changes in certain lipo-protein fractions, although not in cholesterol level. On this account we question whether the possibly lower plane of caloric nutrition exhibited by the subjects in this series is of relevance in regard to the low cholesterol values observed.

Pattern of diet. In relevant publications, of the dietary factors considered, almost exclusive attention has been devoted to the rôles of fat, cholesterol, and calorie intakes, in influencing serum cholesterol concentrations. The *pattern of diet,* however, habitual to population groups displaying hypocholesterolemia, is distinctive, and has not been given the attention that it deserves. During war-time, it is frequent for diets to fall in energy value, protein (particularly animal protein), and fat (particularly cholesterol) contents, and to rise in carbohydrate and crude fiber contents. The consumption of meat, dairy produce, eggs, fatty foods and sugar usually goes down, but increases occur in consumption levels of cereals and their products, legumes, and vegetables. Speaking

generally, this pattern of diet is much the same as that of Non-White dwellers in tropical and semi-tropical regions. One feature of the diet described which may be of relevance is that it usually contains a large amount of crude fibre, thereby constituting a "high residue" diet, which contrasts with a "low residue" diet, *i.e.*, one based on refined foodstuffs. In this respect, attention is drawn to the two Dutch investigations cited above (35, 36); both diets consumed, which were relatively high in fat, although associated with reduced or low mean cholesterol values, were high in bulk-forming capacity. We must point out that knowledge of the effect of pattern of diet on cholesterol synthesis, excretion into the bile, absorption and re-absorption in the intestine, and loss in the stools, is virtually nil. Investigations on certain of these aspects have been initiated.

Summarizing, of the dietary factors, our impression is that, apart from low fat intake, there is a factor or factors in the pattern of diet of the Bantu possibly related to its high fibre content which bears some responsibility for the low serum cholesterol values observed.

Pathology and dysfunction of the liver

Hypocholesterolemia is the rule in advanced forms of cirrhosis (38), a lesion commonly observed among the South African Bantu, as with many other indigenous populations of tropical and semi-tropical countries (39–41). Local observers have the impression that cirrhosis is present in approximately a tenth of the adult hospitalized urban Bantu population. Moreover, intermediate degrees of chronic liver disease are common (42), and certain liver function tests have revealed widespread abnormalities affecting more than half of the Bantu population (43). The question naturally arises, to what extent this commonness of hepatic disease and dysfunction is responsible for the low serum cholesterol values observed. Unfortunately, present evidence does not permit a satisfactory answer to be given. However, regarding cirrhosis, we doubt whether its incidence is sufficiently high to account for our results. In relation to milder hepatic damage and dysfunction, no information is available upon the extent to which such states affect serum cholesterol values, although a detailed investigation on this subject is now in progress at this Institute (43). Two of our findings, however, appear to be relevant. Firstly, among the 69 Bechuanas and Basutos, the biochemical tests undertaken revealed abnormalities in 48 (67 per cent); yet there was no significant difference in mean cholesterol levels between the groups with and without dysfunction. Furthermore, of the Bantu group consuming the Europeanized diet, 37 of the 57 subjects examined (65 per cent) showed abnormality of liver function according to the criteria used; yet the groups with and without dysfunction had mean cholesterol concentrations which did not differ significantly (Table III). We therefore suggest that hepatic dysfunction and mild chronic liver disease are little implicated in the low serum cholesterol values obtained.

Pancreatic disease

Marked atrophy of the parenchymal cells of the pancreas is common among Bantu infants and young children, but such lesions are found to be milder and much less frequent in adults (44). As far as we are aware, no correlation has been postulated between the presence of such lesions and altered serum cholesterol concentrations. Hypercholesterolemia is a feature of diabetes, but the latter occurs very infrequently among the Bantu population generally (39, 41). We thus consider it unlikely that pancreatic disease is implicated in the low serum cholesterol data obtained.

Non-dietary factors

Both Keys (13) and Groen, Tjiong, Kamminga, and Willebrands (35) have referred to subjective factors, such as mental over-exertion, emotional tension, *etc.*, in promoting hypercholesterolemia. We do not know to what extent these factors in the Bantu influence their cholesterol levels.

The uncertainties involved in the subject under discussion are clearly apparent and indicate the extent of the further investigational work required. Nevertheless, we feel justified in believing that an important influencing factor sharing responsibility for the low serum cholesterol concentrations observed in the Bantu is their habitually low fat intake. In addition, however, we have the impression that there is a factor in the pattern of diet,

possibly its bulk-forming capacity, which bears some responsibility.

SUMMARY

1. The South African Bantu, in common with most indigenous populations dwelling in tropical and semi-tropical regions, are habituated to a diet, *inter alia,* of low fat content, averaging less than a half of that consumed in the United States.

2. The age trend of serum cholesterol has been investigated in 218 urban Bantu subjects, of ages ranging from 15 to 93 years. Up to 40 years, the differences between mean values obtained among the Bantu and in Minnesota (Keys and his associates), are not significant; thereafter, the Bantu data are significantly lower ($P < 0.01$). The regression curves of the Bantu and Minnesota data for the group from 17 to 45 years differ significantly in slope ($P < 0.05$).

3. Groups of rural Bantu from other territories likewise accustomed to a low fat intake, and in a good state of health, were also found to have low mean cholesterol values.

4. Urban Bantu consuming a Europeanized diet with an increased fat intake have a significantly higher mean serum cholesterol concentration than corresponding groups of rural (though not urban) Bantu eating their usual diet with its low fat content ($P < 0.05$).

5. Discussion of the relevant evidence suggests that racial differences, state of health, caloric deficiency, low cholesterol intake, and pathology and dysfunction of liver and pancreas bear little responsibility for the low serum cholesterol values observed.

6. The main dietary influencing factor may well be the habitually low fat intake of these people. Impressions suggest, however, that further dietary factor or factors are implicated, possibly related to the high "residue" diet of these people.

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