ATHEROSCLEROSIS—THE CASE AGAINST PROTEIN

J. C. ANNAND, M.A., M.B., Ch.B.
Dundee

It would appear that opinion continues to be divided concerning the role of dietary fat in the etiology of atherosclerosis,[2 3 42 43 72] and no clear cut evidence has yet been produced finally proving that fat *per se* plays the major part in the pathology of this disease process. Nor can it be said that a deficiency of the essential fatty acids[72-73] is the most important factor causing the present increase in the incidence of atherosclerosis. Therefore, as it has already been reported[1] that after a lag period varying up to about one month in duration vitamin B_{12} (cyanocobalamin) makes the signs and symptoms of patients suffering from severe atherosclerosis much worse, and angina pectoris, intermittent claudication and " rest pain " became prominent features, the position of protein, and of animal protein in particular, would seem to deserve closer examination—a contention, which also seems to receive further support from the following work.

Yudkin,[4] who carried out an epidemiological survey for the year 1952 involving 15 countries, recorded that animal protein gave at least as good a correlation as both animal and total fat, not only in the *different countries* examined, but also in the *occupational class* survey for the United Kingdom.

Hilleboe carried out two surveys, one for 12 countries in 1950-52,[5] and another for 22 in 1957.[6] In both it was recorded that animal protein gave a significantly better correlation than fat intake. Here, the work of Keys[7] was strongly criticized as, in his survey, which only covered six countries, he had described an apparent almost exact correlation between fat intake and the mortality rate from atherosclerosis—a correlation which broke down completely when the survey was extended to cover the larger field of 22 countries. (In all these surveys, as in this, the animal protein intake was plotted against the mortality from " Arteriosclerotic and Degenerative Heart Disease ", the " B26" classification of the International Abbreviated List, 1948, and hereafter referred to as the " B26 Mortality Rate ").

In view of these findings, it was decided to carry out yet another survey, this time for the year 1954, and at the same time, to compare the various trends in food consumption for the period 1934-39 with those of the period 1950-54. In this connection, the figures

listed below have been obtained from the food balance sheets provided by the Food and Agricultural Organization of the United Nations and are not those of actual consumption but are the amounts of the various foodstuffs which were available for consumption at that time; thus *only trends in consumption* are being considered.

It was hoped that these same trends might be helpful in throwing some light on the etiology of atherosclerosis for the following reasons: (*a*) It is generally accepted that atherosclerosis is dietary in origin; (*b*) It is an increase in the incidence of atherosclerosis, which we are now investigating, and not a new disease process. (*c*) Therefore, it would seem reasonable to search for an increase in the consumption of a particular food or of a group of foodstuffs—an increase which would be especially evident in those countries which had incurred the highest incidence of the disease.

In the first instance, figure 1 reveals a rough correlation between the amount of animal protein available for consumption and the B26 mortality rate (per 100,000 men aged 55-64), a correlation which is obviously more linear than that obtained from total fat (figure 2) or from butter (figure 3). The graphs thus obtained would appear to correspond fairly closely with those of Yudkin,[4] and the findings of Hilleboe[5] and those of Yerushalmy and Hilleboe.[6]

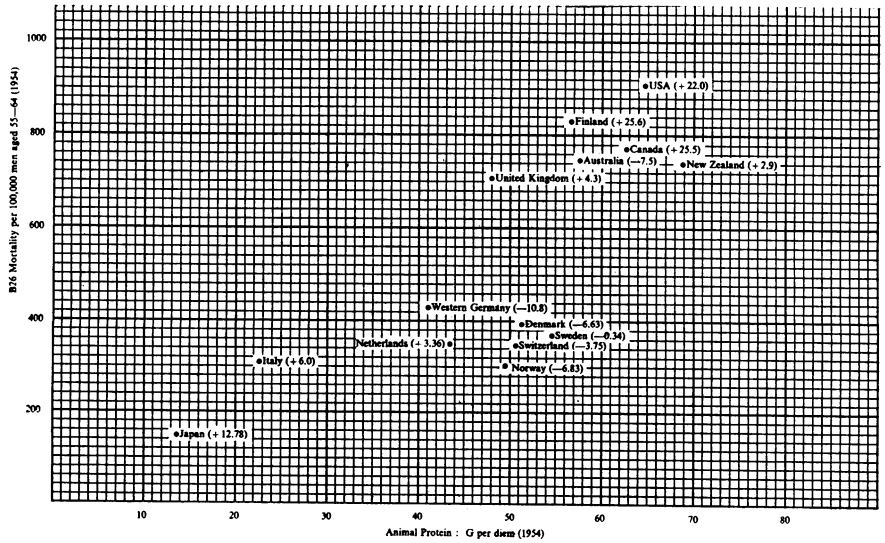

FIGURE 1.
Available animal protein and B26 mortality rates.

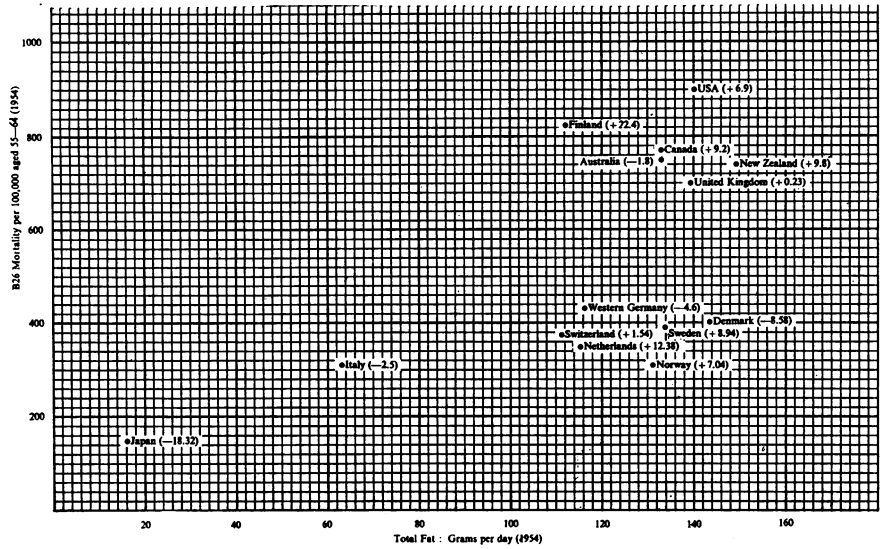

FIGURE 2.
Total available fats and B26 mortality rates.

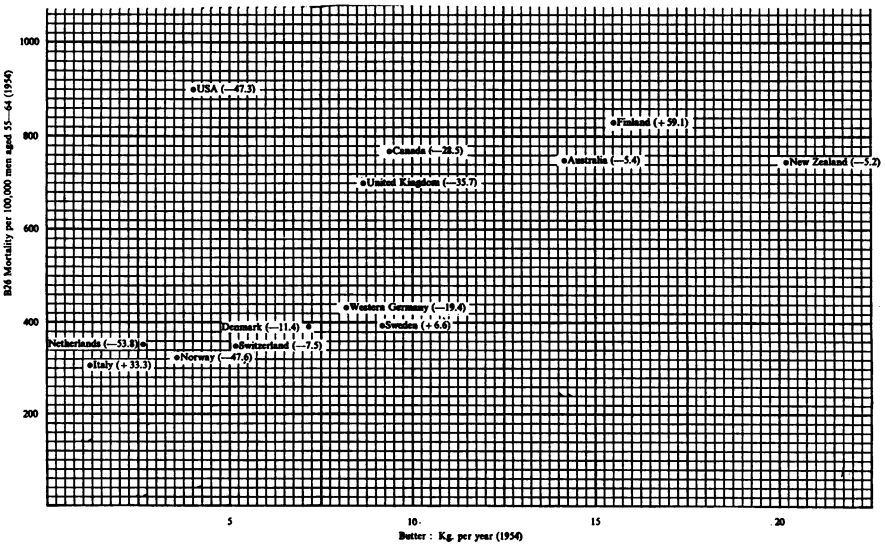

FIGURE 3.
Available butter and B26 mortality rate.

Moreover, when we come to examine the different periods 1934-39 and 1950-54 for changing trends in consumption, we find that, during the period 1950-54, the three countries, U.S.A., Finland, and Canada, which have incurred the highest incidence of atherosclerosis, all demonstrate a significant increase (average 24.4 per cent) over the amount of animal protein available for consumption in the period 1934-39. (In figure 1 the figures in brackets after the name of each country are relevant.) On the other hand, in the case of both total fat (figure 2) and of butter (figure 3), the increases are not significant, except in the case of Finland: nor are they in the case of sugar[4] (figure 4), nor in the case of total calories (figure 5). In figures 2, 3, 4, and 5, the numbers in brackets following the names of the countries are again relevant. As regards animal fat, the increases for the U.S.A., Canada and New Zealand were respectively 2.3 per cent, 0.5 per cent and 2.6 per cent: Australia showed a fall of 3.8 per cent, figures for the remaining countries were not available. Thus the only group of foodstuffs, which was found to correlate in any significant degree under all the necessary conditions so far related, was animal protein.

Another change, occurring within this century, is the marked increase in the consumption of milk and of milk products. The

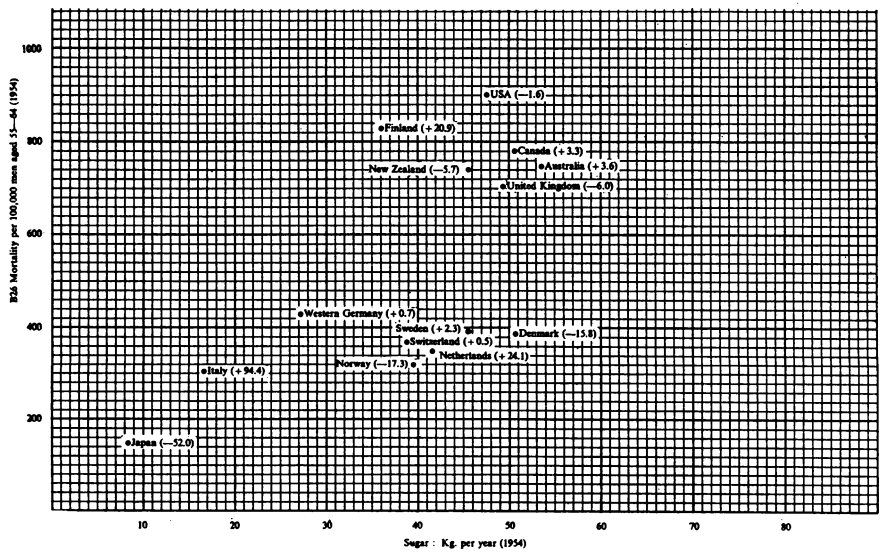

FIGURE 4.
Available sugar and B26 mortality rates.

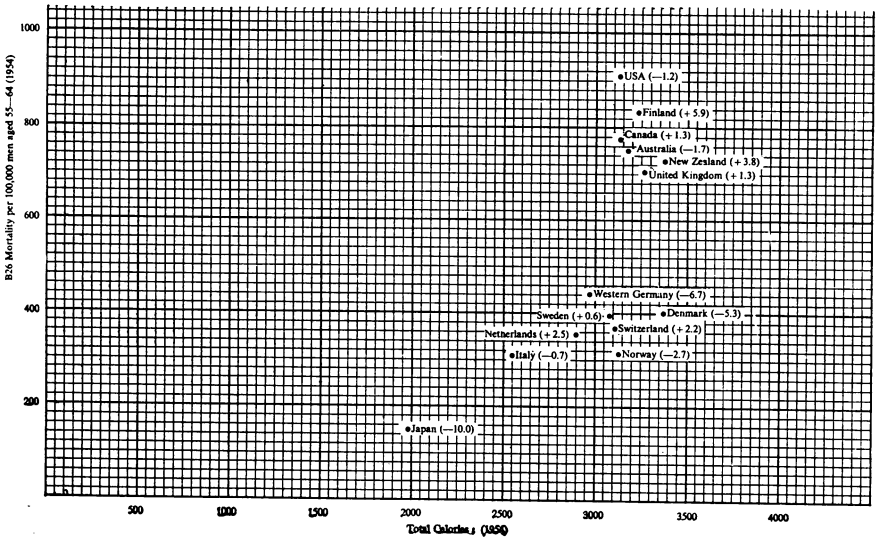

FIGURE 5.
Total calories and B26 mortality rates.

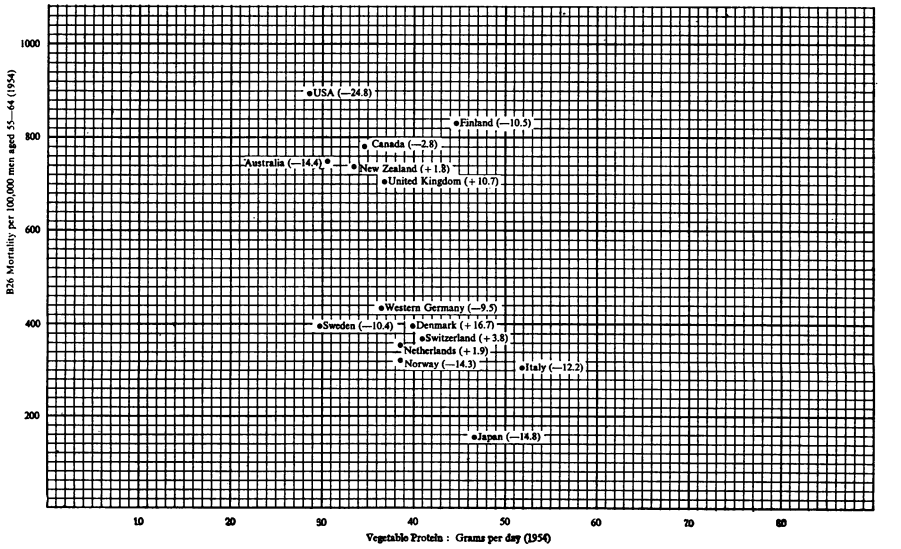

FIGURE 6.
Vegetable protein and B26 mortality rates.

percentage increase in the same two periods in terms of milk protein (grams per day) is shown for the various countries in figure 7: it is at once obvious that all the countries incurring the higher incidence (Group A, figure 7) have also increased their consumption of milk protein by a more significant degree than those incurring the lower incidence (Group B, figure 7) three of whom show a slight decrease.

Another current trend is of course the fact that increasing quantities of milk are now pasteurized before consumption and that milk products also are now mainly manufactured from pasteurized milk. An investigation of all the countries involved was then carried out and it was revealed that, in the year 1954, in all the countries incurring a high incidence of atherosclerosis (Group A, figure 7) practically all milk was pasteurized and all milk products were manufactured from pasteurized milk (figure 7).

In this connection, it is pointed out that the elevation of temperature used in cooking and/or in pasteurization would normally free vitamin B_{12} from its bound state within the protein[45] and thereby, might well assist the absorption of this free vitamin.

Although the data available (for the vitamin B_{12} content of the various animal foods, see appendix 1) did not permit an accurate estimation of the vitamin B_{12} consumption of the various countries, it may be said that in the author's experience, beef, and/or mutton, and/or heated milk, and/or its products, especially cheese, and/or offal, seem to loom large in the diet of the patients suffering from atherosclerosis; and, if the different animal foods are divided roughly into two groups in relation to their vitamin B_{12} content, a high and a low group, the latter to include pig meat, fish, veal, eggs and in one third of the countries unpasteurized milk, the

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Key to Figure 7.

is the amount of animal protein derived from milk and milk products available for consumption in 1954. The percentage stated is the amount of milk and of milk products pasteurized in that country in 1954.

is the amount by which milk and milk products available for consumption in 1954 have increased or decreased in relation to the amount of the same products available in the period 1934—38. If a decrease has occurred, it has been placed at the lower end of the column, and is then not to be included in the total amount of milk and milk products available in 1954.

is the amount of animal protein of relatively *high* Vitamin B12 content available for consumption in 1954: (see Appendix 1), i.e., *beef, mutton, poultry, game and offal.*

is the amount of animal protein of relatively *low* Viatmin B12 content available for consumption in 1954: (see Appendix 1), i.e., *fish, eggs, pig meat and veal.*

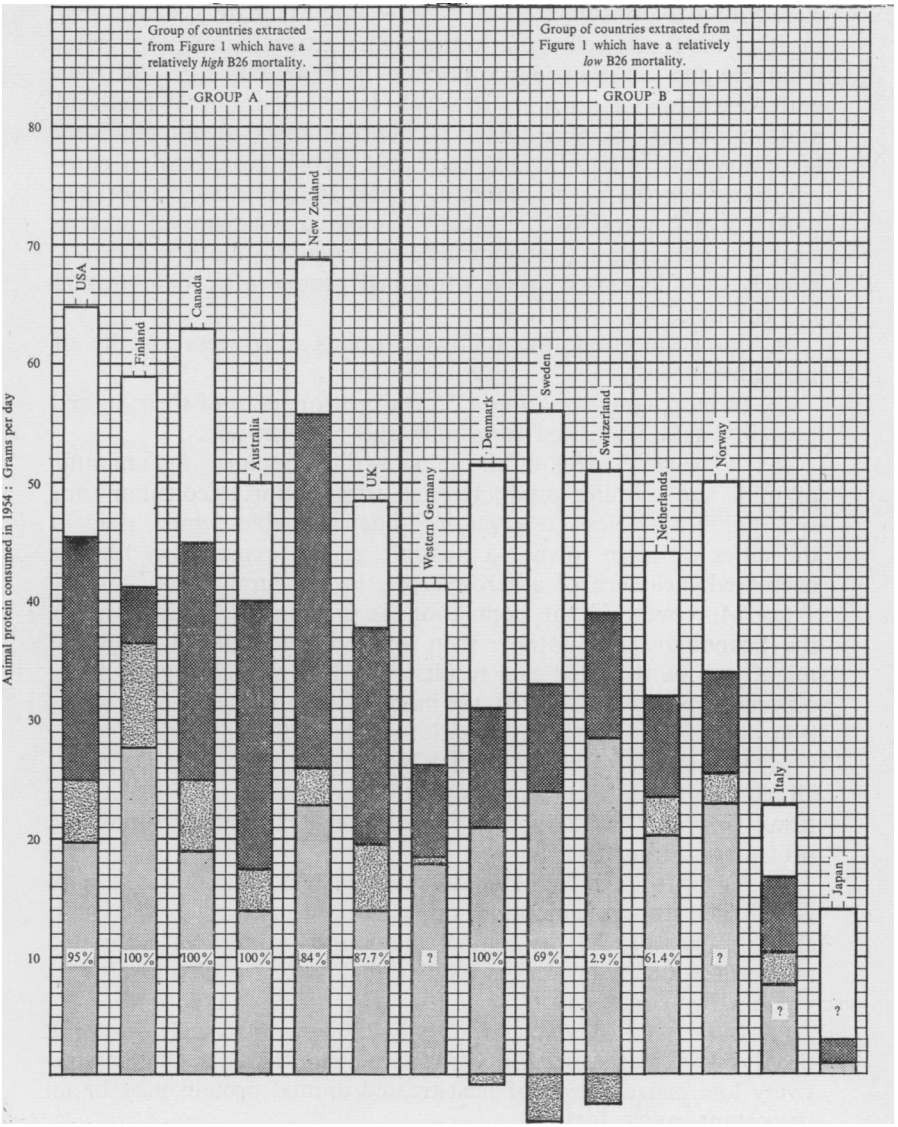

FIGURE 7.

The number of grams per day of animal protein consumed in countries with a relatively *high* B26 mortality (group A), and in countries with a relatively *low* B26 mortality (group B) is represented by the total height of the column. For key to the division of the column see foot of page 370.

group of countries showing the higher incidence (Group A, figure 7), were found to consume, on an average, approximately 50 per cent more of the foods belonging to the higher group than the countries incurring the lower incidence (Group B, figure 7); e.g., Australia and New Zealand consume relatively large quantities of beef and mutton, whilst the Scandinavian countries seem to eat relatively large amounts of fish (mainly cod), and of pig meat, both of comparatively low vitamin B_{12} content. There is thus revealed a possible partial explanation for the greatly increased incidence of atherosclerosis occurring in the countries listed under Group A in figure 7, namely their increased intake of animal protein of a high vitamin B_{12} content.

In this connection, the following points also seem worthy of mention:

(*a*) In most countries, about 35 per cent or more of their animal protein intake is derived from milk and milk products.

(*b*) In the rural areas of all the countries examined, the amounts of milk and of milk products pasteurized prior to consumption, was significantly less to a varying indeterminable degree, than in the cities or large towns—a possible partial explanation for the decreased incidence of atherosclerosis in the rural areas.

(*c*) Moreover, if the population is predominately rural, as in the Scandinavian countries, then an apparent lowered incidence might well be recorded as a result. The B26 mortality of Norway especially may be so affected, the more so as compulsory pasteurization was only instituted in the cities and large towns in 1954.

(*d*) It should also be noted that Norway, Sweden, and Denmark all consume relatively large quantities of marine oils, 38.6mg., 12mg. approx., and 8.8mg. per day respectively—another possible protective factor.[19]

(*e*) As regards the Netherlands, in 1954, 31.2 per cent of the milk was issued for consumption unpasteurized mainly in the rural areas; moreover, 7.4 per cent of the country's milk was subjected to sterilization, a process, which probably destroyed most of its vitamin B_{12} content.[75]

(*f*) In the case of Switzerland, only 2.9 per cent of milk and milk products was pasteurized in 1954; here again therefore, a comparatively low consumption of heat-treated animal protein may be an important saving factor.

(*g*) As regards the Federal Republic of Western Germany, two points may account for their relatively high incidence: (1) pasteurization was confined mainly to the cities, conurbations and large towns, which form the major part of the population and (2) Western Berlin was included in the survey.

(*h*) As regards Finland, another possible explanation for their

unusually high incidence, has recently been submitted by Roine *et al.*[8] and by Votila *et al.*,[9] namely a high incidence of endemic goitre, which like atherosclerosis, seems to predominate, in the eastern half of the country. These dietary surveys, however, also reveal that the eastern half of the country consumed approximately 11—12 per cent more animal protein than the west and 24 per cent less ascorbic acid. Moreover, the highly significant increase in the consumption of milk and milk products, 100 per cent pasteurized, should, of course, not be forgotten.

(*i*) Finally, a sharp decline in the B26 mortality in European countries during the last war, was accompanied by a fall in the consumption of milk, butter, cheese and eggs.[2] In this country, the type of milk especially involved, was " condensed milk ", which was consumed in significantly increasing amounts prior to the war; expressed as a percentage of pre-war consumption levels, there occurred, in the United Kingdom, in the period 1940-41, a 13 per cent drop in the consumption of animal protein generally.[69]

Animal Experiments

In 1915, Knack[10] fed one group of rabbits a normal " rabbit " diet plus 4.5g. cholesterol, and another group, milk, one egg and greens—total daily cholesterol 0.3g. The latter group, although they had consumed much less cholesterol, and for a shorter period, developed much more extensive atherosclerosis.

These findings were confirmed by Newburgh and Clarkson,[11] who fed rabbits cooked beef, fat free and with a low cholesterol content, and recorded a high incidence of atherosclerosis, roughly proportionate to the duration of the experiment and to the amount of animal protein consumed. These findings were reviewed by Freyberg[12] in 1937 and he suggested that the atherosclerosis so produced in rabbits might be due " to a non-protein constituent other than cholesterol in animal flesh ".

Maeker and Kesten[14] also found that casein, freed of its cholesterol content, caused severe atherosclerosis in rabbits, whilst Jones and Huffman[15] recorded that the severity of atherosclerosis produced by casein in rats, did not seem to vary with the amount of fat in the diet. Old rats especially, attained unusually high cholesterol levels when fed 40 per cent casein. Moreover, Mayer and Jones[27] using the obese rat as a yardstick, obtained higher blood cholesterol levels on a high protein (casein) diet than on a high fat (lard) or high carbohydrate diet.

More recently,[16] in further confirmation, the saturated fat, hydrogenated cotton seed oil, produced the most striking elevation of serum cholesterol levels, when casein was the dietary protein.

Finally Chakravarti *et al.*[68] fed six rabbits a stock diet plus 0.5g. cholesterol suspended in 5ml. olive oil, and, gave them daily injections of 10μg vitamin B_{12} for a period of 12 weeks; compared

with five controls only, they showed an apparent decrease in the number of atheromatous plaques but an increase in the relative amounts of free cholesterol.

Human Experiments

It has long been known[17] that a high protein, high fat diet causes hypercholesteremia in the human, but only recently has it become more apparent that animal protein may play a vital part. Thus, in a dietary survey of three Nigerian tribes,[18] the blood cholesterol was found to correlate more closely with the animal protein intake than with the fat intake:

Name of tribe	*Moslem*	*Ibo*	*Pagan*
Intake of animal protein ..	3g.	20g.	6g.
Total fat intake	26g.	45g.	45g.
Total cholesterol .. .. (mg. per 100 ml.)	118.8	129.9	119.0

Moreover, in the South African Bantu[19] fed different types of fat and beef meat, the latter, when compared especially with beef dripping, was found to be responsible not only for the most rapid rise in the blood cholesterol level, but also for the highest cholesterol level thus obtained.

Finally, the work of Groen *et al.*[22] deserves special mention. Sixty normal subjects were fed different diets during three periods of twelve weeks each. It was then found that a high meat diet raised the blood cholesterol level, a vegetable diet lowered the serum cholesterol by a highly significant degree, whilst a low meat diet, typical of the Netherlands, produced an intermediate effect. *Moreover, it was also noted that an exclusively vegetable diet reduced the blood cholesterol even in the presence of a relatively high fat diet 90—125g. per day.*

These findings have been confirmed in part[26] more recently in that all of seven human subjects, five of whom were hypercholesteremic, demonstrated a significant fall in blood cholesterol when fed, for one week only, a diet which contained 25g. protein, most of which was of vegetable origin. In this connection, of course, it has long been known that vegetarians are not generally subject to cardiovascular disease and have significantly low blood cholesterol levels.[20-23]

(It would appear reasonable to postulate that most of the animal protein used in both the animal and the human experiments listed above has been heat treated in some way prior to consumption.)

As part of the evidence against animal protein being a factor in the etiology of atherosclerosis, it has often been said that the meat-eating Masai, an East African tribe, do not develop atherosclerosis. An investigation of the available published work on this point[24 25] produced no evidence showing that this tribe did not in fact suffer

from this disease. Thus the average age of the Masai examined for blood pressure by Orr and Gilks[24] was only 28, and no evidence was brought concerning the incidence of cardiovascular disease among the older age groups. At that time, 1930-31, the diet of the young warrior consisted solely of cooked meat, fresh, unheated milk and fresh blood, whilst the older men, who still maintained a relatively high animal protein diet, also consumed moderate quantities of cereals and fruit.

Although there appears to be some doubt as to whether the Alaskan Eskimo,[28 29] like the Navajo Indian,[30] is or is not relatively immune to atherosclerosis, the work of Rabinowich[31] leaves no doubt about the incidence of this disease amongst the Eskimos on the Eastern Canadian seaboard. Using radiography to detect arterial calcification, the Eskimo, average age 44.4 and domiciled in the more civilized areas of Hudson Bay and Hudson Straits, was found to have an incidence of 48.7 per cent, whilst the Eskimo, average age 50 years and domiciled in the more northerly Baffin and Ellesmere Islands, had only 16.6 per cent. The incidence of cardiovascular disease, as manifested by thickened and tortuous radial and temporal arteries, arterial retinopathy, albuminuria and hemiplegia seemed to diminish as the investigator proceeded North. As one of the principal differences between the northern uncivilized and the civilized southern Eskimo might well be the fact that the latter consumed more cooked meat than the former, who, as is well known, eats his meat mostly in the raw state,[32] a possible parallel to the findings of this survey again comes to light. Moreover protection may also have been afforded in some areas by their well known high consumption of marine oils.

Vegetable Protein

There is an increasing amount of evidence[12 14 39-41 70 71] showing that vegetable protein, in the form of wheat gluten and soya flour, possesses a *powerful protective action in cholesterol fed animals*; it therefore seems reasonable to postulate that a *relative* deficiency of such protein may be a contributory factor in the etiology of atherosclerosis. Moreover, as already mentioned, these findings have been confirmed recently, to some extent, in man.[26 35 36] It may thus be something more than a coincidence that figure 6 reveals a fairly good *negative* correlation between the daily intake of vegetable protein and the B26 mortality for the various countries listed, a finding which confirms that of Yerushalmy and Hilleboe.[6] It may also be significant that, of the 14 countries examined *no less than nine have experienced a fall in their consumption of vegetable protein* over the periods 1934-39 and 1950-54; the U.S.A. experienced the highest drop in consumption, i.e., 24.8 per cent, whilst none of

them, with the possible exception of Denmark, (+16.7 per cent), showed a significant increase.

Discussion

In a previous report,[1] it was found that vitamin B_{12}, after a lag period varying up to one month, appeared to make the symptoms and signs of patients suffering from obvious atherosclerosis much worse; angina pectoris, and/or intermittent claudication and/or "rest" pain became prominent features. It was this lag period which allowed the possibility that these findings might be coincidence. Now however, this gap seems to be closing fast and the following points may well help to complete the closure.

Since this report[1] was first published, all patients who suffered from or were suspected to be suffering from atherosclerosis have been advised to maintain a diet low in animal and high in vegetable protein, in short mainly a lacto-vegetarian diet, about one pint of unheated milk being allowed for all purposes. They were also advised to maintain a high intake of vitamin C in the form of either fruit juices or in tablet form, 25-50mg. thrice daily. After this regime had been maintained for a period varying from 6-8 weeks, depending on the severity and on the progress of the case, small helpings of animal protein of low vitamin B_{12} content were permitted, e.g., bacon, ham, white fish, veal and an occasional egg; the necessity to maintain a high intake of vegetable protein, e.g., pulses and cereals, was again stressed. As a result, not only has coronary thrombosis become a comparative rarity—only one case, proved by electrocardiogram, has occurred during the last two years—a menopausal patient, who, through a misunderstanding, had been consuming considerable quantitites of chicken—but all patients who have maintained the above regime have either improved considerably, or, if their symptoms and signs have been long-standing, have shown no sign of deteriorating. It is emphasized that, if the patient showed any sign of suffering from any other additional pathological condition(s), e.g., myxoedema, thyrotoxicosis, vitamin deficiency, etc., such condition(s) were also corrected as far as possible.

Vitamin B_{12} Metabolism

That vitamin B_{12} can and does reach the tissues in amounts sufficient to be a factor in the etiology of atherosclerosis, seems possible when one remembers (*a*) that heat treatment of animal protein at the temperatures normally used in cooking and pasteurization, frees the vitamin from its bound state,[45] and (*b*) the serum concentration of vitamin B_{12} has been found to vary from as low as 86 to as high as 900mμg per ml. in the "normal" person,[46] [47]

whilst the daily human output of vitamin B_{12} via the bowel seems to average as much as 5.μg[48] Moreover, isotope investigation has shown that the intestinal absorption of vitamin B_{12} varies considerably—from 40—109 per cent—in different persons.[49 52] It may also be significant that the ability of both liver[53] and of normal serum[50] to bind vitamin B_{12} has been found to be somewhat limited.

(*c*) The urinary excretion rate of vitamin B_{12} appears to diminish with the advancing age of the patient,[54] whilst the serum cholesterol level, which vitamin B_{12} seems to control,[55 56] increases concurrently.[57]

(*d*) Both vitamin B_{12}[67] and animal protein[65 66] (not devitaminized) have been found to increase membrane permeability.

(*e*) Both reduced glutathione[58 59] which vitamin B_{12} appears to maintain catalytically in the reduced state, and vitamin B_{12} block the action of the glucocortiocoids in both the animal [61] and the human,[62] thereby possibly permitting the opposing action of the mineralocorticoids. (In this connection, it may well be relevant that nitro-glycerine and erythrotetranitrate appear to oxidize reduced glutathione both *in vitro* and *in vivo*.[63]

Summary

An epidemiological survey covering 14 countries, and an examination of various food trends have both produced strong evidence suggesting that (*a*) an increased intake of heat treated animal protein and (*b*), in some countries, a decreased intake of vegetable protein, may both have played an important part in causing the present increase in the incidence of atherosclerosis.

Supporting evidence, obtained from published work, is also related.

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REFERENCES

1. Annand, J. C., *Lancet*, 1957, **1**, 789.
2. Bronte-Stewart, B., *Brit. med. Bull.*, 1958, **14**, 243.
3. Poole, J. C. F., *Ibid.*, 1958, **14**, 253.
4. Yudkin, J., *Lancet*, 1957, **2**, 155.
5. Hilleboe, H. E., *J. chron. Dis.*, 1957, **6**, 210.
6. Yerushalmy, J., Hilleboe, H. E., *N.Y. St. J. Med.*, 1957, **57**, 2343.
7. Keys, A. J., *J. Mt. Sinai Hosp.*, 1953, **20**, 134.
8. Roine, P., Pekkarinem, M., Karvonem, M. J., Kihlberg, J., *Lancet*, 1958, **2**, 173.
9. Votila, V., Raekallio, J., Ehrnrooth, W., *Lancet*, 1958, **2**, 171.
10. Knack, A. V., *Virchows Arch. path. Anat.*, 1915, **220**, 36.
11. Newburgh, L. H., Clarkson, S., *Arch. intern. Med.*, 1923, **31**, 653.
12. Freyberg, R. H., *Ibid.*, 1937, **59**, 660.
14. Meeker, D. H., Kesten, H. D., *Arch. Path.* (*Chicago*), 1941, **31**, 147.
15. Jones, R. J., Huffman, S., *Proc. Soc. exp. Biol.* (*N.Y.*), 1956, **93**, 519.

16. Portman, O. W., Hegsted, D. M., Stare, F. J., Bruno, D., Murphy, R., Sinisterra, L., *J. exp. Med.*, 1957, **104**, 817.
17. Tolstoi, E. J., *J. biol. Chem.*, 1927, **83**, 753.
18. Maun, G. V., Nicol, B. M., Stare, F. J., *Brit. med. J.*, 1955, **2**, 1008.
19. Bronte-Stewart, B., Antonis, A., Eales, L., Brock, J. F., *Lancet*, 1956, **1**, 521.
20. Hardinge, M. G., Stare, F. J., *J. clin. Nutr*, 1954, **2**, 83.
21. Donath, W. F., Fischer, I. A., van der Meulen, van Eysbergen, H. C., de Wijn, J. F., *Voeding*, 1953, **14**, 153.
22. Groen, J., Tjiong, B. K., Kamminga, C. E., Willebrands, A. F., *Ibid.*, 1952, **13**, 556.
23. Nath, H. P., Gupta, N. K., Iyer, P. V. K., *Indian J. med. Res.*, 1957, **45**, 217.
24. Orr, J. B., Gilks, J. L., *Spec. Rep. Ser. med. Res. Coun.* (*Lond.*), 1931, 155.
25. Thomson, J., *Through Masai Land*, Low, Marston, Searle & Rivington, London, 1885.
26. Olson, R. E., Vester, J. W., Gursey, D., Longman, D., *J. clin. Invest.*, 1957, **36**, 917.
27. Mayer, J., Jones, A. K., *Amer. J. Physiol.*, 1953, **175**, 339.
28. Rodahl, K., *Hawaii med. J.*, 1956, **16**, 131.
29. Scott, E. M., Griffith, I. V., Hoskings, D. D., Whaley, R. D., *Lancet*, 1958, **2**, 667.
30. Page, I. H., Lewis, A. L., *Circulation*, 1956, **13**, 675.
31. Rabinowitch, I. M., *Canad. med. Ass. J.*, 1936, **34**, 487.
32. Thomas, W. A., *J. Amer. med. Ass.*, 1927, **88**, 1559.
33. Peterson, D. W., Nicols, C. W., Jr., Scheour, E. A., *J. Nutr.*, 1952, **47**, 57.
34. Pollak, O. J., *Circulation*, 1953, **7**, 696.
35. Pollak, O. J., *Ibid.*, 1953, **7**, 702.
36. Bloem, T. F., van Handel, E., Neumann, H., *Bull. Schweiz. Akad. med. Wiss.*, 1957, **13**, 348.
39. Nikkila, E. A., Ollila, O., *Acta. path. microbiol. scand.*, 1957, **40**, 177.
40. Nishida, T., Takenaka, F., Kummerow, F. A., *Circulat. Res.*, 1958, **6**, 194.
41. Kokatnur, M., Rand, N. T., Kummerow, F. A., Scott, H. M., *J. Nutr.*, 1958, **64**, 177.
42. Duguid, J. B., *Lancet*, 1954, **1**, 891.
43. Keys, A. J., Anderson, J. T., Mickelson, O., Adelsen, S. F., Fidanza, F., *J. Nutr.*, 1956, **59**, 39.
44. Kelly, F. C., Snedden, W. W., Prevalence and Geographical Distribution of Endemic Goitre, *Bull. Wld. Hlth. Org.*, 1958, **18**, 5.
45. Ross, G. I. M., *Nature*, 1950, **166**, 270.
46. Pitney, W. R., Beard, M. F., *J. clin. Nutr.*, 1954, **2**, 89.
47. Mollin, D. L., Ross, G. I. M., *J. clin. Path.*, 1952, **5**, 129.
48. Girdwood, R. H., *Blood*, 1950, **5**, 1009.
49. Chow, B. F., Okuda, K., *Fed. Proc.*, 1955, **14**, 430.
50. Pitney, W. R., Beard, M. F., van Loom, E. J., *J. biol. Chem.*, 1954, **207**, 143.
51. Miller, O. N., *Arch. Biochem.*, 1957, **68**, 255.
52. Glass, G. B. J., Laughton, R. W., *Proc. Soc. exp. Biol.* (*N.Y.*), 1957, **95**, 325.
53. Pitney, W. R., Beard, M. F., van Loon, E. J., *J. biol. Chem.*, 1955, **212**, 110.
54. Watkin, D. M., Lang, C. A., Chow, B. F., Shock, N. W., *J. Nutr.*, 1953, **50**, 341.
55. Ling, C. T., Chow, B. F., *J. biol. Chem.*, 1954, **206**, 797.
56. Register, U. D., *Ibid.*, 1954, **206**, 705.
57. Keys, A., Mickelson, O., Miller, E. V. O., Hayes, E. R., Todd, R. L., *J. clin. Invest.*, 1950, **29**, 1347.
58. Cornforth, J. W., Long, D. A., *Lancet*, 1952, **1**, 950.
59. Anderson, G. E., Wiesel, L. L., Hillman, R. W., Stumpe, W. M., *Proc. Soc. exp. Biol.* (*N.Y.*), 1951, **76**, 829.
60. Dubnoff, J. W., *Fed. Proc.*, 1951, **10**, 178.
61. Venkataraman, P. R., Dublin, A., Fiedell, M. T., *Metabolism*, 1954, **3**, 502.
62. Lovgren, O., Norman, A., Winqvist, G., *Acta rheum. scand.*, 1955, **1**, 106.
63. Heppel, L. A., Hilmoe, R. J., *J. biol. Chem.*, 1950, **183**, 129.

64. Oliver, M. F., Boyd, G. S., *Lancet*, 1956, **2**, 1273.
65. Wilhelmj, C. M., Gunderson, D. E., Shaput, D., McCarthy, H. H., *J. Lab., clin. Med.*, 1955, **45**, 516.
66. Wilhelmj, C. M., Racher, E. M., Milani, D. P., McCarthy, H. H., *Idem.* 1954, **43**, 888.
67. Annand, J. C., *Practitioner*, 1955, **175**, 725.
68. Chakravarti, R. N., De, U. N., Mukerji, B., *Indian J. med. Res.*, 1957, **45**, 315.
69. *The Working-class Household Diet*, 1940-49, H.M.S.O., 1951.
70. Nath, N., Harper, A. E., Elvehjem, C. A., *Arch. Biochem.*, 1958, **77**, 234.
71. Moyer, A. W., Kritchevsky, D., Logan, J. B., Cox, H. R., *Proc. Soc. exp. Biol. (N.Y.)*, 1956, **92**, 736.
72. Larsen, N. P., *Arch. intern. Med.*, 1957, **100**, 436.
73. Mann, G. V., *Ibid.*, 1957, **100**, 77.
74. Perrault, M., Gautier, J.—CC., *Progr. Méd. (Paris)*, 1958, **11**, 211.
75. Tomarelli, R. M., Linden, E., Bernhart, F. W., *Pediatrics*, 1952, **9**, 88.

APPENDIX 1

VITAMIN B12 CONTENT OF ANIMAL PROTEIN (μg per 100g.)

The values expressed were obtained by bacterial assay and are the averages of the results found by different workers; they gave wide variations, perhaps due to different species and/or to different seasons.

Beef	1.4	Salmon	2.89
Beef kidney	10.5	Salmon (canned)	2.07
Beef liver	49.0	Mackerel	4.85
Beef heart	2.4	Tuna	1.48
Beef tongue	1.9	Cusk	0.30
Horse meat (canned)	1.8	Crab	0.47
Lamb	2.9	Lobster	0.47
Pork	0.7	Shrimp	0.91
Veal	0.5	Clam	62.30
Cod	0.45	Oyster	14.60
Haddock	0.53	Smelts	3.44
Haddock roe	5.10	Poultry	3.2??
Halibut	0.71	Egg yolk	0.918
Herring meal	0.66	Egg white	0.0
Pilchard meal	0.56		

Milk

Whole milk	1.6—6.6 ug per litre
National dried milk	2.23 μg per 100g.
Sweetened condensed milk	0.46 μg per 100g.
Commercial evaporated milk	0.14 μg per 100g.

Cheese

Natural cheddar	1.03 μg per 100g.
Processed cheddar	0.80 μg per 100g.
Natural swiss	2.08 μg per 100g.
Processed swiss	1.22 μg per 100g.
Cottage cheese	0.88 μg per 100g.
Processed cheddar cheese food	0.65 μg per 100g.