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THE NEWER KNOWLEDGE OF ATHEROSCLEROSIS*

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In North America and northern Europe the degenerative arterial disease atherosclerosis has become a major cause of death—indeed, possibly the leading cause of death. There may be some consolation in the fact that the increasing death rate from this disease is partly a reflection of our increasing average life span, but far from reassuring is the high incidence of death and disability due to atherosclerosis among men in the prime of life. For this is not just a disease of old age. Coronary artery disease (about 95% of which is due to atherosclerosis) reaches its peak incidence as a cause of sudden death in men aged 50 to 55 (Rabson and Helpert, 1948), and, as is well known, death and disability from coronary disease in men below 50 are by no means rare.

An attempt will be made to sketch here the main outlines of our present knowledge of atherosclerosis, but several intriguing problems will have to be omitted or merely mentioned without elaboration.

Terminology and Pathology

The term "arteriosclerosis" is unsatisfactory because it is used in various senses. To some it is synonymous with atherosclerosis; others use it to mean degenerative vascular disease in general. Occasionally, certain inflammatory arterial diseases are included under this heading. As the term arteriosclerosis has been applied to various diseases which differ pathologically and aetiologically, it is probably best, in the interests of clarity, to avoid it altogether and use a more specific terminology (Boyd, 1950).

Medial sclerosis involves mainly medium-sized arteries. Degeneration and calcification occur primarily in the medial coat of the vessel. Except as a rare cause of death in infants, this disease runs a benign and symptomless course.

Arteriolar sclerosis is a diffuse hyperplastic disease of the arterioles and is usually associated with hypertension.

Atherosclerosis affects mainly the large and small arteries. It predisposes to occlusion—particularly of the coronary, cerebral, and lower-limb vessels—and to haemorrhage, particularly from the cerebral vessels. The disease is characterized by a patchy and irregular deposition of lipids in the arterial intima, by intimal proliferation, and later by intimal and medial degeneration and calcification.

Incidence and Mortality

How common is atherosclerosis? Unfortunately, during life the presence of the disease cannot be detected until lesions are well advanced and some clinical complication occurs. Even then the diagnosis of atherosclerosis is only presumptive. Palpable peripheral arteries indicate medial, not intimal, sclerosis. Calcification demonstrable by radiographs may indicate advanced atherosclerosis or only primary medial disease. The occurrence of myocardial infarction almost always indicates severe coronary atherosclerosis, but, because of the patchy and irregular distribution of the lesions, the full extent of the disease can be determined only at necropsy.

Fig. 1 shows the incidence of severe coronary atherosclerosis found in recent studies at the Mayo Clinic on the hearts of men and women who died between 30 and 90 years of age (Ackerman *et al.*, 1950; White *et al.*, 1950). The hearts of the men and women in each age group were examined consecutively regardless of the cause of death. The incidence in men reached a maximum in the 50 to 60 age group; but, beyond the age of 60, the percentage with severe lesions decreased, probably, of course, because the presence of severe coronary disease decreases the chances of survival to older ages. The incidence in women was lower than in men, but it tended to approach the male incidence beyond age 70.

How important is atherosclerosis as a cause of death? This disease is not listed as a cause of death in official statistics, but from necropsy data one can estimate that about 95% of deaths from coronary disease (Wartman and Hellerstein, 1948), about 50% of deaths from cerebrovascular disease (Aring and Merritt, 1935), and about 50% of deaths assigned to diabetes mellitus (Millard and Root, 1948) are due to it. Atherosclerosis is a rare cause of death in men and women below age 25, but the mortality rises quite steeply with age and is not inconsiderable in men under 50.

FIG. 1.—The incidence of severe coronary atherosclerosis at necropsy. Data of White *et al.* (1950) and Ackerman *et al.* (1950).

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Aetiological Factors : (1) Time

What is the cause of atherosclerosis, and what factors influence its development? Although we have no definite answers to these questions there is a great deal of information available, and it is not accurate to state, as many have, that virtually nothing is known.

It is now generally agreed that atherosclerosis is not a natural or inevitable concomitant of ageing; it is a pathological process. It occurs in children with familial hypercholesterolaemia (Cook *et al.*, 1947) or with the hypercholesterolaemia associated with the nephrotic syndrome (Block *et al.*, 1948), and sudden death associated with severe coronary atherosclerosis, demonstrated at necropsy, has occurred in children as young as 11 years of age (Cook *et al.*, 1947). On the other hand, persons dying at advanced ages may have minimal or no atherosclerotic lesions (Allbutt, 1915). The incidence of the disease increases with age probably because the development of lesions is usually slow, and because hypercholesterolaemia (Keys *et al.*, 1950), obesity (Brozek and Keys, 1950) and hypertension (Robinson and Brucer, 1939) become more prominent with increasing age.

(2) Hypercholesterolaemia

There are seven main lines of evidence suggesting that the plasma cholesterol is implicated in the development of atherosclerosis.

In the first place, the lesions have a high cholesterol content (Hirsch and Weinhouse, 1943).

Secondly, atherosclerosis has a high incidence and severity in a variety of diseases associated with prolonged hypercholesterolaemia: myxoedema (Bartels and Bell, 1939), diabetes mellitus (Millard and Root, 1948), the nephrotic syndrome (A. Steiner and Domanski, 1942), and familial hypercholesterolaemia (Bloom *et al.*, 1942).

Thirdly, the average concentration of cholesterol, particularly of esterified cholesterol, in the sera of men who have experienced myocardial infarction is significantly higher than in the sera of men in the same age group without clinical evidence of coronary disease (Gertler *et al.*, 1950).

Fourthly, as illustrated in Fig. 2, the average serum-cholesterol level of men increases with age and passes through a maximum at about 55 years. It will be remembered that this is approximately the age of maximum incidence of severe coronary atherosclerosis in males at necropsy and of the maximum relative incidence of coronary disease as a cause of death in males. Fig. 2 is based, in part, on the results of a recent investigation by Keys *et al.* (1950) in Minnesota. Reviewing the literature, they have found a similar trend in data from other parts of the United States and from Europe.

Gram and Leverton (1949) have reported that the average serum-cholesterol level of women increases with age. From their data and from the data of Keys *et al.* (1950), it would appear that the average levels are similar in men and women up to about age 40, but thereafter the average serum-cholesterol level in women is less than in men until about

age 60. The highest average level was found in women 60 to 70 years of age—the oldest group examined by Gram and Leverton. The similarity of this trend to that of the incidence of coronary atherosclerosis in women is apparent, but the data for women are much less extensive than for men and must be interpreted with caution. The higher incidence of severe coronary atherosclerosis in men is unexplained. Dock (1950) believes that the greater depth of the coronary intima in males is a factor.

A fifth piece of evidence is that persons living on low-cholesterol diets, such as the Chinese, Okinawans, and Europeans subjected to food rationing, show, or did show, a low incidence of the disease (Sachs, 1949). Of course, this sort of evidence is only suggestive: dietary cholesterol is not the only variable factor involved.

P. E. Steiner (1946) examined 150 Okinawans, 18 days to 95 years of age, at necropsy; 40 were over 50 years old. Only one case of coronary sclerosis with calcification was seen; there were five cases of slight coronary sclerosis. No complications of vascular disease were seen in heart, brain, or kidneys. This low incidence of degenerative vascular disease is in striking contrast to American and European experience. Steiner reported that the diet of these people was largely vegetable in origin. Fat intake was low; meat and milk were taken infrequently. Their colons were large, long, and tortuous, similar to those of herbivorous animals.

The consumption of foods rich in cholesterol and the incidence of atherosclerotic complications both fell sharply in northern Europe during the 1939–45 war (Malmros, 1950; Strøm and Jensen, 1951). Data compiled by Malmros (1950) show a marked difference between the trends in the United States and Sweden. In Sweden the consumption of eggs and dairy products fell during the war; the consumption of eggs (the most important dietary source of cholesterol) and the death rate from "arteriosclerosis" (including diseases of the coronary arteries) both reached a minimum in 1942. In the United States, on the other hand, both the consumption of eggs and dairy products and the death rate from "arteriosclerosis" continued to increase throughout the war.

Obviously, there is no proof that the low cholesterol intake was responsible for these findings in Okinawa and Sweden. A low incidence of hypertension and especially of obesity probably played an important part.

A sixth line of evidence implicating cholesterol in atherosclerosis is the fact that typical atherosclerotic lesions have never been produced in animals without the administration of cholesterol either by mouth or by injection.

Cholesterol, a constituent of all animal cells, is synthesized in the body from relatively simple precursors (Bloch, 1950). In spite of this fact, there is no evidence that increased synthesis of cholesterol plays a part in the development of atherosclerosis in mammals. Birds differ from mammals in that the concentrations of the blood lipids, including cholesterol, are markedly elevated following the injection of oestrogens. Lipid-containing plaques may be produced in the arteries of chickens, even on a diet very low in cholesterol, if oestrogen is administered for several months (Chaikoff *et al.*, 1948; Horlick and Katz, 1948). However, these lesions differ from those produced in chickens by cholesterol feeding; they contain relatively little cholesterol in proportion to other lipids, and they exhibit relatively little intimal thickening (Chaikoff *et al.*, 1948). The lesions of so-called "spontaneous arteriosclerosis" seen in the coronary and other arteries of chickens maintained on a low-cholesterol diet contain very little cholesterol or other lipid; Paterson *et al.* (1948) believe that the coronary lesions result from primary medial disease.

Shortly after Windaus, in 1910, had demonstrated the high cholesterol content of human atherosclerotic lesions. Anitschkow showed that the disease could be produced in rabbits by cholesterol feeding. Anitschkow (1933) believed that the ingestion of cholesterol was the first and essential

FIG. 2.—The relation between age and mean serum total cholesterol level. Data of Gram and Leverton (1949) and Keys *et al.* (1950).

event in the train of events leading to the development of lesions in animals and in man. Thus began a controversy which has continued for over thirty years. Leary (1949) and Dock (1950) have repeatedly defended Anitschkow's basic concept, but most authors have remained unconvinced.

In 1935 Duff discussed this subject in an excellent critical review which has had profound influence on subsequent work. He pointed out that the disease had been produced only in herbivorous animals, which normally eat a cholesterol-free diet, and that these animals develop a more marked hypercholesterolaemia than do omnivorous or carnivorous animals when fed cholesterol. He admitted that the experimental lesions are very similar to human lesions, but he stressed the fact that they are distributed differently. In 1942 Dauber and Katz produced atherosclerosis in an omnivorous animal—the chicken; and in 1946 A. Steiner and Kendall produced the disease by cholesterol feeding in a carnivore—the dog. The lesions in chickens and dogs are distributed very much as are human lesions. The disease has now been produced by cholesterol feeding in rabbits, guinea-pigs, chickens, dogs, and hamsters (Altschul, 1950), but it has never been seen in the rat (Horlick and Havel, 1948).

In the dog, it requires about a year of cholesterol feeding to produce gross lesions, but, if thiouracil is also given (with consequent depression of thyroid activity), hypercholesterolaemia is much more marked, and lesions develop in a few months. Thiouracil alone does not produce the disease (A. Steiner *et al.*, 1949). Similarly, in rabbits fed cholesterol, thyroidectomy increases hypercholesterolaemia and atherosclerosis, while thyroid administration inhibits both (Turner, 1933).

A seventh and final piece of evidence is that the severity of atherosclerosis in experimental animals is generally proportional to the degree and duration of the hypercholesterolaemia. Discrepancies do occur, but this does not necessarily mean that hypercholesterolaemia has nothing to do with atherosclerosis; it may only indicate the operation of other independent factors. One of these modifying factors is obesity or something associated with it.

(3) Obesity

The high incidence of fatal atherosclerotic complications in the obese has been known to life insurance statisticians for a long time. Wilens (1947a, 1947b) concluded from a careful study of the findings in over 1,300 necropsies on men and women over 35 years of age that severe atherosclerosis is at least ten times as common in the obese as in the lean in each age group. Subjects of average adiposity had an intermediate incidence of severe lesions. Wilens (1947b) also found evidence that weight loss before death was associated with a resorption of lipid from the lesions in some subjects, but there was no good evidence that hyalinization or calcification was reversible.

This evidence that atherosclerosis is less severe in the lean suggested to us that variations in the degree of obesity, or some factor associated with obesity, might account in some part for the poor correlation often observed between blood-cholesterol level and severity of atherosclerosis. In order to examine this possibility, we have investigated the effect of food restriction on the development of atherosclerosis in rabbits.

Personal Observations

Some of the animals were allowed, throughout the experiment, all the Purina Rabbit Chow Checkers they would eat; others were restricted to 60, 50, or 40% of the food eaten by a freely fed animal of similar initial body weight. Each rabbit received daily by stomach-tube 1 g. of semi-purified cholesterol in aqueous suspen-

sion. Daily food consumptions and weekly body weights were recorded, and blood total cholesterol levels were determined weekly by the Schoenheimer-Sperry method. The animals were killed after four to seven weeks of cholesterol administration, and the sudan-stained aortae were graded as unknowns into five groups according to the area of intima involved by gross lesions.

The data obtained are summarized in the accompanying Table, with the animals arranged in order of increas-

Results of Experiment on Rabbits

Rabbit No.	Cholesterol Dosage (g. or Days)	Average Blood Total Cholesterol (mg./100ml.)	Initial Weight (kg.)	Weight Change (kg.)	Degree of Atherosclerosis	
					Found	Predicted
1	X ₁	X ₂	X ₃	X ₄	Y	Ŷ
2	49	139	2.20	+0.79	0	—1
3	44	141	2.36	+0.68	1	2
4	51	150	2.10	+0.11	0	1
5	45	151	3.39	—0.12	3	3
6	44	156	2.58	—0.14	0	2
7	51	224	2.15	+0.39	3	3
8	35	243	2.75	—0.15	2	2
9	44	245	2.36	—0.06	1	1
10	49	245	2.05	+0.26	4	4
11	44	278	2.49	+0.24	4	5
12	45	280	2.25	+0.17	4	5
13	45	297	2.56	+0.27	3	5
14	45	310	2.62	—0.16	2	1
15	30	313	2.39	—0.21	0	4
16	45	316	2.45	+0.78	4	4
17	44	346	2.55	+0.67	3	4
18	44	349	2.52	—0.30	1	2
19	43	356	2.74	—0.57	2	1
20	35	365	2.19	—0.11	2	4
21	45	370	3.57	—0.44	1	2
22	49	373	2.15	—0.25	4	3
23	45	379	1.98	+0.03	1	2
24	44	395	2.15	+0.03	3	3
25	43	396	2.67	—0.01	2	2
26	30	424	2.46	—0.24	4	4
27	51	424	2.10	+0.83	3	3
28	45	463	2.06	+0.10	3	3
	51	677	2.10	+0.33	4	5

ing average blood-cholesterol level. Notice their wide variation, even among animals receiving cholesterol for the same length of time. There was no significant correlation between average blood-cholesterol level and weight change or initial weight. As might be expected, weight change was closely correlated with average food consumption. The initial weights of the animals were probably roughly proportional to their ages, but unfortunately their exact ages were unknown.

In a previously published account of this work (Firstbrook, 1950a), the weight changes were expressed as per kilogram of initial weight. Since, as Tanner (1949) has pointed out, the use of per-weight ratios may lead to spurious correlation, the weight changes are here expressed directly in kilograms and subsequent calculations have been made from these values. However, this change in the manner of expressing the results makes little difference to the correlations in this case.

Upon what does the degree of atherosclerosis depend? At first glance there seems to be no relation between atherosclerosis and the other variables. Actually, the degree of atherosclerosis is positively correlated with each of the other variables, but only in the case of average blood cholesterol is there a statistically significant correlation—that is, the chances that as high a correlation should arise from random variation alone are less than 1 in 20. However, on looking at the data, one is certainly not impressed by the relation between blood-cholesterol level and atherosclerosis.

It may be, though, that the severity of lesions is influenced by all four factors independently. If this is so, one would expect atherosclerosis to increase, in

general, with increasing blood-cholesterol level, but would expect modifications because of deviations in cholesterol dosage, in initial weight, and in weight change. For example, rabbit No. 4 had more atherosclerosis than animals of similar blood-cholesterol level; one notices that its initial weight was high—the second highest in the entire series, in fact. On the other hand, rabbit No. 25 had less atherosclerosis than might be expected; one notices that it lost considerable weight compared with animals with similar blood-cholesterol values.

The linear equation which best describes the relations between atherosclerosis and the other four variables can be calculated by the method of least squares. This equation is shown in Fig. 3. According to it the degree

Estimated Atherosclerosis	Dose	Blood Cholesterol	Initial Weight	Weight Change
$\hat{Y} = -6.67 + 0.043X_1 + 0.0069X_2 + 1.92X_3 + 1.85X_4$				
	n.s.	**	**	**
1 Degree	23 g. or days	144 mg./100 ml.	0.52 kg.	0.54 kg

FIG. 3.—The linear equation of best fit for the data of the Table (n.s.=not statistically significant; **=statistically significant at the 1% level).

of atherosclerosis may increase, independently, with increasing duration of cholesterol feeding, with increasing average blood-cholesterol level, with increasing initial weight, and with increasing weight gain. The coefficients in the equation express the effect of each of the four independent variables on the degree of atherosclerosis. The coefficient for duration of cholesterol feeding is not statistically different from zero—that is, it may easily have arisen from random variation. This does not mean, of course, that increased duration of cholesterol feeding may not increase the severity of atherosclerosis; it means only that this experiment has failed to establish a significant relationship. This failure is probably due to insufficient spread in the total amounts of cholesterol administered. The other three coefficients are highly significant. The chances that they arise from random variation are, in each case, less than 1 in 100.

On the average, other things being equal, an increase in average blood-cholesterol level of about 144 mg. per 100 ml. is associated with a one-degree increase in atherosclerosis. Similarly, with other factors constant, an increase in initial weight of about 0.5 kg., or an increase in weight gain of about 0.5 kg., is also associated with a one-degree increase. It can be seen that, when all factors vary, the correlation between atherosclerosis and any one factor is likely to be poor. From a knowledge of dosage, blood cholesterol, initial weight, and weight change we can predict the degree of atherosclerosis quite accurately. Predicted values are entered in the last column of the table. There is a highly significant correlation between predicted and observed values; on the average, agreement as good as this would occur by chance alone less than once in 100 trials.

The rate at which aortic atherosclerosis develops in rabbits depends, then, not only on the degree of hypercholesterolaemia attained but also on the body weights of the animals at the beginning of cholesterol administration and on the amount of body weight gained or lost during the experiment. Other things being equal, the atherosclerotic process is inhibited in animals of relatively low initial weight, and it is inhibited in animals which lose weight or gain relatively little. This opens the way to control of the process.

Inhibition of Experimental Atherosclerosis

Duff and McMillan (1949) and, independently, McGill and Holman (1949) have reported that the development of atherosclerosis in rabbits is inhibited by the induction of alloxan diabetes prior to cholesterol administration. A rapid loss of body weight is characteristic of severe uncontrolled diabetes in the rabbit. It is possible that the inhibition of atherosclerosis is largely associated with this loss of weight rather than with a more specific effect of the diabetes. An examination of the data of Duff and McMillan lends support, I believe, to this view. In animals receiving the same amount of cholesterol for the same length of time there is a general increase in atherosclerosis with increasing average serum-cholesterol level. An animal with less than the expected severity of lesions is found, in general, to have a low initial weight or a low or negative weight gain, or both. The diabetic animals tend to have lower initial weights than the controls, and they tend to show negative or small weight gains. Duff and Payne (1950) have recently reported that treatment with insulin partially removes the inhibition of atherosclerosis seen in diabetic rabbits. This is possibly due to the fact that insulin diminishes glycosuria and prevents or lessens loss in body weight.

Several groups of workers have reported that the administration of choline mixed with the food is associated with an inhibition of atherosclerosis in rabbits. It occurred to us that this inhibition might be due to appetite depression, with consequent depression of weight gained. In an experiment in which cholesterol-fed rabbits received 1 g. of choline chloride a day in gelatin capsules instead of mixed with the food, we found the degree of atherosclerosis to be closely proportional to the degree of hypercholesterolaemia; but we found no significant difference between choline-treated and control animals in average blood-cholesterol level or in weight change, and no evidence of an inhibition of atherosclerosis (Firstbrook, 1950b). Moses and Longbaugh (1950) have reported that, although the administration of choline with the food is associated with inhibition of atherosclerosis in rabbits, it is also associated with inhibition of weight gain.

(4) "Unstable" Plasma Cholesterol

The fact that the degree of atherosclerosis is not always proportional to the degree and duration of hypercholesterolaemia has suggested to many workers that the physical state of the plasma cholesterol may be more important than its concentration.

All, or almost all, the cholesterol in plasma is bound to protein in large lipoprotein molecules. Gofman *et al.* (1950) have reported an analysis of these giant molecules in the ultracentrifuge. They have found in the sera of cholesterol-fed rabbits a type of lipoprotein molecule which is absent from the sera of normal rabbits, and the concentration of which approximately parallels the degree of atherosclerosis which develops. Similar molecules are found in many human sera. The percentage of men having detectable concentrations (5 mg. per 100 ml. or over) of these S_f 10–20 molecules increases up to about age 60, and then declines somewhat—a trend similar to that shown by the average serum-cholesterol level; over age 40, about 50% of clinically normal persons harbour these molecules. Over 90% of myocardial infarction cases exhibit these particular lipoproteins, and their incidence and average concentration are increased not only in myocardial infarction but also in cases of coronary insufficiency, diabetes mellitus, hypothyroidism, familial hypercholesterolaemia, nephrotic syndrome, and hypertension.

The effect of a low-fat, low-cholesterol diet on them is of interest. Gofman *et al.* examined the effect of diets devoid of egg yolk and allowing only skimmed milk and 100–200 g. of lean meat daily as sources of animal fat. The diets contained less than 200 mg. of cholesterol a day—the average hen's egg contains about 300 mg. of cholesterol (Okey, 1945). When the diet was strictly followed, the concentration of S_f 10–20 molecules fell in clinically normal persons and in persons with a variety of diseases. However, in common with serum-cholesterol levels (Wilkinson *et al.*, 1950; Keys, 1950), they showed no consistent relation to cholesterol intake among persons eating ordinary diets. Gofman *et al.* have found that some persons can tolerate large amounts of dietary cholesterol before these molecules appear in the serum, but, in others, the molecules decrease or disappear only when a low-cholesterol diet is strictly adhered to. Similarly, it would appear that drastic restriction of dietary cholesterol is necessary in many persons to effect a fall in the serum-cholesterol level (Keys, 1950).

It would seem to be a reasonable hypothesis that only the cholesterol present in plasma in a particular form, or forms, is involved in the pathogenesis of atherosclerosis, but it has not been satisfactorily demonstrated that the concentration of these special molecules is a more accurate measure of this "unstable" plasma cholesterol than is the total cholesterol concentration. Studies of free and esterified serum-cholesterol levels as extensive and carefully controlled as the ultracentrifuge studies of Gofman and his colleagues might produce equally striking results.

The relation of lipids other than cholesterol to hypercholesterolaemia and atherosclerosis is not clear. A high-fat diet may be expected to facilitate cholesterol absorption, but Keys (1950) has reported the aggravation of hypercholesterolaemia by feeding vegetable oil in a human subject on a diet free of cholesterol. Since the subject had familial hypercholesterolaemia, this finding may not be a general one. The administration of vegetable oil to rabbits does not produce hypercholesterolaemia or atherosclerosis, but further investigation in human subjects is desirable.

It has been suggested that a high ratio of serum phospholipid level to serum total cholesterol level is associated with inhibition of the development of atherosclerosis. This ratio is high in subjects with biliary obstruction, and, although there is marked hypercholesterolaemia, the incidence of severe atherosclerosis at necropsy is not increased (Ahrens, 1950). However, since phospholipid level is closely related to free cholesterol level (Popják, 1946), a high ratio of phospholipid to total cholesterol will occur whenever the free cholesterol is elevated to a greater extent than is the esterified cholesterol. In biliary obstruction there is a great increase in serum-free cholesterol and phospholipid concentrations, but a relatively small increase in esterified cholesterol. The low incidence of atherosclerosis in cases of biliary obstruction may be related to the low level of cholesterol esters rather than to the high phospholipid level. The fact that phospholipid plays a part in maintaining non-turbidity of serum may have no connexion with the genesis of atherosclerosis. Duff and McMillan (1949) found that inhibition of the development of atherosclerosis in diabetic rabbits was associated with increased, rather than decreased, serum turbidity.

The relation of lipid deposition to the production of degeneration in the arterial wall has long been a controversial question. Leary (1949) has presented evidence for his belief that crystalline esters of cholesterol cause a foreign-body reaction similar to that produced by silica in tissues.

(5) Hypertension

Severe atherosclerosis is about twice as common in hypertensives as in non-hypertensives of the same age, sex, and adiposity (Wilens, 1947a). This is probably partly due to mechanical effects on the arterial wall, but the average serum total cholesterol (Watkin *et al.*, 1950) and S_f 10–20 (Gofman *et al.*, 1950) levels are also higher in hypertensives. On the Kempner rice diet, which is free of cholesterol, the average serum-cholesterol level of hypertensives decreases to below the average level found in clinically normal persons on ordinary diets (Watkin *et al.*, 1950).

(6) Arterial Damage

Mechanical or toxic damage to arteries predisposes to atherosclerosis at the site of injury (Duff, 1935), but there is no evidence that arterial damage is a prerequisite to lipid deposition.

Conclusion

On the basis of present evidence, it would seem that the initiating cause of experimental atherosclerosis is the administration of cholesterol. However, because of the operation of modifying factors, not all animals receiving the same amount of cholesterol for the same length of time develop the same degree of lesions. In the first place, there is a wide variation in the degree of hypercholesterolaemia which develops. This variation depends probably on varying thyroid activity and possibly on other factors. In the second place, not all animals with the same degree of hypercholesterolaemia develop lesions at the same rate. Although, in general, the greater the degree of hypercholesterolaemia the more rapidly does atherosclerosis develop, variations in the degree of obesity, or some factor associated with obesity, can greatly modify the response. Finally, the rate of development of lesions is influenced by the arterial blood pressure and by pre-existing damage to the arterial wall.

What can be said about the aetiology and possible prevention of this disease in man?

So far as dietary cholesterol is concerned, although the *circumstantial* evidence is considerable, there is no *direct* evidence for the hypothesis that dietary cholesterol is involved in human atherosclerosis. Direct evidence for or against this hypothesis will be very difficult to obtain because of obvious difficulties in controlling a great diversity of factors, including diet, in a long-term investigation in human subjects. In the meantime, however, because of the high incidence of atherosclerosis as a cause of death and disability, it is important to consider whether dietary cholesterol should be restricted, and, if so, to what degree. It seems probable, in view of the experimental and clinical evidence, that it should be restricted, but the degree of restriction necessary to prevent the development of lesions will probably vary from person to person. Some persons, like some rabbits, can tolerate large amounts of dietary cholesterol before hypercholesterolaemia (or a detectable serum S_f 10–20 level) develops, but others possess a low tolerance. In addition, of course, there is good evidence that the serum-cholesterol (or S_f 10–20) level is not the only factor affecting the rate of development of lesions. Obviously, the question of restricting dietary cholesterol presents many difficulties.

Where myxoedema exists, its treatment would be expected to inhibit the progress of atherosclerosis; but desiccated thyroid must be administered very cautiously in the presence of coronary disease (Bartels and Bell, 1939). Conversely, the induction of hypothyroidism for the relief of angina pectoris would seem to be an unwise procedure because of probable acceleration of the underlying atherosclerotic process.

There is strong evidence from clinical, pathological, and experimental studies that an eminently desirable measure in the prevention of atherosclerosis is the prevention or correction of obesity. The virtues of a low-fat diet in this connexion are well known. A diet particularly low in animal fat has what may prove to be an additional virtue—a low-cholesterol content.

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BACTERIAL FLORA AND BACTERIAL COUNTS OF INFANTS' BOTTLE FEEDS

BY

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An account is given of the bacterial flora and bacterial counts of 300 infants' bottle feeds sampled in London during 1946-8. The feeds were obtained from children's hospitals, an infectious diseases hospital, residential nurseries, the out-patient department of a children's hospital, and an infant welfare clinic. Most of the samples were collected from the feeding-bottles immediately before the feeds were given; some, however, were obtained from bulk feeds prepared in milk kitchens. The feeds were of various composition and included reconstituted dried milk preparations, liquid cow's milk, expressed breast milk, thickened feeds, and clear fluids. The bacterial content may not have been representative of infants' feeds in general. Feed samples brought by mothers to out-patient and infant welfare clinics may have had their bacterial content increased during transport to them; on the other hand, in hospitals and nurseries, advice was often sought, and given, with regard to improved technique in feed preparation, and in two institutions at least this led to a reduction in the bacterial counts.

Sources of the Feed Samples

Children's and Infectious Diseases Hospitals.—Hospital A (66 samples) and hospital B (22 samples) are children's hospitals. Most of the samples were collected in the wards and some in the central milk kitchens. In both hospitals, feeds sufficient for 24 hours were prepared, and were then stored in a refrigerator until required. Feeding-bottles in hospital A were autoclaved and in hospital B they were boiled; in both hospitals teats were boiled between each use. Hospital C (38 samples) is an infectious diseases hospital, and the samples were collected in the gastro-enteritis unit. Feeds were freshly prepared, as required, in the ward kitchen, except that certain thickened feeds were prepared in bulk, boiled, and stored either at room temperature or in the refrigerator for periods depending on the demand. Bottles and teats were boiled between each use.

Residential Nurseries.—Nursery X (22 samples) and nursery Y (32 samples) had central milk kitchens. Nursery Z (37 samples) had a milk kitchen on each infant floor, and practice regarding feed preparation varied in detail from floor to floor and from time to