Vitamin C Deficiency, Cholesterol Metabolism and Atherosclerosis

Emil Ginter, Ph.D., D.Sc.[1]

Introduction

Despite a persistent decline in the cardiovascular mortality rate of about 2 per cent per year since 1968, nearly one million Americans will die this year from cardiovascular diseases. What is more, about 30 million people in the United States are already afflicted by atherosclerosis. There are many coronary heart disease risk factors, e.g. smoking, sedentary habits, high blood pressure and high level of cholesterol in the blood plasma. Due to the simplification of this fact, a great cholesterol myth dominates in the United States, The American population is being fooled into believing that they must stay almost entirely away from dietary cholesterol. This anti-cholesterol fad ignores two important points:

1) the influence of the dietary cholesterol on the cholesterol concentration in blood plasma is not very pronounced in the human organism

2) no matter how successfully we deal with high blood cholesterol, cardiovascular diseases will not disappear, because high cholesterol concentration in blood plasma is by no means the only causative factor of atherosclerosis.[1]

The aim of this review is to show the complexity of cholesterol metabolism in the human body and to stress the fact, that in the diet there are besides cholesterol and saturated fatty acids, many other biologically active substances which play an important role in the prevention of atherosclerosis.

Cholesterol Metabolism

Cholesterol is fatty alcohol which has a cyclic structure with a high stability. This important component of cell membranes is of vital necessity to cell growth and survival. On the other hand, an excessive presence of cholesterol in blood plasma leads to atherosclerosis. Hence, human cells are faced with a double problem. On the one hand, they must have an adequate quantity of cholesterol for the formation and renewal of cell membranes; on the other, they must control the excessive accumulation of cholesterol in blood plasma. Therefore, cholesterol metabolism is rather complex and internally controlled by a series of feedbacks. The cholesterol level in the blood and in tissues is the resultant of a whole series of mutually interconnected processes. Cholesterol reaches the organism through absorption from diet, and is also synthesized *de novo* in numerous organs from two-carbon fragments. Cholesterol is removed from the organism in two principal ways: part of it is released unchanged from the liver into the gastrointestinal tract and is excreted in the stool changed by intestinal microflora into neutral sterols. The second part is transformed in the liver into bile acids which are released with the bile into the intestine and also leave in the stool. A small quantity of cholesterol is excreted through skin and urine. Cholesterol is transformed also into steroid hormones, but this quantity is too low to be considered in the total balance. Plasma cholesterol is exchanged at various rates with cholesterol of various tissues.

Cholesterol as a substance practically insoluble in water must be transported in the blood plasma in the form of stable complexes of lipids with proteins, named lipoproteins. Lipoproteins are spherical particles the outer membrane of which consists of water-soluble substances, particularly of proteins and phospholipids. The core of lipoproteins consists of hydrophobic lipids, of cholesterol esters and triglycerides. In human blood plasma there are four main classes of lipoproteins:

1. Institute of Preventive and Clinical Medicine, UPKM, Limbova 14, 833 01 Bratislava, Czechoslovakia.

chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The quantity of cholesterol in chylomicrons and in VLDL is in healthy people rather low and these particles do not play the decisive role in the deposition of cholesterol into arterial walls. LDL, the major cholesterol carrier in human blood plasma, is a spherical particle with a diameter of 22 millionths of a millimeter. Its oily core consists of some 1,500 molecules of cholesterol esters. The LDL core is shielded from the blood plasma by a detergent coat composed of 800 molecules of phospholipid, 500 molecules of free, unesterified cholesterol and one very large protein molecule, named apoprotein B-100. When blood cholesterol is elevated, increasing the risk of atherosclerosis, the reason is almost always in increasing the number of these LDL-particles.[2]

Apoprotein B-100 of the LDL particle is recognized and bound by a glycoprotein, named LDL-receptor. The LDL-receptor carries a binding site that protrudes from the cell surface. Its action is very effective: LDL receptor can pick out a single LDL particle from more than a billion molecules of water. LDL-receptor binds only lipoproteins carrying apoprotein B-100 or related protein designated apoprotein E. In man and in most laboratory animals about 75 per cent of the receptor mediated removal of LDL particles takes place in the liver. Many other tissues also have LDL-receptors, but those of the liver, adrenal gland and ovary, i.e. the organs with particularly high requirements for cholesterol, have the highest amount of LDL-receptors.[3] LDL particle bound to the receptor is carried into the cell. Digestive enzymes break down the LDL's coat and liberate amino acids from apoprotein B. Lysosomal esterases liberate free cholesterol, the amount of which controls by feedback three processes:

1) it reduces the endogenous cholesterol synthesis by turning off the synthesis of a key enzyme of cholesterol production, 3-hydroxy 3-methylglutaryl CoA reductase (HMG CoA reductase),

2) the free cholesterol promotes the storage of cholesterol by activating acyl-CoA: cholesterol acyltransferase (ACAT). This enzyme transforms excess of free cholesterol into cholesterol esters that are deposited in the cell in the form of storage droplets,

3) the accumulation of free cholesterol within a cell drives a feedback that makes the cell stop synthesizing new LDL receptors. Cells thereby adjust the number of receptors so that enough cholesterol is brought in to meet their demands (e.g. the formation of new membranes, synthesis of hormones and bile acids) but not enough to overload them.[2 3]

Vitamin C Deficiency and Cholesterol Turnover

Our research group has shown that this excellent control mechanism is damaged by vitamin C deficiency.[4] For the research on the relationship between vitamin C and cholesterol, a model of chronic marginal vitamin C deficiency in guinea pigs proved successful. In this model guinea pigs are fed a vitamin C-free diet, provided with a small maintenance dose of ascorbic acid. Such deficient animals normally consume food, their weight curves are normal, and they seem to be quite healthy like a man suffering from subclinical vitamin C deficiency. Besides the fact that this model approaches the contemporary nutritional situation of many population groups, it also has another advantage: it enables us to carry out long-term experiments which is of great importance, especially in the study of chronic diseases, e.g. of atherosclerosis. Our longest experiments with marginally deficient guinea pigs were longer than one year which represents one third of the life span of a guinea pig. If apparently healthy guinea pigs are at the state of marginal vitamin C deficiency for three months, cholesterol accumulation in their blood and liver takes place, because vitamin C deficiency retards cholesterol transformation into bile acids. The rate of this process is graded in dependence of the vitamin C concentration in the liver: slow in vitamin C-deficient animals and maximal in guinea pigs saturated with vitamin C.[4]

Cholesterol transformation into bile acids is quantitatively the most important process by means of which the human organism removes cholesterol. This complex 14-stage process takes place gradually

in the endoplasmic reticulum, mitochondria and cytosole of the liver cell. The first reaction of this process, incorporation of a hydroxy-group into the cholesterol nucleus in the 7-alpha position is the rate-limiting reaction of the whole process of cholesterol transformation into bile acids. Vitamin C is specifically required for this key reaction, cholesterol 7-alpha hydroxylation.[4 5] (Fig. 1) The enzyme catalyzing this reaction, cholesterol 7-alpha hydroxylase, belongs to the group of enzymes, so called mixed-function oxygenases, which activate molecular oxygen by means of cytochrome P-450. One of oxygen atoms is during this reaction reduced to water, the other one is incorporated in the form of hydroxy-group into different substrates, such as cholesterol and various foreign substances. The hydroxylated substrates are more polar and therefore more water-soluble and they could be more easily excreted in bile or in urine. These, by cytochrome P-450 catalyzed reactions are the main way in which man gets rid of various foreign substrates, e.g. industrial poisons, pesticides and various drugs. Many research groups have found slowed-down degradation of both cholesterol and various xenobiotics in vitamin C-deficient laboratory animals.

Retarded cholesterol degradation results first in the cholesterol accumulation in the liver and later in the blood plasma of deficient animals. The increase in plasma cholesterol in vitamin C-deficient guinea pigs is conditioned entirely by the increase of cholesterol content in the LDL-fraction. It is probable that chronic vitamin C deficiency results in lowered degradation of LDL-particles due to lowered activity of LDL-receptors.[6] The activity of LDL-receptors could be assessed by injecting LDL labeled with radioactive iodine and measuring the decline of radioactivity in blood samples. The loss of radioactivity reflects the cellular uptake of LDL and hence indirectly the activity of LDL-receptors. We have found that the removal of radioactive LDL from the circulation was slowed-down in vitamin C-deficient animals.[6] The mean life span of an LDL particle was evidently longer in deficient guinea pigs. LDL particles transport the majority of cholesterol into the blood vessel wall and into the atherosclerotic lesions. Our experiments based both on the chemical analysis of the aorta and on the determination of ^{14}C-labeled cholesterol deposited in the aorta have shown that an evident accumulation of cholesterol takes place in the arterial walls of vitamin C-deficient guinea pigs.[7] In guinea pigs with chronic vitamin C deficiency manifest atherosclerotic lesions were observed.

Vitamin C and Cholesterolemia in Man

To provide exact evidence of the role of chronic marginal vitamin C deficiency in the development of human atherosclerosis is a difficult task indeed, for ethically, it is just unthinkable to provoke experimentally chronic vitamin C deficiency in man. However, also a reversed procedure, resa-turation of vitamin C-deficient organisms with ascorbic acid is possible. In the countries where year seasons are changing, the winter is long and the import of fresh fruits is low, a high percentage of inhabitants are frequently in the state of vitamin C deficiency. In Czechoslovakia it is possible to find vitamin C-deficient levels in blood of as much as 50 per cent of inhabitants. We have used this for the study on the relationship between vitamin C and cholesterol metabolism in man.[7] Persons with biochemically proven vitamin C deficiency were given ascorbic acid at doses of 300 to 1,000 mg per day. In cases of persons with high initial cholesterol level and low vitamin C status a distinct decrease of cholesterolemia was observed in cca 60 per cent of subjects. The most striking cholesterol-lowering effect was achieved in elderly persons and in diabetics.[7] In the long-term studies, however, we noted that in the first months of vitamin C treatment, cholesterol declined quite evidently, but after six months the cholesterol level tended to return back to higher values. Probably mechanism of this phenomenon is as follows (Fig. 2): an increased intake of ascorbic acid speeds up the hydroxylation of cholesterol, causing an increase in the bile acid pool. The increased number of bile acid molecules returning by recirculation back into the liver, affects by a negative feedback the rate of cholesterol hydroxylation, as a result of which, the stimulating action of vitamin C on cholesterol degradation cannot become so manifest. Such

negative feedback can be eliminated by substances capable of binding bile acids in the intestine.

Synergism Between Vitamin C and Pectin

Certain natural substances, components of dietary fiber, particularly pectin, possess this property. Pectin is a polymer of methoxylated D-galacturonic acid forming the walls of plant cells. Pectin is resistant to the digestive enzymes and in the intestine it forms a gel capable to bind bile acids. Due to this, pectin prevents the return of bile acids back to the liver, interrupts enterohepatic circulation and results in increased removal of bile acids in the stool. In this way pectin blocks the feedback by which bile acids coming back into the liver, inhibit the transformation of cholesterol to bile acids (the so-called end-product inhibition).[7] After experiments with various kinds of laboratory animals we have designed a preparation containing 15 grams of citrus pectin and 800 mg of ascorbic acid in a daily dose. The cholesterol-lowering effect of this preparation was tested in outpatients with different forms of hypercholesterolemia. The combination of vitamin C and pectin reduced the level of LDL-cholesterol, and at the same time, it did not change or even slightly increase the level of protective HDL-cholesterol.

The mechanism of the synergic action of vitamin C with pectin is probably as follows (Fig. 3): vitamin C stimulates in the liver 7 alpha-hydroxylation of cholesterol and thus the degradation of cholesterol into bile acids. Bile acids leaving the liver through bile into the intestine are bound here to pectin gel. Their return to the liver is retarded and bile acids as the end product of cholesterol catabolism are excreted in the stool. The advantage of such preparation based on vitamin C and pectin is that its components are natural substances which are present in vegetables and fruits. There is little probability that a combination of vitamin C with pectin, on the contrary to synthetic hypolipemic drugs, could induce adverse side effects even after long-term therapy.

Vitamin C and Lipoperoxidation

There is another important mechanism through which vitamin C prevents atherogenesis. The system of vitamin C in human blood and tissues consists of three biologically active forms:

1) reduced ascorbate;
2) monodehydroascorbate, which is a radical form of vitamin C;
3) dehydroascorbate.

This system is able to reduce peroxides, lipid hydroperoxides and free radicals, e.g. superoxide anion, hydroxyl radical and radicals of polyunsaturated fatty acids. According to definition, free radicals are atoms, ions or molecules which contain one or more unpaired electrons. They are highly reactive by taking or giving on electrons from or to other molecules. Particular feature of free radical formation is the tendency to create chain reactions. Aggressive oxidants such as hydroxyl radical can damage nucleic acids, membrane phospholipids or lipoproteins and impair their metabolism and functions. Therefore free radicals are implicated in the etiology of many diseases. The list of the "free radical diseases" is very long and besides atherosclerosis it contains cancer, essential hypertension, senile dementia, arthritis, senile cataract, Parkinson's disease, diabetes mellitus type 1 and acute pancreatitis.

Recent investigations help to clarify the role of oxygen free radicals in the development of atherosclerotic lesion. In pigtail monkeys within 12 days of the initiation of a high-fat diet, clusters of leukocytes (principally monocytes) were attached to the surface of the arterial endothelium. Many of these monocytes were often found between endothelial cells, they accumulated lipids and took on the appearance of foam cells.[8] The earliest recognized lesion in atherosclerosis is the fatty streak, characterized by an accumulation of cells loaded with cholesterol esters (foam cells). Most foam cells arise from circulating monocyte cells and contain cholesterol transported into them by low density lipoproteins.

The classic studies of Brown and Goldstein[2 3] showed the critical importance of LDL receptors in the metabolism of LDL particles. However, recent studies show that the formation of foam cells occurs through pathways independent of LDL receptors.[1] LDL particles must be modified to be atherogenic and oxygen radicals play

an important role in such modification. When LDL is incubated with cells, the LDL particles undergo structural changes that dramatically alter their metabolism. These changes depend upon a common initiating step, the peroxidation of poly-unsaturated fatty acids in the detergent coat composed of phospholipids and in the oxidative fragmentation of apoprotein B-100. Oxidatively modified LDL particles are not recognized by LDL-receptors in the liver, but they are recognized by another type of receptors in macrophages. It was shown that modified form of LDL was taken up by macrophages 10 times more rapidly than native LDL. This type of receptors is not controlled by a feedback and therefore macrophages become overloaded with cholesterol and change themselves into foam cells. An attractive hypothesis was proposed concerning the development of the fatty streaks that represent the first step in atherogenesis.[9] This hypothesis includes four atherogenic effects of oxidatively changed LDL particles:

1) chemotactic factor present in oxidized LDL attracts circulating monocytes from the blood into the vessel wall;

2) oxidized LDL inhibit the motility of resident macrophages and therefore their ability to leave the intima;

3) enhanced uptake of oxidized LDL by resident macrophages, leading to the generation of foam cells;

4) cytotoxic effects of oxidized LDL, leading to the loss of the integrity of endothelial cells.

The loss of the control upon propagation of oxygen free radicals and lipid peroxidation may be the key process involved in the initiation of atherosclerosis. This clearly implicates lipid peroxidation as a probable cause of both ischemic heart disease and stroke. It was shown in many laboratories that oxidative modification of LDL particles is prevented by antioxidant vitamins and related substances.[10] The combination of vitamin C with tocopherols (vitamin E) is most effective. The cardiovascular death rate of persons supplemented with relatively large amounts of vitamin C and vitamin E was significantly below the expected value; this was also true for cancer.

Epidemiological Research

In various epidemiological studies evidence is being accumulated that high intake of antioxidant vitamins and related substances (vitamin C, tocopherols, beta-carotene, bioflavonoids, selenium and some further microelements) and of dietary fibre is effective in the prevention of hypercholesterolemia and cardiovascular disease.[11 12] The main source of vitamin C, pectin and of some further antioxidants are vegetables and fruits. If the conception of synergism between vitamin C and pectin is right, the population groups with high consumption of vegetables and fruits should have lower levels of plasma LDL-cholesterol and lower incidence of cardio-vascular diseases. It has been proved repeatedly that the vegetarians have lower levels of blood cholesterol. The reduction of total cholesterol levels is conditioned in the vegetarians particularly by a sharp decrease of atherogenic LDL-fraction to almost half of the values found in the persons of the same age consuming the common diet. The level of protective HDL-cholesterol was influenced only little. In vegetarians, also distinctly lower values of serum triglycerides were found. It is probable that one of the reasons for low cholesterol levels in vegetarians is high intake of vitamin C. In Czechoslovakia we have studied 600 blood donors. The occurence of higher levels of serum lipids was often associated with low levels of vitamin C in white blood cells. After dividing this set of healthy people into subgroups with low, medium and high vitamin C levels, graded levels of serum lipids were observed. Persons with the lowest vitamin C-status had the highes cholesterol and triglyceride levels, and vice versa; the persons best supplied with vitamin C had the lowest lipid concentrations.[13] A long-term research was performed on a group of Seventh-Day Adven-tists, the members of which were vegetarians. Mortality due to myocardial infarc-tion in this group was considerably lower than the mortality of average California population. In the younger age group of male adventists it reached only one quarter and in older age groups one half of the coronary mortality of the whole California population.[14] Similar results were published

in Great Britain and in Germany. Surprisingly close indirect correlation between the consumption of fresh vegetables and coronary mortality has been observed in Great Britain.[15] In the northern regions, particularly in Scotland, the consumption of vegetables is low and the coronary mortality is the highest. In the southern regions the consumption of fresh vegetables is significantly higher and coronary mortality is the lowest here. The above mentioned relationship was observed in both the subgroups of women and men. In the United States more than 20 years ago the threatening increase of coronary mortality was stopped and since about 1968 coronary mortality is continuously decreasing. There are several factors responsible for this phenomenon, e.g. better clinical care, better control of hypertension and of hypercholesterolemia, programmes of physical activity and lowered consumption of cigarettes and animal fats. At the same time, consumption of vegetables and fruits has been increased, and production and consumption of synthetic ascorbic acid raised, too. Similarly, the fall in cerebrovascular mortality observed in the last 30 years in Great Britain was well correlated with increased consumption of fruits and vegetables.

Consumption of fruits is very low in some Middle-European countries. Fig. 4 based on the World Health Organization data illustrates an alarming trend in the death rate from cardiovascular diseases and cancer in men during the last 20 years in Czechoslovakia, Hungary and Poland. This phenomenon has evidently a political aspect because we do not see any such trend in the neighbouring democratic countries (Austria, West Germany and Switzerland). It can be assumed that the negative mortality trend was influenced by psychological factors (e.g. depression and frustration caused by the long-lasting dictatorship) and by extremely high environmental pollution. Another negative factor is the high consumption of animal fat, sugar, alcohol and salt and on the other hand the low consumption of fruits, vegetables and fish. The food in Czechoslovakia, Hungary and Poland is rich in calories but it contains too little vitamin C and other protective antioxidants.[16] These countries with chronically low vitamin C intake could serve as an ideal object for a long-term interventional trial that might answer the question if the optimal intake of vitamin C and other protective antioxidants (tocopherols, carotenes, bioflavo-noides, selene, etc.) could significantly lower the incidence of cardiovascular diseases.

References

1. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL: Beyond cholesterol. Modification of low-density lipoprotein that increase its atherogencity. *N. Engl. J. Med.* 320: 915-924, 1989.
2. Brown MS, Goldsten JL: How LDL receptors influence cholesterol and atherosclerosis. *Scientific American* 251: 58-66, 1984.
3. Brown MS, Goldstein JL: A receptor-mediated pathway for cholesterol homeostasis. *Science* 232: 34-47, 1986.
4. Ginter E: Ascorbic acid in cholesterol and bile acid metabolism. *Ann. N.Y. Acad. Sci.* 258: 410-421, 1975.
5. Holloway DE, Rivers JM: Influence of chronic ascorbic acid deficiency and excessive ascorbic acid intake on bile acid metabolism and bile composition in the guinea pig. /. *Nutr.* 111:412-424, 1981.
6. Ginter E, Jurcovicova M: Chronic vitamin C deficiency lowers fractional catabolic rate of low-density lipoproteins in guinea pigs. *Ann. N.Y. Acad. Sci.* 498: 473-475, 1987.
7. Ginter E, Bobek P, Kubec F, Vozar J, Urbanova D: Vitamin C in the control of hypercholesterolemia in man. *Intern. J. Vit. Nutr. Res., Suppl.* 23: 137-152, 1982.
8. Ross R: The pathogenesis of atherosclerosis — an update. *N. Engl. J. Med.* 314: 488-500, 1986.
9. Quinn MT, Parthasaraty S, Fong LG, Steinberg D: Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc. Natl. Acad. Sci. USA* 84: 2995-2998, 1987.
10. Esterbauer H, Dieber-Rotheneder M, Striegl G, Waeg G: Role of vitamin E in preventing the oxidation of low-density lipoprotein. *Am. J. Clin. Nutr.* 53: 314S-321S, 1991.
11. Trout DL: Vitamin C and cardiovascular risk factors. *Am. J. Clin. Nutr.* 53: 322S-325S, 1991.
12. Gey KF, Puska P, Jordan P, Moser UK: Inverse correlation between plasma vitamin E and mortality from ischemic heart disease in cross-cultural epidemiology. *Am. J. Clin. Nutr.* 53: 326S -334S, 1991.
13. Cerna O, Ginter E: Blood lipids and Vitamin C

status. *Lancet* 1: 1055-1056, 1978.
14. Phillips RL, Lemon FR, Beeson WL, Kuzma JW: Coronary heart disease mortality among Seventh-Day Adventists with differing dietary habits: a preliminary report. *Am. J. Clin. Nutr.* 31: 191S-198S, 1978.
15. Armstrong BK, Mann JI, Adelstein AM, Eskin F:Commodity consumption and is ischemic heart disease mortality with special reference to dietary practices. /. *Chron. Dis.* 28: 455-469, 1975.
16. Ginter E: Ascorbic acid in cholesterol metabolism and in detoxification of xenobiotic substances: problem of optimum vitamin C intake. *Nutrition* 5: 369-374, 1989.

Figure 1.
Interference of chronic marginal vitamin C deficiency with reactions transforming cholesterol to bile acids in the liver cell.
(1) cholesterol
(2) 5-cholestene-3beta, 7alpha-diol (7 alpha-hydroxycholesterol)
(3) 5beta-cholestane-3alpha, 7alpha-diol
(4) chenodeoxycholic acid
The process of cholesterol transformation into bile acids is intentionally simplified in the diagram.

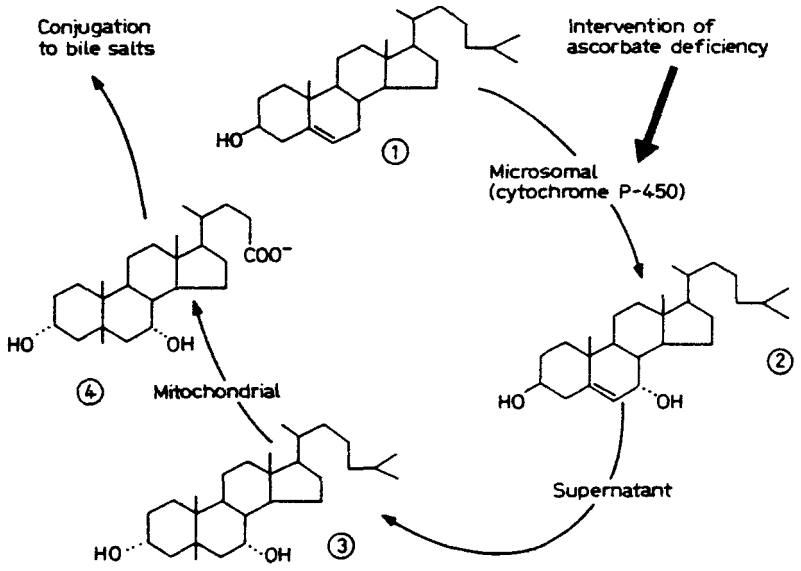

Figure 2.
Schematic presentation of the feedback control of bile acid synthesis.

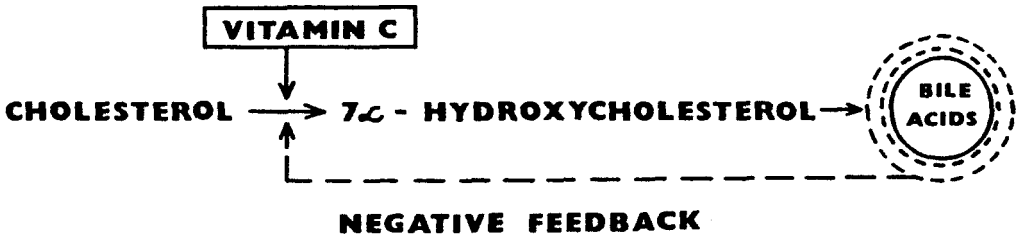

Figure 3.
Presumed mechanism of synergic effect between ascorbic acid and pectin in the control of blood plasma cholesterol concentration.

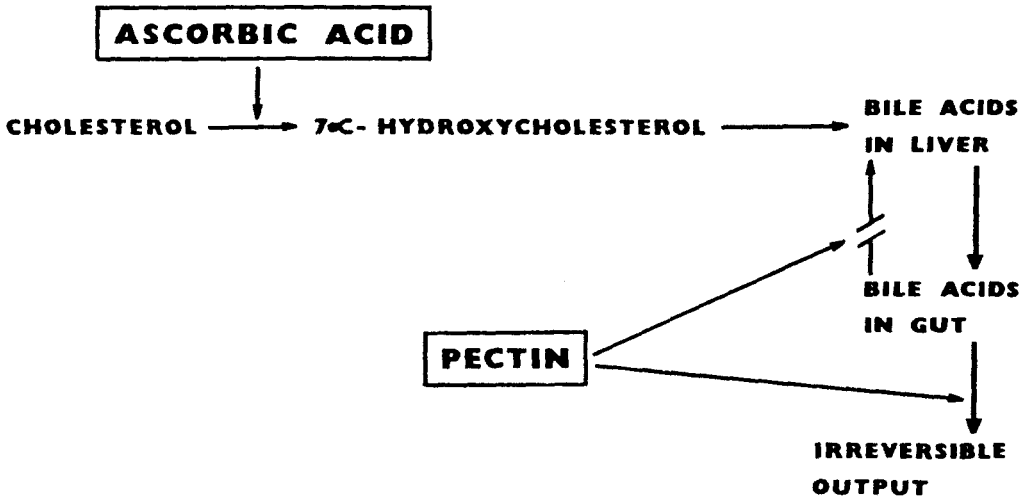

Figure 4.
Trends in premature, age specific mortality rates from cardiovascular diseases and cancer in men *in* Middle Europe.