

# Marginal Vitamin C Deficiency, Lipid Metabolism, and Atherogenesis

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## I. Introduction

Vitamin C (ascorbic acid, ascorbate) holds a special position among the vitamins, since most vertebrates synthesize it in the glucuronic acid pathway of glucose metabolism and are therefore not dependent on a supply from external sources. In amphibians, reptiles, and phylogenetically older species of birds, ascorbate is synthesized in the kidneys, while in developmentally higher birds and mammals, vitamin C is synthesized in the liver (Chatterjee, 1973). For reasons which are still a mystery, some birds and mammals (guinea pigs, bats, monkeys, man) have lost the

ability to synthesize ascorbic acid. The liver microsomes of these species lack the enzyme of the last stage of ascorbate biosynthesis, gulonolactone oxidase (Burns, 1957). This is assumed to be a specific genetic limitation, mutation of the operon responsible for synthesis of this enzyme. For most of these species, this genetic disturbance is not a real danger, as they are herbivores inhabiting tropical or subtropical regions with an abundant supply of vitamin C the whole year round. The species most seriously affected by this mutation is *Homo sapiens*, who inhabits the whole of the globe, including regions where the supply of food rich in vitamin C is limited for part of the year. Although advances in agriculture, transport and storage techniques have largely abolished the danger of scurvy (acute vitamin C deficiency), there are probably still, even now, in the second half of the 20th century, millions of people who suffer from marginal vitamin C deficiency for at least part of the year. The aim of this review is to sum up data on the effect of vitamin C deficiency on the metabolism of cholesterol, triglycerides, and various components of the blood vessel wall and to draw attention to the fact that latent vitamin C deficiency (hypovitaminosis C) must be regarded as a risk factor in association with atherosclerosis.

## II. Disorders of Lipid Metabolism in Acute Scurvy

Practically all studies of the effect of acute scurvy on lipid metabolism have been carried out with guinea pigs and few with monkeys. The guinea pig model of acute scurvy is attractive because of its simplicity, since guinea pig is extremely sensitive to alimentary vitamin C deficiency. The biological half time of ascorbic acid in the guinea pig is substantially shorter than in man, being in the region of 4 days (Burns *et al.*, 1951; Ginter *et al.*, 1971b). If a guinea pig is put on a vitamin C-free diet, distinct signs of deficiency (lack of appetite, a drop in body weight) are observed within 3 weeks and the animal dies in 4 weeks with typical signs of scurvy. This seemingly convenient "express" model of avitaminosis C makes it very hard to interpret the results, however, since acute vitamin C deficiency is a dynamic process, in which the character of the metabolic disorders alters with the development of scurvy. The administration of a vitamin C-free diet to guinea pigs is immediately followed by a drop in tissue ascorbate levels and as avitaminosis develops they steadily fall still further (Fig. 1). As a result, the individual phases of acute avitaminosis often differ completely in respect of even such basal parameters as nitrogen balance, for example (Ginter, 1970b). The terminal phase of acute avitaminosis C is an immensely complicated pathological state, very hard to define metabolically, in which, alongside vitamin C deficiency, a decisive

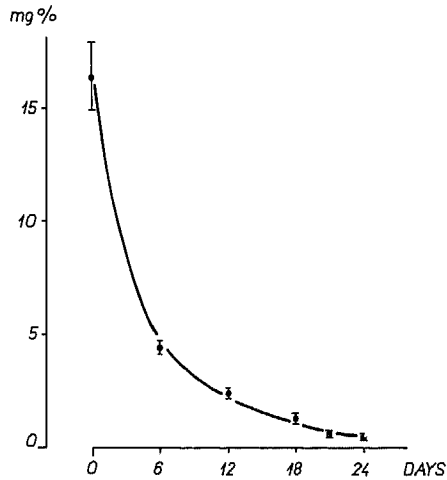


FIG. 1. Drop in vitamin C concentration in guinea pig liver during development of acute scurvy. The vertical bars represent  $\pm$  the standard error of the mean. The same method of expressing the scatter of the values is used in all the other figures and in the tables.

role is played by many nonspecific factors, such as the abrupt drop in body weight, a negative nitrogen balance, and hemorrhage in various parts of the body.

It is therefore not surprising that the relatively numerous data on disorders of lipid metabolism in acute scurvy are very contradictory. Some authors (Murray and Morgan, 1946; Banerjee and Ghosh, 1960) claim that tissue lipid levels fall in scurvy, while others (Tomlinson, 1942; Baldwin *et al.*, 1944) found no changes and others again (Bessey *et al.*, 1934; Sheppard and McHenry, 1939) described an increase in the concentration of lipid substances in the tissues of scorbutic animals. There is likewise a lack of unanimity on the question of the effect of acute vitamin C deficiency on the plasma-cholesterol levels. Some authors (Mouriquand and Leulier, 1925; Banerjee and Singh, 1958; Banerjee and Bandyopadhyay, 1963; Naydu and Nath, 1968) found no significant changes in scorbutic guinea pigs, while others (Bolker *et al.*, 1956; Banerjee and Ghosh, 1960) described hypercholesterolemia in vitamin C-deficient guinea pigs. In vitamin C-deficient monkeys and humans, a tendency for the serum-cholesterol level to fall was observed (Banerjee and Bal, 1959; Bronte-Stewart *et al.*, 1963; Hodges *et al.*, 1969; Kotzé *et al.*, 1974b). Most authors found a significant drop in the cholesterol concentration in the adrenals of severely scorbutic guinea pigs and monkeys (Mouriquand and Leulier, 1925; Banerjee and Deb, 1951; Belavady and Banerjee,

1954; Banerjee and Singh, 1958; Banerjee and Kawishwar, 1964). Baldwin *et al.* (1944), on the other hand, found no changes in the adrenal-cholesterol level in scorbutic guinea pigs and Oesterling and Long (1951) actually described an increase in the adrenal cholesterol concentration in guinea pigs with mild vitamin C deficiency. Study of the dynamics of cholesterol levels in guinea pigs adrenals in different phases of avitaminosis C (Ginter *et al.*, 1965) showed that the discrepancies in these results are only apparent. In the first stage of avitaminosis C, very pronounced accumulation of esterified (and hence of total) cholesterol occurs in guinea pig adrenals. As scurvy develops, the cholesterol level returns to normal and it is not until the terminal stages, when body weight falls, that the adrenal-cholesterol concentration drops below the control values (Fig. 2).

Acute scurvy interferes markedly with metabolism of the acetate pool. In avitaminosis C, the function of the tricarboxylic acid cycle is impaired (Takeda and Hara, 1955; Guchhait and Ganguli, 1961; Banerjee and Kawishwar, 1964), resulting in slower oxidation of acetate to  $\text{CO}_2$ . The incorporation of  $[1-^{14}\text{C}]$ acetate into the liver glycogen (Kumar and Venkatasubramanian, 1964) and adipose tissue fatty acids of scorbutic guinea pigs (Kumar and Venkatasubramanian, 1963; Guchhait *et al.*, 1964) is also slower. On the other hand, significantly more  $[1-^{14}\text{C}]$ acetate is incorporated into the liver and (especially) the adrenal cholesterol of severely scorbutic guinea pigs (King *et al.*, 1953), although in mild vitamin C deficiency the amount of labeled acetate incorporated into the cholesterol in the liver, adrenals, aorta, and epididymal adipose tissue is the

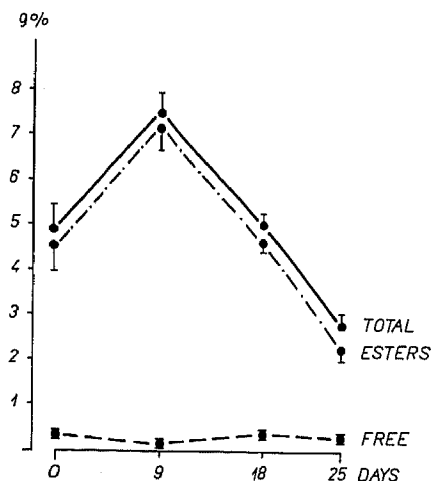


FIG. 2. Free, esterified, and total cholesterol concentration in guinea pig adrenals in different phases of acute scurvy. From Ginter (1970b).

same as in the controls (Becker *et al.*, 1953; Bolker *et al.*, 1956; Kumar and Venkatasubramanian, 1963). Some authors attribute elevated cholesterol accumulation in the body of scorbutic guinea pigs to raised utilization of the acetate pool for cholesterol synthesis or to reduced transformation of cholesterol to bile acids in their liver (Banerjee and Ghosh, 1960; Guchhait *et al.*, 1963). The availability of acetate does not seem to be rate limiting in cholesterol synthesis, however (Gould and Swyryd, 1966). The extremely intricate situation in lipid metabolism is complicated still further by the fact that hypoinsulinism develops in guinea pigs with acute scurvy (Banerjee and Ghosh, 1947).

Last but not least, objections to the preceding model of acute scurvy are based on the fact that it does not give a realistic picture of the nutritional situation in modern man. Acute scurvy is rare in civilized human societies, whereas latent vitamin C deficiency is very common. Since acute scurvy and latent vitamin C deficiency are two metabolically very different states, we felt the need for the elaboration of a new model of chronic ascorbic acid deficiency which would have none of the shortcomings of the acute scurvy model and would be closer to the situation in human nutrition.

### III. Model of Chronic Latent Vitamin C Deficiency

After a series of preliminary experiments, a standardized technique for inducing chronic latent vitamin C deficiency was evolved (Ginter *et al.*, 1968b). For 14 days, guinea pigs are fed on a modified form of Lund's scorbutogenic diet (Ginter, 1975b), without adding ascorbic acid. In this period the body pool of vitamin C abruptly diminishes, but no discernible sequelae of ascorbate deficiency can yet be detected. After 2 weeks the peroral administration of ascorbic acid is started, in doses of 0.5 mg/animal/day. The controls are fed on the same diet, but their peroral doses of ascorbic acid are much larger (usually 10 mg/animal/day). Body weight, appearance, behavior, and food consumption in guinea pigs with latent vitamin C deficiency follow the same course as in the controls. In this way, guinea pigs can be kept in a state of marginal vitamin C deficiency for a very long time, e.g., 1 year.

Figure 3 shows changes in ascorbate concentration in the spleen during the development of latent vitamin C deficiency. In the 2 weeks when an ascorbate-free diet is given, the vitamin C concentration falls abruptly. In the next phase, when a maintaining dose of ascorbic acid is administered, the ascorbate level remains at approximately the same low value, irrespective of the duration of marginal vitamin C deficiency. The given model

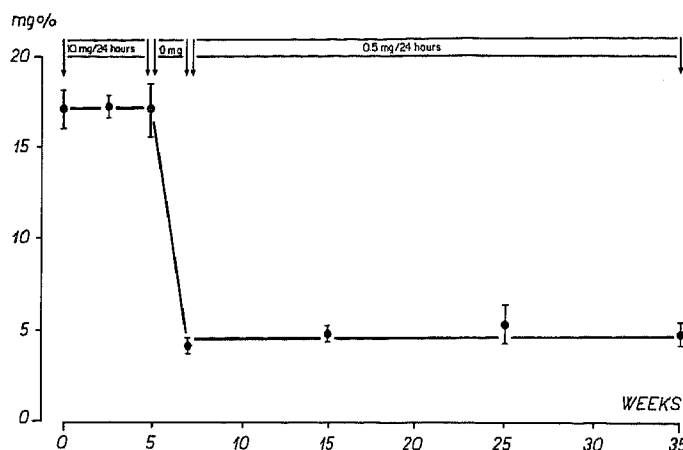


FIG. 3. Vitamin C concentration in the spleen during development of chronic latent vitamin C deficiency in guinea pigs. The ascorbic acid dosage is shown at the top of the figure.

thus creates a situation of equilibrium characterized by a stable low tissue ascorbate level close to the concentrations found in guinea pigs with incipient scurvy. On the other hand, the continuous administration of maintaining doses of ascorbic acid prevents the development of acute scurvy, so that this state, like subclinical vitamin C deficiency, can be described as hypovitaminosis C. An important feature in this model is that when evaluating the effect of hypovitaminosis C, the only variable that has to be taken into account is the substantial depletion of body pool in vitamin C while various secondary phenomena (e.g., loss of body weight) are here immaterial. If any biochemical disturbance is found in deficient animals, the decrease in vitamin C concentration in blood and tissues can be unequivocally denoted as the causal factor. In long-term experiments, during which the experimental animals' body weight increases severalfold, the doses of ascorbic acid can be modified on a body weight basis. So far, however, we still do not know the optimum dose of vitamin C either for man or for guinea pigs.

#### IV. Model of Alimentary Hypercholesterolemia and Atherosclerosis in Guinea Pig

In most earlier studies investigating the influence of vitamin C on cholesterolemia and atherosclerosis, the experimental animal was the rabbit (Flexner *et al.*, 1941; Chakravarti *et al.*, 1957; Myasnikov, 1958; Zaitsev

*et al.*, 1964; Sokoloff *et al.*, 1967; Pool *et al.*, 1971), i.e., the species in which atherosclerosis was first successfully induced (Anitschkow, 1912). Unlike man, the rabbit synthesizes ascorbate, however, and actually reacts to a supply of exogenous cholesterol by an increase in vitamin C synthesis (Ginter, 1970a; Novitskii, 1971). One of the causes of the different results of different teams investigating the possibility of influencing cholesterolemia and atherosclerosis with vitamin C was the choice of species synthesizing ascorbate, such as the rat, rabbit, chicken, and pig (Flexner *et al.*, 1941; Myasnikov, 1958; Fernández-Gimeno *et al.*, 1960; Chang, 1965; Cajola, 1968; Hutagalung *et al.*, 1970; Cromwell *et al.*, 1970; Rolek and Dale, 1972). Homeostasis of ascorbate levels in the blood and tissues is exceptionally highly developed in the rat, so that neither the addition of 1% ascorbic acid to a balanced diet, nor its omission from the diet, affects the ascorbate levels in the organs except the kidneys (Ginter, 1975b). Under these conditions, serum- and tissue-cholesterol concentrations are naturally likewise unaffected.

The effect of the addition of 0.2% ascorbic acid to the diet on the turnover of [4-<sup>14</sup>C]cholesterol was studied in rabbits with cholesterol atherosclerosis (Ginter, 1974). The results of a kinetic analysis of the die-away curves of plasma cholesterol specific activity, in terms of the two-pool model (Goodman and Noble, 1968; Nestel *et al.*, 1969), are given in Table I. Experimental atherosclerosis induced significant enlargement of both cholesterol pools and led to an increased cholesterol turnover rate, irrespective of whether no ascorbic acid or large doses had been administered. The pool and kinetic parameters in rabbits with a zero and a high ascorbic acid intake were found to be practically identical. The course of hypercholesterolemia and cholesterol accumulation in the liver, adrenals, and thoracic aorta in the two groups likewise followed a similar pattern. Owing to endogenous ascorbate synthesis, the vitamin C level in several organs was also the same in the two groups. In keeping with these biochemical findings the degree of atheromatous changes in the aorta and coronary arteries in the two groups corresponded.

For studying the influence of vitamin C deficiency on hypercholesterolemia and atherosclerosis, it is thus obviously necessary to use animals dependent, like man, on exogenous vitamin C. The best would be non-human primates, but experiments on large series of monkeys are very exacting. Although Anitschkow (1922) long ago drew attention to the possibility of inducing atherosclerosis in guinea pigs and other authors (Altschul, 1950; Bernick *et al.*, 1962) confirmed it, the guinea pigs were not used for a long time for studying atherogenesis, because a high cholesterol (1–2%) diet induces hemolytic anemia in them (Okey and Greaves, 1939; Ostwald and Shannon, 1964) and the lesions in the blood vessels

**Table I**  
**SIZE OF POOLS AND KINETIC PARAMETERS OF CHOLESTEROL TURNOVER IN RABBITS**  
**WITH ALIMENTARY ATHEROSCLEROSIS ON ZERO AND HIGH INTAKE OF ASCORBIC ACID**

Parameter	Experimental atherosclerosis		
	Control	Vitamin C: 0	Vitamin C: 0.2% in diet
$t_{1/2\alpha}$ : half-life of first exponential (days)	1.6 ± 0.1 <sup>a</sup>	2.1 ± 0.2	2.0 ± 0.2
$t_{1/2\beta}$ : half-life of second exponential (days)	19.0 ± 1.2	26.8 ± 3.2	27.8 ± 3.1
$M_A$ : size of pool A (mg/animal)	1,113 ± 90	4,174 ± 451	3,900 ± 406
$M_{Bmin}$ : minimum size of pool B (mg/animal)	1,635 ± 40	4,392 ± 688	4,569 ± 715
$PR_A$ : production rate in pool A = turnover rate (mg/animal/day)	124 ± 7	254 ± 27	247 ± 32
$k_A$ : rate constant for irreversible excretion from pool A (day <sup>-1</sup> )	0.116 ± 0.012	0.061 ± 0.001	0.063 ± 0.004

<sup>a</sup> Mean ± SEM.



are not very marked (Cook and McCullagh, 1939). Guinea pigs fed on a high cholesterol diet were used with success to study questions associated with familial lecithin-cholesterol acyl transferase deficiency (Glomset and Norum, 1973).

By modifying their diet, we succeeded in demonstrating that guinea pigs can be used as suitable model animals for producing alimentary hypercholesterolemia and atherosclerosis (Babala and Ginter, 1968; Ginter *et al.*, 1968a, 1970a). For inducing atheromatous lesions, three factors are important: the cholesterol level in the diet, the fatty acid composition of the diet, and the length of time for which the atherogenic diet is administered. Not more than 0.3% cholesterol should be added to the diet, as larger doses cause serious anemia and high mortality. The diet should have a high content of saturated fatty acids and a low ascorbate and polyunsaturated fatty acid content (milk lipids proved to be a suitable source of fats in the diet). If the guinea pigs were fed on this diet for a sufficient length of time (about 4 months), marked atheromatous lesions developed in their vascular system, mainly in the coronary arteries (Fig. 4).

In guinea pigs, the atheromatous lesions displayed certain morphological differences which depended on the anatomical structure of the individual

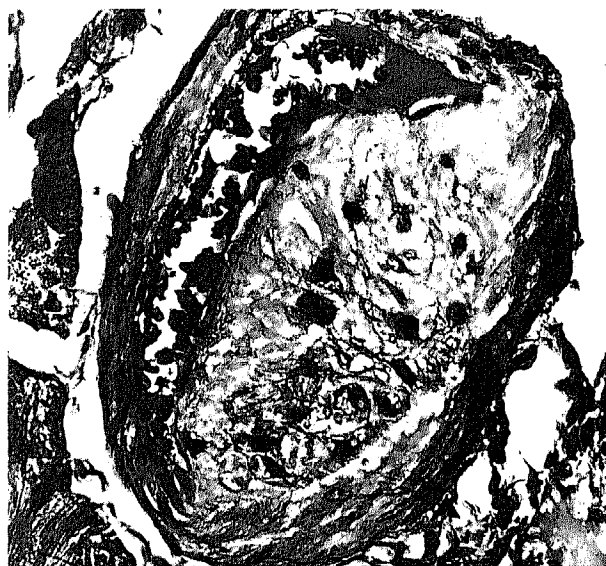


FIG. 4. Endothelized atheromatous material pervaded with histiocytic elements in branch of coronary artery of hypovitaminous guinea pig fed 202 days on cholesterol diet. Hematoxylin and eosin;  $\times 400$ .

vessels and particularly on the proportion of muscular and elastic components. In vessels with a rather small muscular and elastic component, the vascular wall, in the early phases, displayed edema throughout its entire extent, either in the form of foci or, less frequently, round the whole of the periphery. Focal injury and destruction of endothelial cells were observed and at such sites there was parietal adhesion of masses of the character of coagulated lipemic plasma with disintegrated thrombocytes. These parietal thrombotic masses were successively covered with endothelium and cholesterol later crystallized inside them. In larger vessels of the muscular or elastic type, the process remained limited to the intima, which was separated from the internal elastic membrane; here agglomeration of lipophages or monocytes with minutely vacuolated and

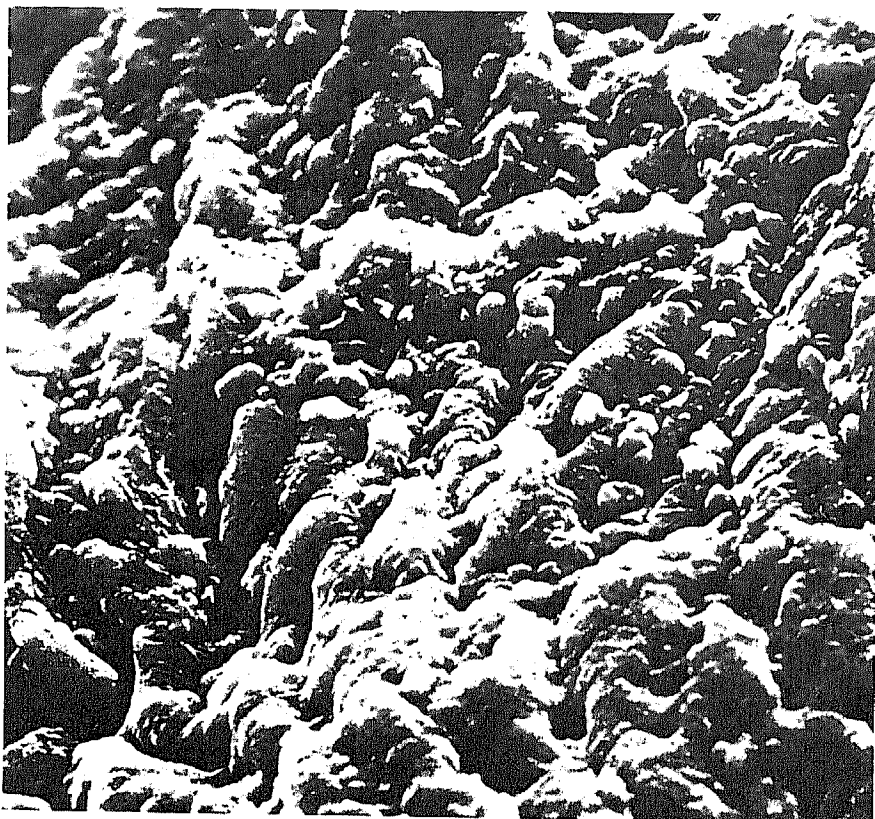


FIG. 5. Intimal surface of aorta of guinea pig fed 4 months on cholesterol diet. Erythrocytes and platelets are scattered over the amorphous covering or are incorporated into it.  $\times 1000$ . From Weber and Tosi (1971).

finely granular cytoplasm was found. The lipids present in the lesions were mainly of the hydrophobic type (cholesterol and its esters). Of the hydrophylic lipids, sphingomyelin and a variable amount of lecithin could be demonstrated. The mucosubstances demonstrated were mainly neutral; the amount of acid mucosubstances was very small. Oxidoreductase activities very very low. Lysosomal enzyme activities, which are greatly enhanced during the formation of plaques in other animals, showed a less marked increase in the guinea pig (Horáková *et al.*, 1973). Scanning electron microscope studies showed that two phases could be distinguished in the development of cholesterol atherosclerosis in the guinea pigs (Weber and Tosi, 1971). In the first phase, an amorphous substance, diffusely covering the intimal surface of the aorta, was deposited. In the second phase (after about 4 months of cholesterol feeding), erythrocytes and platelets were scattered over this amorphous covering (Fig. 5) and intimal plaques became recognizable. Coronary lipohyalinosis was described in guinea pigs given large doses of cholesterol (Manning *et al.*, 1974).

Cholesterol atherosclerosis in guinea pigs, as distinct from rabbits, develops in the presence of relatively low plasma-cholesterol levels (about 300 mg%) reminiscent of human hypercholesterolemia. There is also a parallel with the pathogenesis of human atherosclerosis in the slow development of atherosclerotic lesions in guinea pigs. On the other hand, the lipoprotein metabolism of guinea pigs is very different from that of man since normal guinea pig plasma contains no detectable high-density lipoproteins and no lipoproteins with alpha mobility (Puppione *et al.*, 1971). It will thus manifestly be more satisfactory to use monkeys for studying the influence of vitamin C on lipoprotein metabolism.

#### V. Vitamin C in Regulation of Cholesterol Turnover

On using our model of latent ascorbic acid deficiency (a diet containing 10% butter, without additional cholesterol), short-term hypovitaminosis C did not have a marked effect on cholesterol levels in guinea pig blood and tissues. If latent vitamin C deficiency lasted longer than 3 months, however, cholesterol always accumulated in the guinea pig liver and hypercholesterolemia developed (Ginter *et al.*, 1965, 1969c, 1971a, 1973b) (Table II). The hypercholesterolemic action of vitamin C deficiency depends on the lipid composition of the diet. A vitamin C-free diet containing 12.5% cottonseed oil leads to hypercholesterolemia in guinea pigs in only 2 weeks, while the addition of 5% coconut oil potentiates the hypercholesterolemic effect of ascorbate deficiency still further (Fujinami *et al.*, 1971). If a diet containing 4% groundnut oil is given, the hyper-

Table II  
 INFLUENCE OF CHRONIC LATENT VITAMIN C DEFICIENCY ON TOTAL CHOLESTEROL  
 CONCENTRATION IN BLOOD PLASMA AND LIVER OF MALE GUINEA PIGS<sup>a</sup>

Duration of deficiency (weeks)	Blood plasma		Liver	
	Control	Deficiency	Control	Deficiency
16-20	118 ± 14 <sup>b</sup>	171 ± 18	368 ± 29 <sup>c</sup>	659 ± 40
22	99 ± 6	132 ± 6	456 ± 56	627 ± 65
23	95 ± 7	140 ± 8	395 ± 35	616 ± 70
24	94 ± 7	140 ± 7	411 ± 33	592 ± 77
26	88 ± 7	139 ± 8	325 ± 14	586 ± 106
28	110 ± 6	135 ± 5	357 ± 22	661 ± 96

<sup>a</sup> Data from Ginter (1975a).

<sup>b</sup> Milligrams per 100 ml plasma ± SEM.

<sup>c</sup> Milligrams per 100 g wet tissue ± SEM.

cholesterolemic effect of latent vitamin C deficiency is still not manifested after 4 months, but the administration of a diet containing 15% coconut oil and 0.3% cholesterol, under the same conditions, results in significant elevation of the serum cholesterol level in vitamin C-deficient guinea pigs compared with groups given large doses of ascorbic acid (Nambisan and Kurup, 1975). The cholesterol concentration in the other organs of vitamin-deficient guinea pigs remains unchanged, except for an increase in the amount of Liebermann-Burchardt-positive sterols in the skin (Ginter *et al.*, 1973b). If 0.3% cholesterol is added to the diet, however, hypovitaminosis C causes cholesterol to accumulate in various organs, including the thoracic aorta (Ginter *et al.*, 1969a,b) (Table III). In some tissues, cholesterol levels are graduated in correlation to the dose of ascorbic acid, and in some tissues there is a significant negative correlation between the cholesterol concentration and the ascorbate level, i.e., the higher the ascorbate level, the lower the cholesterol concentration, and vice versa (Ginter *et al.*, 1969b). Similar results were reported by Nambisan and Kurup (1975).

#### A. LOCALIZATION OF INTERFERENCE OF VITAMIN C DEFICIENCY WITH CHOLESTEROL METABOLISM

Serum and tissue cholesterol concentrations are the outcome of a great number of processes mutually bound by feedback mechanisms, such as cholesterol distribution between blood and tissues, endogenous cholesterol synthesis, the absorption of exogenous cholesterol, cholesterol excretion, and the transformation of cholesterol to bile acids.

We followed the passage of labeled cholesterol from the blood plasma to 14 different guinea pig tissues forming the major part of cholesterol pools in the body (liver, kidney, adrenal, small intestine, large intestine, stomach, lung, myocardium, brain, testis, epididymal fat, skeletal muscle, thoracic aorta, and skin). The results obtained from the controls and the vitamin-deficient animals did not differ significantly. Since the total amount of cholesterol in these tissues in the two groups is the same (excepting the liver and the skin), the accumulation of cholesterol in liver and plasma of vitamin C-deficient animals cannot be accounted for by lower cholesterol deposition in other parts of the body.

##### 1. Vitamin C and Endogenous Cholesterol Synthesis

Study of cholesterol biosynthesis in the liver of hypovitaminous guinea pigs at different intervals after the administration of [1-<sup>14</sup>C]acetate did not yield completely conclusive results, but indicated that marginal

**Table III**  
**TOTAL CHOLESTEROL CONCENTRATIONS IN THE TISSUES OF CHOLESTEROL-FED GUINEA PIGS**  
**GIVEN VARIOUS DOSES OF ASCORBIC ACID <sup>a</sup>**

Duration of experiment (weeks)	Tissue	Doses of ascorbic acid (mg/animal/day)		
		0.5 (deficiency)	5.0	50.0
12	Liver	4,017 ± 485 <sup>b</sup>	3,652 ± 310	3,404 ± 42
	Adrenal	10,774 ± 1,621	10,646 ± 1,047	8,651 ± 527
	Small intestine	387 ± 19	345 ± 35	272 ± 32
20	Thoracic aorta	548 ± 48	545 ± 96	409 ± 29
	Liver	6,622 ± 548	5,611 ± 416	3,509 ± 350
	Adrenal	7,942 ± 890	7,782 ± 671	5,186 ± 840
	Small intestine	364 ± 23	317 ± 17	282 ± 20

<sup>a</sup> Data from Ginter (1975a).

<sup>b</sup> Milligrams of total cholesterol per 100 g of wet tissue.

vitamin C deficiency did not markedly affect endogenous cholesterol synthesis in the liver (Ginter *et al.*, 1965; Ginter and Nemeč, 1969). In guinea pigs, the rate of endogenous cholesterol synthesis in the ileum is much higher than in the liver (Swann *et al.*, 1975; Turley *et al.*, 1975, 1976). We found in an *in vivo* experiment (Fig. 6) that the incorporation of [ $1-^{14}\text{C}$ ]acetate into [ $^{14}\text{C}$ ]digitonides was at least one order higher in the guinea pig ileum than in the other tissues studied and that latent vitamin C deficiency did not influence this process in the ileum. In the other tissues, we observed a tendency to higher values in the vitamin-deficient group, but it is questionable whether these differences could markedly influence total cholesterol biogenesis. The incorporation of labeled acetate and mevalonate into cholesterol was lower in liver homogenates prepared from vitamin C-deficient baboons (Weight *et al.*, 1974). *In vivo* experiments were rather indicative of elevated cholesterol synthesis in ascorbate-deficient baboons, however (Kotzé *et al.*, 1974b). In animals which synthesize ascorbate (the rat, the rabbit), vitamin C, in

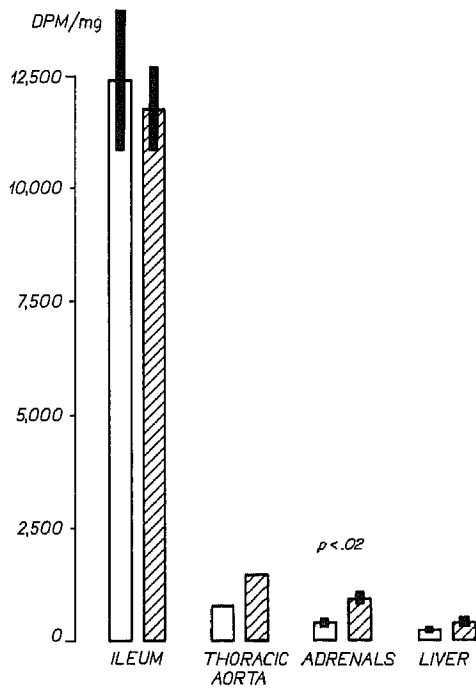


FIG. 6. Specific activity of [ $^{14}\text{C}$ ]digitonides isolated from different guinea pig tissues 20 min after i.p. injection of [ $1-^{14}\text{C}$ ]acetate. Control: white columns, hypovitaminosis C: shaded columns.

given circumstances, can stimulate cholesterol synthesis (Popják *et al.*, 1958; Novitskii, 1969; Misra and Srivastava, 1974), while in hamsters fed on a lithogenic diet, even large doses of ascorbic acid do not markedly affect cholesterol biosynthesis (Section V,C). Although the influence of ascorbic acid on endogenous cholesterol synthesis has not been completely elucidated, it is unlikely that the accumulation of cholesterol in the body of hypovitaminous guinea pigs could be due to raised synthesis of endogenous cholesterol.

## 2. Ascorbic Acid and Absorption and Excretion of Cholesterol

Following an intragastric application of [4-<sup>14</sup>C]cholesterol, hypovitaminous guinea pigs had a significantly higher <sup>14</sup>C activity in the gastrointestinal tract and stool and, on the other hand, a substantially lower activity in the blood and tissues (Ginter, 1970b). Raised accumulation of cholesterol in the blood and liver of vitamin C-deficient guinea pigs thus cannot be ascribed to raised absorption of exogenous cholesterol. Marginal vitamin C deficiency tends rather to inhibit this process. Similar results were obtained in humans with acute vitamin C deficiency (Bronte-Stewart *et al.*, 1963).

Myasnikov (1958) drew attention to the possibility that the hypocholesterolemic effect of ascorbic acid might be associated with stimulated secretion of cholesterol from the liver into the bile. The results obtained in studies of the influence of vitamin C on cholesterol levels in rabbit bile are contradictory, however (Kolmakov, 1957; Novitskii, 1969). Some interesting effects of ascorbic acid-2-sulfate on cholesterol excretion have been reported. Verlangieri and Mumma (1973) found sulfation of cholesterol by ascorbic acid-2-sulfate *in vivo*; the resultant product, cholesterol sulfate, was excreted in the stools. Hayashi *et al.* (1974, 1976) reported that sodium ascorbic acid-2-sulfate had a hypocholesterolemic effect on hyperlipemic rats, rabbits, and guinea pigs. On the other hand, Hornig, *et al.* (1974) observed no stimulation of the fecal excretion of cholesterol or cholesterol sulfate by ascorbic acid-2-sulfate in rats. According to our results, marginal ascorbate deficiency does not markedly influence cholesterol excretion in the form of neutral sterols. Guinea pigs were given an intraperitoneal injection of [4-<sup>14</sup>C]cholesterol and the excretion of [<sup>14</sup>C]-neutral sterols and [<sup>14</sup>C]bile acids in their stools was measured for 20 days (Ginter *et al.*, 1971a). The excretion of [<sup>14</sup>C]sterols by the controls and vitamin-deficient animals was found to be practically the same, so that this factor likewise failed to explain cholesterol accumulation in the blood and liver of hypovitaminous guinea pigs. This experiment was instrumental in discovery of the key to the problem, however; the excretion



of [ $^{14}\text{C}$ ]bile acids in the stools was smaller in vitamin-deficient guinea pigs. This result indicated that the rate of cholesterol transformation to its principal catabolic product, bile acids, is slowed down in marginal vitamin C deficiency.

#### B. THE ROLE OF VITAMIN C IN CHOLESTEROL TRANSFORMATION TO BILE ACIDS

The transformation of cholesterol to bile acids can be studied by two isotope methods. If cholesterol labeled with  $^{14}\text{C}$  in position 4 of the cyclic structure is administered, the activity that occurs in the bile acid fraction is the criterion of the rate of the process, as mammals do not possess an enzymatic system capable of splitting the cholesterol nucleus (Chaikoff *et al.*, 1952). If cholesterol labeled in position 26 on the side chain is given, the isopropyl fragment is split off from the side chain during cholesterol catabolism and  $^{14}\text{C}$  is released in the form of carbon dioxide. The rate of cholesterol catabolism is measured from the amount of  $^{14}\text{C}$  in the expired  $\text{CO}_2$ . The bile acids isolated from the liver and gallbladder bile three days after injection of [ $4\text{-}^{14}\text{C}$ ]cholesterol were labeled to a lesser extent in hypovitaminous guinea pigs (Table IV). When [ $26\text{-}^{14}\text{C}$ ]cholesterol was injected, the amount of  $^{14}\text{CO}_2$  recovered in 10 days was significantly smaller in guinea pigs with marginal vitamin C deficiency than in the control group (Ginter *et al.*, 1971a). Furthermore, the resaturation of vitamin C-deficient guinea pigs with large doses of ascorbic acid significantly stepped up the rate of [ $26\text{-}^{14}\text{C}$ ]cholesterol oxidation to  $^{14}\text{CO}_2$  (Ginter *et al.*, 1972) (Fig. 7).

**Table IV**  
INFLUENCE OF CHRONIC HYPOVITAMINOSIS C ON THE DISTRIBUTION OF  $^{14}\text{C}$   
IN NEUTRAL STEROLS AND BILE ACIDS 3 DAYS AFTER INTRAPERITONEAL INJECTION  
OF [ $4\text{-}^{14}\text{C}$ ]CHOLESTEROL <sup>a</sup>

Sample	Fraction	Control	Hypovitaminosis C
Liver (dpm/g wet tissue)	Neutral sterols	23,970 $\pm$ 2,292	26,505 $\pm$ 2,353
	Bile acids	3,397 $\pm$ 635	2,017 $\pm$ 173
	Bile acids/ neutral sterols	0.157 $\pm$ 0.030	0.080 $\pm$ 0.007
Gallbladder bile (dpm/g bile)	Neutral sterols	1,273	1,367
	Bile acids	132,307	108,755
	Bile acids/ neutral sterols	103.9	79.6

<sup>a</sup> Data from Ginter *et al.* (1971a).

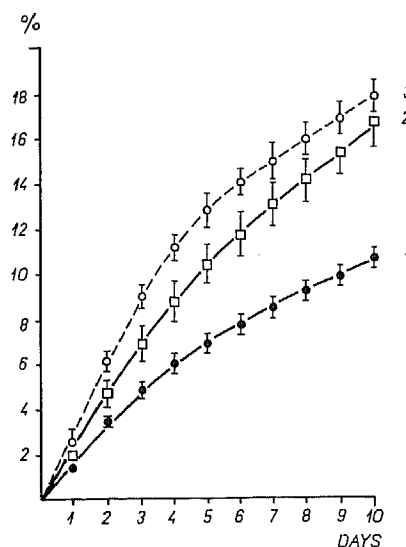


FIG. 7. Oxidation of  $[26-^{14}\text{C}]$ cholesterol to  $^{14}\text{CO}_2$  as percentage of dose administered to vitamin C-deficient (1), resaturated pair-fed (2), and resaturated *ad libitum*-fed (3) guinea pigs. From Ginter *et al.* (1972).

Simultaneous determination of the amount of expired  $^{14}\text{CO}_2$  and of the specific activity of liver or serum cholesterol after the administration of  $[26-^{14}\text{C}]$ cholesterol allows quantification of the rate of cholesterol transformation to bile acids (Myant and Lewis, 1966; Ginter *et al.*, 1973a). Application of this technique indicated that chronic latent ascorbate deficiency significantly reduced the rate of cholesterol transformation to bile acids in guinea pigs (controls:  $11.8 \pm 0.6$ ; hypovitaminosis C:  $8.3 \pm 0.4$  mg cholesterol/24 hours/500 g body weight) (Ginter, 1973). Cholesterol is transformed to bile acids in the liver and the rate of this process very probably depends on the ascorbate concentration in the liver cells, since there is a relatively close linear correlation between the rate of bile acid synthesis and the ascorbic acid concentration in the liver (Fig. 8). In guinea pigs given small doses of ascorbic acid, reduced bile acid biosynthesis leads to a decrease in the size of the body pool of bile acids (Hornig and Weiser, 1976). There is a significant direct correlation in guinea pigs between the amount of ascorbate in the liver and the size of the bile acid pool (Fig. 9), which is reminiscent of the correlation between the liver-ascorbate level and the rate of bile acid biosynthesis (Fig. 8).

Cholesterol transformation to bile acids is a multistage process taking place successively in the liver cell microsomes, supernatant fraction, and mitochondria. It involves hydroxylation, dehydrogenation, saturation of a

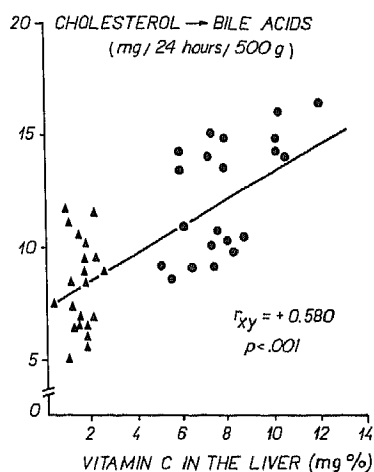


FIG. 8. Linear correlation between liver ascorbic acid concentration and the rate of cholesterol transformation to bile acids in control guinea pigs (●) and guinea pigs with chronic latent vitamin C deficiency (▲). From Ginter *et al.* (1973b).

double bond in the nucleus, 3-ketone reduction, and  $\omega$ - and  $\beta$ -oxidation of the cholesterol side chain. The transformation of cholesterol to the principal bile acid of guinea pigs, chenodeoxycholic acid, entails two hydroxylations: at position  $7\alpha$  in the cholesterol nucleus and at position 26 on its side chain (Fig. 10). In contrast to ovarian and adrenal tissue (Sulimovici and Boyd, 1968; Shimizu, 1970), ascorbate does not seem

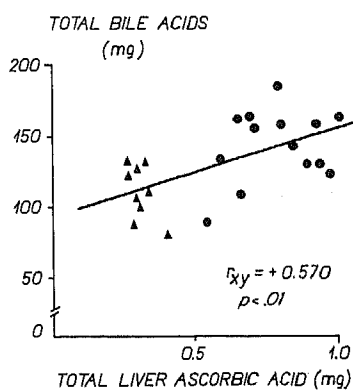


FIG. 9. Linear correlation between amount of ascorbic acid in liver and size of bile acid pool (liver + gallbladder + small intestine) in guinea pigs with a low (0.75 mg twice daily, ▲) and a higher (5 mg twice daily, ●) Na ascorbate intake. The graph was constructed on the basis of data given by Hornig and Weiser (1976).

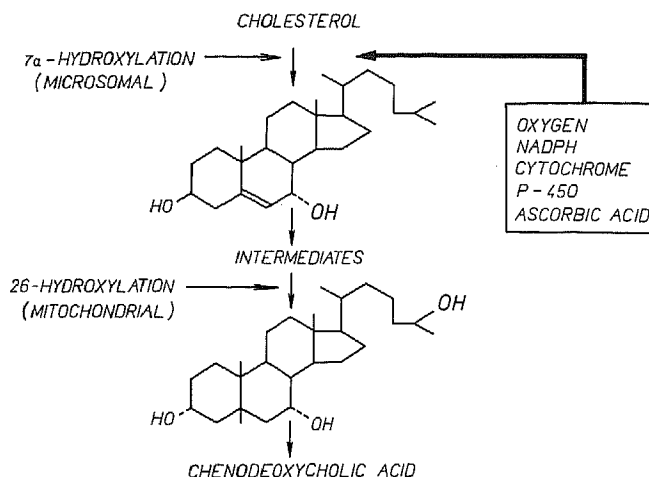
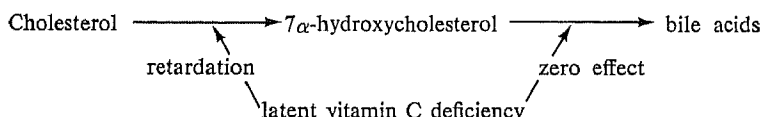


FIG. 10. Scheme of hydroxylation reactions during cholesterol conversion to chenodeoxycholic acid. Ascorbic acid participates in the first reaction, i.e., 7 $\alpha$ -hydroxylation of cholesterol to cholest-5-ene-3 $\beta$ ,7 $\alpha$ -diol and does not participate in 26-hydroxylation (synthesis of 5- $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ -26-triol).

to affect oxidation of the side chain (i.e., 26-hydroxylation) of cholesterol in liver mitochondria (Kritchevsky *et al.*, 1973).

The first step in cholesterol transformation to C<sub>24</sub> bile acids is the production of 7 $\alpha$ -hydroxycholesterol and this reaction is rate limiting for cholesterol catabolism. If the interference of ascorbate deficiency with the biosynthesis of bile acids is localized solely at 7 $\alpha$ -hydroxylation level, then 7 $\alpha$ -hydroxycholesterol catabolism, as distinct from cholesterol catabolism, ought not to be affected by latent vitamin C deficiency:



This assumption was verified by synthesizing [26-<sup>14</sup>C]-7 $\alpha$ -hydroxycholesterol [26-<sup>14</sup>C]cholest-5-ene-3 $\beta$ ,7 $\alpha$ -diol) and following its oxidation to <sup>14</sup>CO<sub>2</sub> *in vivo* (Ginter, 1975a). The results (Fig. 11) show that, unlike the significantly retarded oxidation of [26-<sup>14</sup>C]cholesterol, the oxidation of [26-<sup>14</sup>C]-7 $\alpha$ -hydroxycholesterol is not significantly affected by latent vitamin C deficiency. In *in vitro* experiments, Kritchevsky *et al.* (1973) did not find statistically significant increase in 7 $\alpha$ -hydroxylation of [1,2-<sup>3</sup>H]-cholesterol in the liver microsomes of normal guinea pigs after the addition of ascorbic acid, but a relatively close correlation was found between the amount of added ascorbate and the rate of 7 $\alpha$ -hydroxylation ( $r_{xy} = +$

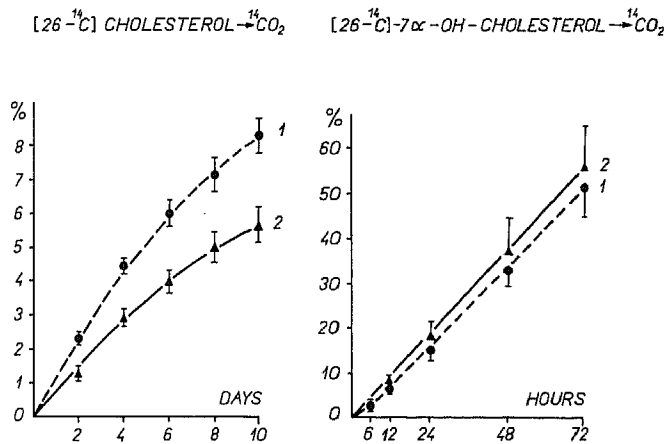


FIG. 11. Oxidation of  $[26-^{14}\text{C}]$ cholesterol and  $[26-^{14}\text{C}]$ - $7\alpha$ -hydroxycholesterol to  $^{14}\text{CO}_2$  as percentage of dose administered to control (1) and vitamin C-deficient (2) guinea pigs. Latent vitamin C deficiency significantly reduced the oxidation of  $[26-^{14}\text{C}]$ cholesterol, while the oxidation of  $[26-^{14}\text{C}]$ - $7\alpha$ -hydroxycholesterol was not significantly affected. From Ginter (1975a).

0.899). Recently Björkhem and Kallner (1976) reported that the extent of conversion of endogenous cholesterol into  $7\alpha$ -hydroxycholesterol, as determined by mass fragmentography, was more than 10 times lower in incubations with microsomal fraction from vitamin C-deficient guinea pigs than in those from guinea pigs treated with ascorbate. On the other side, 25- and 26-hydroxylation of  $5\beta$ -cholestane- $3\alpha,7\alpha$ -diol was not significantly affected by the ascorbate status of animals (Fig. 12). It seems therefore highly probable that marginal vitamin C deficiency interferes with the biosynthesis of bile acids solely at the stage of  $7\alpha$ -hydroxylation of the cholesterol nucleus.

Cholesterol- $7\alpha$ -hydroxylase is localized in the microsomal fraction of the liver cell and requires NADPH and oxygen for maximum activity. The cholesterol  $7\alpha$ -hydroxylating system consists of cytochrome P-450 and NADPH cytochrome P-450 reductase (Wada *et al.*, 1968; Scholan and Boyd, 1968). The cytochrome P-450 concentration in guinea pig liver microsomes falls within 24 hours after discontinuing the supply of ascorbic acid (Degkwitz *et al.*, 1972, 1975). After administration of ascorbic acid to vitamin C-deficient guinea pigs, the cytochrome P-450 level in the liver microsomes (Leber *et al.*, 1970) rises parallel with  $[26-^{14}\text{C}]$ -cholesterol oxidation to  $^{14}\text{CO}_2$  (Ginter and Nemeč, 1972) (Fig. 13). It seems feasible that the stimulant effect of ascorbate on the  $7\alpha$ -hydroxylation of cholesterol is mediated through its action on the cytochrome P-450

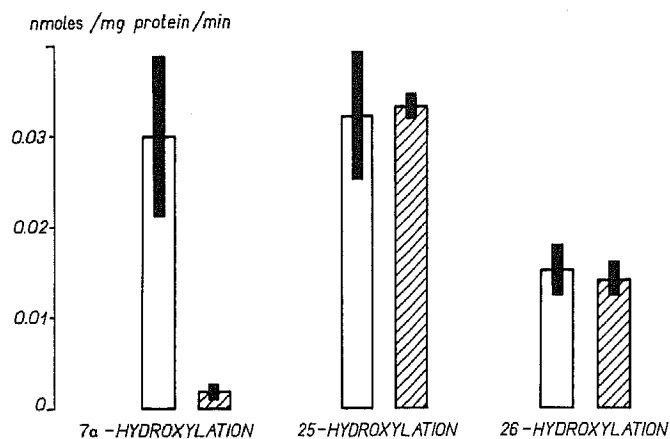


FIG. 12. 7 $\alpha$ -hydroxylation of endogenous microsomal cholesterol and 25- and 26-hydroxylation of 5 $\beta$ -cholestane-3 $\alpha$ ,7 $\alpha$ -diol by microsomal fraction of liver homogenate from control (unshaded columns) and vitamin C-deficient (shaded columns) guinea pigs. The figure was constructed from the data of Björkhem and Kallner (1976).

level in the liver microsomes. Degkwitz and Kim (1973) assumed that ascorbate played a role in biosynthesis of the heme part of the cytochrome P-450. Partially purified cytochrome P-450 from hepatal microsomes of vitamin C-deficient guinea pig in the presence of excess NADPH-cyto-

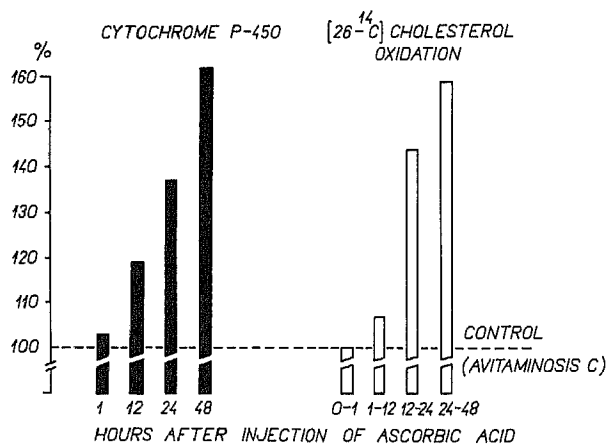


FIG. 13. Parallel increase in [26-<sup>14</sup>C]cholesterol oxidation and the cytochrome P-450 level in the liver microsomes of vitamin C-deficient guinea pigs after the administration of ascorbic acid (values in vitamin C-deficient animals = 100%). The left part of the figure was constructed from the data of Leber *et al.* (1970). From Ginter and Nemeč (1972).

chrome P-450 reductase had a much lower capacity to  $7\alpha$ -hydroxylate cholesterol than a corresponding system containing cytochrome P-450 from liver of normal guinea pig (Björkhem and Kallner, 1976). These authors suggested that vitamin C deficiency lowered the specific type of cytochrome P-450 involved in  $7\alpha$ -hydroxylation of cholesterol. It is also possible that the ascorbic-monodehydroascorbic-dehydroascorbic acid redox system is linked up in the transport of electrons from NADPH to oxidized cytochrome P-450. In this association, the finding of elevated catabolism of [ $1-^{14}\text{C}$ ]ascorbic acid to  $^{14}\text{CO}_2$ , and [ $^{14}\text{C}$ ]oxalate in cholesterol-fed guinea pigs (Ginter, 1975b), i.e., under conditions where a continuous supply of large amounts of exogenous cholesterol raises requirements for the reduction of oxidized cytochrome P-450, is interesting. In cholesterol-fed guinea pigs, vitamin C requirements rise at the same time (Ginter and Zloch, 1972). It is probable, however, that the effect of ascorbate on the  $7\alpha$ -hydroxylation of cholesterol is not an effect on the enzyme activity *per se* since addition of ascorbate to the microsomal fraction had no effect on the rate of  $7\alpha$ -hydroxylation (Kritchevsky *et al.*, 1973; Björkhem and Kallner, 1976).

The strong influence of vitamin C on the liver is manifested not only in the transformation of cholesterol to bile acids, but also in detoxication of a variety of pharmacological agents and environmental chemicals (Zannoni and Sato, 1975; Street and Chadwick, 1975), in which microsomal cytochrome P-450 plays a role, as in  $7\alpha$ -hydroxylation of cholesterol. Chronic latent ascorbate deficiency is associated with morphological, as well as functional, changes in the liver cell, the chief one being marked reduction and replacement of the granular endoplasmic reticulum (Sulkin and Sulkin, 1975) (Fig. 14). In protracted marginal vitamin C deficiency, guinea pig liver displays centrolobular fatty degeneration, moderate hyperplasia of the bile ducts and occasionally discrete signs of fibroplasia (Fig. 15). Fatty cirrhosis, with bile duct proliferation, was even observed in the liver of hypovitaminous guinea pigs fed on a diet containing 0.3% cholesterol (Fig. 16). The pathological changes in animals given the same cholesterol diet, plus 100 mg ascorbic acid/animal/day, were less striking (Ginter, 1975b).

### C. VITAMIN C AND CHOLESTEROL TURNOVER

Chronic marginal vitamin C deficiency is associated with a sharp decline in the liver ascorbate concentration, with a consequent decrease in the rate of cholesterol transformation to bile acids. Lowered bile acid synthesis is associated with lowered absorption of exogenous cholesterol from gastrointestinal tract, but this homeostatic mechanism is not suffi-

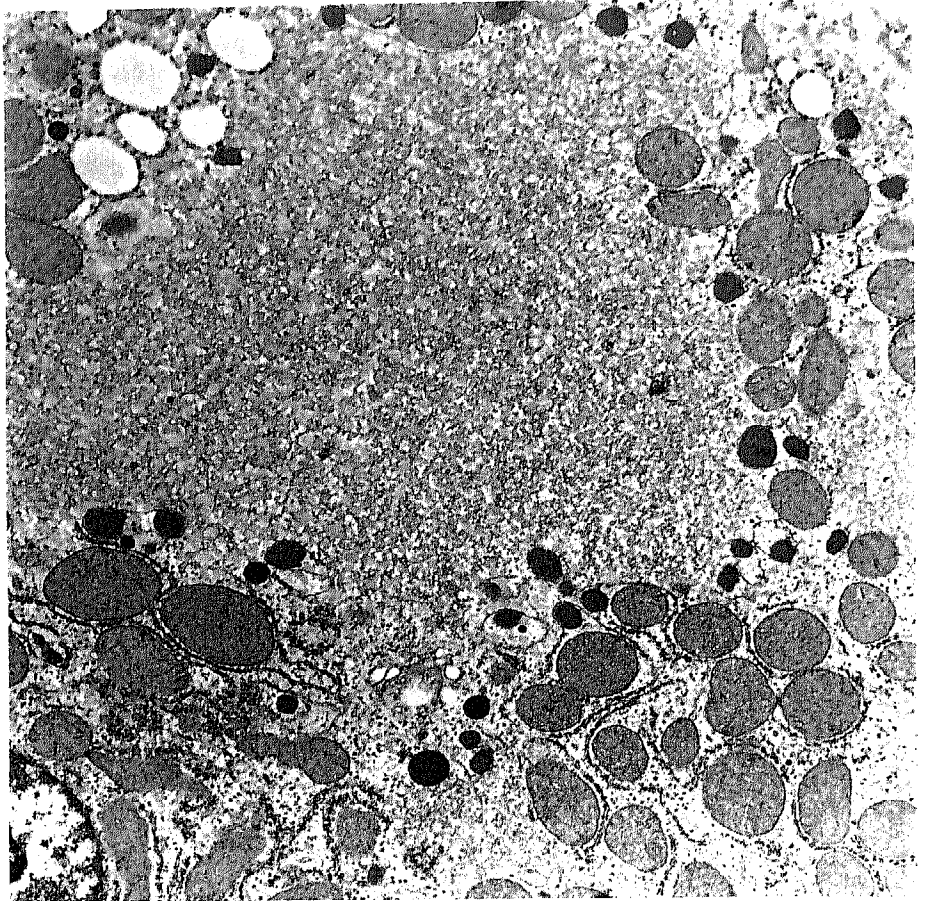


FIG. 14. Electron micrograph of a section of a hepatic cell from a guinea pig that had been on a marginally vitamin C-deficient diet for a period of 104 days. Note the reduction of the granular endoplasmic reticulum and the encirclement of these organelles around the mitochondria when they are present. The sharp proliferation of smooth endoplasmic reticulum to the extent of displacing other organelles is of special significance. From Sulkin and Sulkin (1975).

ciently effective to be able to compensate slower cholesterol catabolism in full. This creates a state of imbalance, in which the supply of cholesterol to the system exceeds the rate of its removal, resulting in the accumulation of cholesterol in the liver and plasma. If marginal vitamin C deficiency is protracted (5-6 months), the guinea pig liver-cholesterol



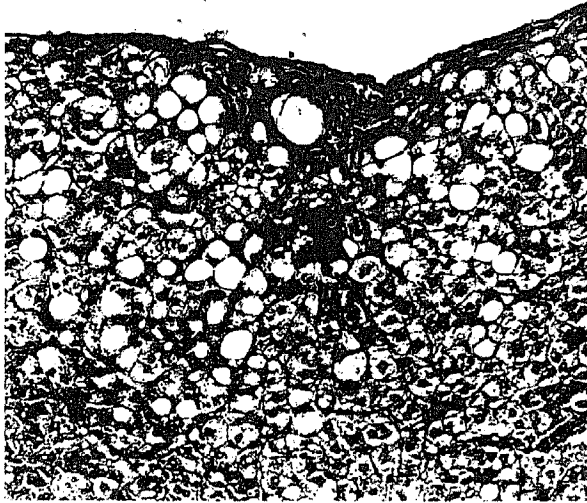


FIG. 15. Fatty degeneration and subcapsular peribiliary fibroplasia causing retraction of surface of liver of guinea pig kept 11 months in a state of latent vitamin C deficiency. Hematoxylin and eosin;  $\times 160$ .



FIG. 16. Fatty cirrhosis of liver of vitamin C-deficient guinea pig fed 6 months on cholesterol diet. Hematoxylin and eosin;  $\times 32$ .

level rises by 50–70% and plasma level by about 40% (Table II, Section V) above the control values. This increase creates a new state of equilibrium, in which the plasma-cholesterol level of deficient guinea pigs does not undergo any more marked changes, but the kinetic parameters of cholesterol turnover, determined by the two-pool analysis (Nestel *et al.*, 1969), are greatly altered (Table V). The half time of the linear part of the hyperbolic curve in hypovitaminous guinea pigs was significantly prolonged, while the values of the rate constant for irreversible cholesterol excretion and total cholesterol turnover rate were significantly lowered (Ginter, 1974). In golden hamsters, which are normally independent of exogenous vitamin C, relative ascorbate deficiency was induced by ad-

**Table V**  
KINETIC PARAMETERS OF CHOLESTEROL TURNOVER IN GUINEA PIGS AND GOLDEN HAMSTERS FED DIFFERENT DOSES OF VITAMIN C

Guinea pigs		
Parameter	Control (10 mg ascorbate/day)	Hypovitaminosis C (0.5 mg ascorbate/day)
Half time of first exponential (days)	8.0	7.1
Half time of second exponential (days)	24.0	30.1
Turnover rate: production rate in pool A (mg/animal/day)	37.8	31.8
Fractional turnover rate (% of pool A renewed in 24 hours)	3.6	3.1
Cholesterol transformation to bile acids (mg/24 hours/500 g body weight)	11.8	8.3
Golden hamsters		
Parameter	Ascorbic acid intake	
	0.5% in diet	Zero
Half-life of plasma cholesterol (days)	15.7	21.5
Half-life of liver cholesterol (days)	16.3	21.5
Size of total cholesterol miscible pool (mg/animal)	128	162
Turnover rate (mg/animal/day)	5.6	5.2
Fractional turnover rate (% of pool renewed in 24 hours)	4.4	3.2
Cholesterol transformation to bile acids (mg/24 hours/100 g body weight)	2.53	2.16

ministering a physiologically unbalanced fat-free, ascorbate-free, high-glucose diet (Ginter *et al.*, 1976). In these animals, just as in vitamin C-deficient guinea pigs, the plasma and liver cholesterol concentration rose compared with the group given the same diet plus 0.5% ascorbic acid, the half-life of plasma and liver cholesterol was prolonged, the size of the body pool of miscible cholesterol increased, the fractional turnover rate fell and the rate of cholesterol transformation to bile acids diminished (Table V, lower part). Total turnover (which equals endogenous cholesterol synthesis on administering a cholesterol-free diet) was not greatly affected.

Latent vitamin C deficiency thus produced in guinea pigs and hamsters changes similar to those produced by alimentary hypercholesterolemia and atherosclerosis in rabbits (Table I, Section IV). All these changes (an increased number of cholesterol molecules per plasma volume unit, prolongation of the mean length of time for which the cholesterol molecules persist in the circulating blood, the overall slowing down of cholesterol turnover) speak for the probability of cholesterol being accumulated in the blood vessel walls of vitamin C-deficient animals. Figure 17 furnishes evidence of the negative correlation between the amount of [ $^{14}$ C]-cholesterol which accumulates in the thoracic aorta and the cholesterol turnover rate, i.e., a decrease in the turnover rate leads to a significant increase in the accumulation of labeled cholesterol in the guinea pig aorta.

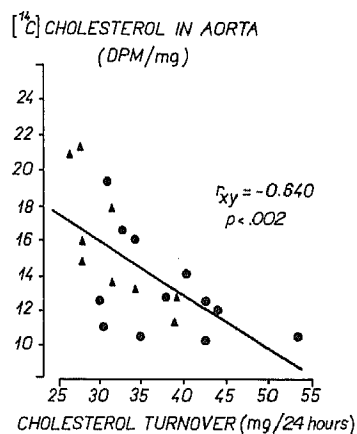


FIG. 17. Negative correlation between cholesterol turnover rate and amount of labeled cholesterol deposited in guinea pig thoracic aorta 42 days after i.p. administration of [ $4\text{-}^{14}\text{C}$ ]cholesterol. (●) Control animals; (▲) guinea pigs kept 27 weeks in state of latent vitamin C deficiency. From Ginter (1975b).

## VI. Further Effects of Vitamin C on Lipid Metabolism

### A. VITAMIN C AND TRIGLYCERIDE METABOLISM

Fujinami *et al.* (1971) found that the serum triglyceride concentration in guinea pigs rises slightly after only 2 weeks' administration of a vitamin C-free diet. If guinea pigs are given a diet to which 0.3% cholesterol is added, marginal vitamin C deficiency leads in 4 months to marked hypertriglyceridemia and to the accumulation of triglycerides in the liver and the aorta (Nambisan and Kurup, 1975). If latent ascorbic acid deficiency is prolonged still further, the blood triglyceride level rises by 50%, even when the guinea pigs are given a diet with no additional cholesterol (Ginter *et al.*, 1976) (Table VI). A raised vitamin C intake causes triglyceride levels to fall not only in guinea pigs (Nambisan and Kurup, 1975) but also in weanling rats (Nambisan and Kurup, 1974), cholesterol-fed rabbits and rats (Sokoloff *et al.*, 1967), and baboons (Kotzé *et al.*, 1975). Marked hypertriglyceridemia can be induced in golden hamsters by administering an ascorbate-free, fat-free, high-glucose diet (Ginter *et al.*, 1976); the addition of 0.5% ascorbic acid to this diet restores the situation to practically normal (Table VI). The hypotriglyceridemic action of vitamin C in humans will be discussed in Section VIII.

The mechanism of the hypotriglyceridemic action of ascorbic acid is not altogether clear, but it is very probably associated with the effect of ascorbic acid on lipolytic enzyme activities. Normalization of hypertriglyceridemia in cholesterol-fed rabbits and rats was associated with an increase in lipoprotein lipase activity in the blood (Sokoloff *et al.*, 1967). Large doses of ascorbate similarly reduced the blood triglyceride level in guinea pigs and simultaneously raised the lipoprotein lipase activity of the plasma (Fujinami *et al.*, 1971). The effect of large doses of vitamin C on lipoprotein lipase in the heart muscle of baboons (Kotzé *et al.*, 1974a) and in the liver and heart of guinea pigs (Nambisan and Kurup, 1975) is just the reverse, i.e., lipoprotein lipase activity in animals given large doses of ascorbate was much lower than in vitamin C-deficient animals. Ascorbic acid affects myocardial and adipose tissue lipoprotein lipase differently: higher serum ascorbate levels (over 0.35 mg%) appear to inhibit the heart muscle enzyme and to stimulate the adipose tissue enzyme (Kotzé, 1975). Heart and adipose tissue lipoprotein lipase also react to experimental hypertriglyceridemia in the same different manner (Shafrir and Biale, 1970; Vrána *et al.*, 1974). In insulin deficiency, lipoprotein lipase activity decreases in adipose tissue and increases in the heart (Kessler, 1963). In this association it is interesting to note that vitamin C deficiency which leads to hypoinsulinism (Banerjee and Ghosh, 1947) also stimulates

**Table VI**  
**INFLUENCE OF VITAMIN C ON BLOOD PLASMA TRIGLYCERIDES IN GOLDEN HAMSTERS AND GUINEA PIGS**

Kind of experimental animal	Control	High-glucose fat-free diet
Golden hamsters	207 ± 11 <sup>a</sup>	Vitamin C: 0      251 ± 13 Vitamin C: 0.5% in diet
Guinea pigs		Hypovitaminosis C
Duration of experiment: 6.5 months	67 ± 7	105 ± 11
Duration of experiment: 11 months	105 ± 10	159 ± 10

<sup>a</sup> Milligrams per 100 ml plasma ± SEM.

lipoprotein lipase activity in the heart (Kotzé *et al.*, 1974a; Nambisan and Kurup, 1975).

The finding that ascorbate had a marked inhibitory effect on hormone-sensitive lipase from adipose tissue (Tsai *et al.*, 1973) led to consideration (Anonymous, 1974) that large doses of vitamin C might inhibit the mobilization of nonesterified fatty acids from depot tissues and in this way lead to obesity. *In vivo* experiments, however, showed that, on the contrary, the mobilization of nonesterified fatty acids is inhibited by vitamin C deficiency (Mueller and Cardon, 1961). Scorbutic guinea pigs do not even respond to the injection of adrenaline by mobilization of nonesterified fatty acids (Mueller, 1962); ascorbic acid, on the contrary, stimulates this process (Mathur *et al.*, 1974). The stimulating action of vitamin C on hormone-induced lipolysis is localized somewhere before the activation of lipase by adenosine 3',5'-cyclic monophosphate. One possible mechanism is the inhibition of phosphodiesterase activity in adipose tissue (Hynie *et al.*, 1970). In baboons ascorbic acid significantly raised the plasma adenosine 3',5'-cyclic monophosphate level, but reduced guanosine 3',5'-cyclic monophosphate levels (Van Wyk and Kotzé, 1975). The preincubation of guinea pig lung fragments with ascorbic acid significantly raised prostaglandin  $F_{2\alpha}$  production (Hitchcock, 1975). Although the hypotriglyceridemic effect of vitamin C is unquestionable, the mechanism by which ascorbic acid deficiency interferes with triglyceride metabolism is evidently complex and requires further research.

#### B. VITAMIN C AND GALLSTONE FORMATION

The initial phase of gallstone formation is a disorder of liver cell metabolism, the production of bile supersaturated with cholesterol (Admirand and Small, 1968). Bile acids and lecithin are the major solubilizing agents for biliary cholesterol. In subjects with cholesterol gallstones, the size of the bile acid pool decreases (Vlahcevic *et al.*, 1970). The reduction of bile acid pool size could lead to a decrease in the proportion of bile acids in relation to cholesterol and this could result in the precipitation of cholesterol and the aggregation of cholesterol crystals into gallstones. A similar metabolic situation develops in guinea pigs with marginal vitamin C deficiency: the rate of cholesterol transformation to bile acids is lowered and so is the [ $^{14}$ C]bile acid content of the gallbladder bile of hypovitaminous guinea pigs injected with [4- $^{14}$ C]cholesterol (Table IV, Section V,B). In guinea pigs given small doses of ascorbate, the size of the bile acid pool is also reduced (Hornig and Weiser, 1976). In agreement with these data, a frequent incidence of gallstones was observed in guinea pigs with acute scurvy (Pavel *et al.*, 1969; Bellmann *et al.*, 1974). These findings indicate

that chronic marginal vitamin C deficiency could play a role in the pathogenesis of cholelithiasis and that the intake of large doses of ascorbate could play a positive role in preventing gallstone formation.

In recently completed experiments we studied the effect of the addition of 0.5% ascorbic acid to a lithogenic diet (Dam, 1964) on the metabolism of [26-<sup>14</sup>C]cholesterol and on gallstone formation in golden hamsters. Vitamin C markedly accelerated cholesterol turnover (Table V, Section V, C) and significantly reduced both the incidence and the extent of experimental cholelithiasis and the accumulation of [<sup>14</sup>C]cholesterol in the gallstones (Table VII). We also succeeded, using the same diet, in inducing gallstone formation in guinea pigs (Fig. 18). When we administered mounting doses of vitamin C which raised liver ascorbate levels, the number of guinea pigs free from gallstones rose significantly (Table VII, lower part).

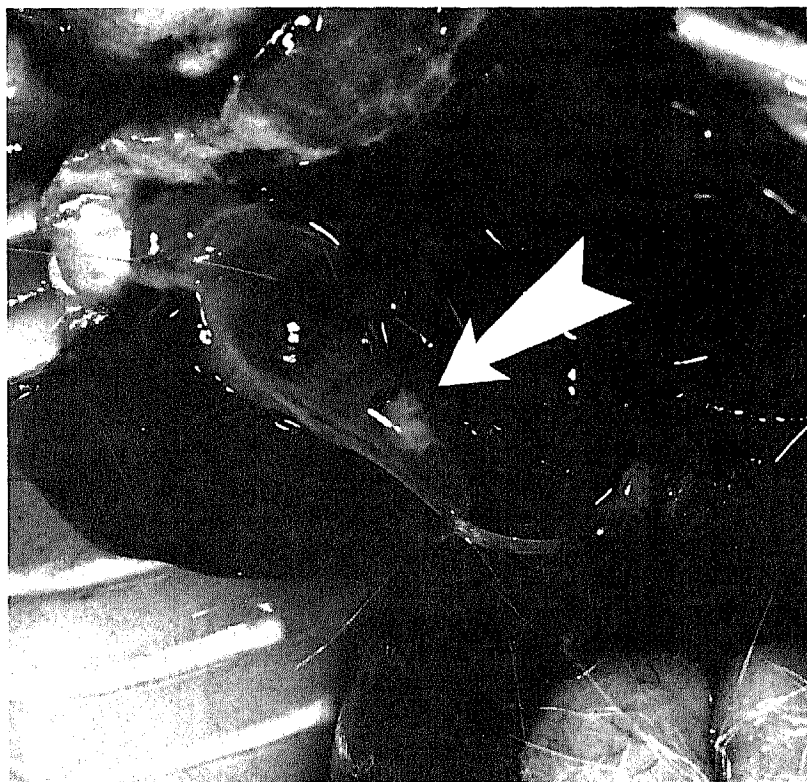


FIG. 18. Gallstone in gallbladder of vitamin C-deficient guinea pig fed 4 weeks on lithogenic diet.

**Table VII**  
**EFFECT OF VITAMIN C ON THE INCIDENCE OF GALLSTONES AND VITAMIN C CONCENTRATION IN THE LIVER**  
**OF GOLDEN HAMSTERS AND GUINEA PIGS FED A FAT-FREE HIGH-GLUCOSE DIET**

Kind of experimental animal	Ascorbic acid supplementation			
	Zero	0.05% in diet	1% in diet	0.5% in diet + 1% in diet + 0.5% in water
Golden hamsters				
Animals free of gallstones (%)	32			56
Animals with incipient cholelithiasis (%)	26			20
Animals with advanced cholelithiasis (%)	42			24
<sup>14</sup> C activity in gallstones (dpm/total gallstones)	768			356
Vitamin C in the liver (mg/100 g wet tissue)	14.7			26.8
Guinea pigs				
Animals free of gallstones (%)		29	50	63
Vitamin C in the liver (mg/100 g wet tissue)		4.4	15.0	43.8



Whether the incidence of human cholelithiasis can be reduced by preventing latent vitamin C deficiency is still an open question. Large doses of vitamin C did not markedly alter the chemical composition of the bile in human subjects (Pedersen, 1975), but in these experiments ascorbic acid was unfortunately administered only over a very short period (7–15 days).

## VII. Vitamin C, Metabolism of Blood Vessel Wall, and Experimental Atherosclerosis

Chronic marginal vitamin C deficiency induces hypercholesterolemia and hypertriglyceridemia in experimental animals. In addition to these two factors promoting atheromatous reconstruction of the vascular system, there is a third important factor, changes in the walls of the blood vessels of vitamin C-deficient animals.

### A. METABOLIC AND STRUCTURAL CHANGES IN BLOOD VESSEL WALL IN VITAMIN C DEFICIENCY

#### 1. Collagen

The role of vitamin C in collagen synthesis is the longest-known biochemical function of ascorbic acid and it has been elaborated in very great detail. Vitamin C participates in the synthesis of collagen hydroxyproline and hydroxylysine, both of which are formed by the hydroxylation of prolyl and lysyl residues previously incorporated into peptide linkage during the process of ribosomal collagen protein synthesis (Barnes, 1975). Intensive investigations are being carried out to elucidate the precise mode of action of ascorbate in these hydroxylations (Hurych *et al.*, 1973; Rokosova and Chvapil, 1974; Alfano *et al.*, 1975; Barnes, 1975; Cardinale *et al.*, 1975; Levene and Bates, 1975); unfortunately, the interest of these teams is not centered on the connective tissue of the blood vessel wall.

In earlier morphological studies, we already find descriptions of atrophy of collagen in vessel walls (Hojer, 1924), breakdown of collagen fibrils adjacent to capillaries (Wolbach and Bessey, 1942), weakening of collagen bundles in atonic dilated venules (Lee and Lee, 1947), and degeneration of the connective tissue within the vessel walls and perivascular areas (Stolman *et al.*, 1961) in vitamin C-deficient guinea pigs. Electron microscopy revealed depletion of aortic subendothelial collagen (Gore *et al.*, 1965b). Except for just one study (Banerjee and Ghosh, 1961), a decrease in collagen measured as hydroxyproline was also observed in the aorta of scorbutic guinea pigs (Gore *et al.*, 1965a; Kishikawa, *et al.*, 1971); it was particularly pronounced in protracted marginal ascorbate deficiency

(Higuchi *et al.*, 1975). Vitamin C deficiency thus probably slows down collagen synthesis in the blood vessel wall also, resulting in collagen depletion in the blood vessels in the presence of chronic ascorbate deficiency.

## 2. *Glycosaminoglycans*

Vitamin C deficiency interferes with yet another important component of the connective tissue of the blood vessel wall, i.e., the glycosaminoglycans of the ground substance. The extreme heterogeneity in origin, structure, and turnover of the various glycosaminoglycans makes problems of the influence of ascorbic acid on mucopolysaccharide metabolism tremendously complicated. They have been solved mainly in tissue cultures and in tendonectomized or otherwise injured guinea pigs (Bates and Levene, 1969), but the results are often at variance.

Histochemical and chemical methods showed an increase in total mucopolysaccharides in the aorta of scorbutic guinea pigs (Weber, 1955; Banerjee and Ghosh, 1961), which was caused mainly by an increase in the hyaluronic acid level (Gore *et al.*, 1965a; Kishikawa *et al.*, 1971). The chondroitin sulfate B (in the latest terminology, dermatan sulfate) level in the aorta of guinea pigs with acute scurvy fell, however (Gore *et al.*, 1965a; Kishikawa *et al.*, 1971). When hyperlipemia was induced by coconut oil feeding, the total glycosaminoglycan level in the aorta of guinea pigs kept in a state of latent ascorbate deficiency for 8 weeks fell, owing to a drop in hyaluronic acid, heparan sulfate, and chondroitin-6-sulfate levels (Higuchi *et al.*, 1975). Similar changes were described by Nambisan and Kurup (1975) in the aorta of guinea pigs kept in a state of marginal ascorbate deficiency for 4 months; when a diet with added cholesterol was given, these changes were even more pronounced (Table VIII).

We observed an increase in  $\beta$ -glycosidase activities in the thoracic aorta of guinea pigs kept for an exceptionally long time (11 months) in a state of marginal vitamin C deficiency (Table IX). A significant increase in  $\beta$ -glucuronidase activity was also described in the aorta of guinea pigs kept in a state of latent ascorbic acid deficiency for a shorter length of time (Fujinami *et al.*, 1975; Nambisan and Kurup, 1975). This enzyme degrades polysaccharide fragments split off from acid mucopolysaccharides by hyaluronidase. The cause of the decrease in the glycosaminoglycan content of the aorta of vitamin-deficient guinea pigs could thus be an increase in  $\beta$ -glucuronidase and other hydrolytic enzyme activities (hyaluronidase and  $\beta$ -hexosaminidase) in the aorta of deficient animals (Nambisan and Kurup, 1975).  $\beta$ -Glucuronidase activity in the blood vessels increases with age and rises markedly in lipid plaques (Kirk, 1969; Lojda, 1974). The finding of raised  $\beta$ -glucuronidase activity in the aorta of vitamin

**Table VIII**  
**GLYCOSAMINOGLYCAN LEVELS IN THE AORTA OF GUINEA PIGS FED VARIOUS DOSES OF ASCORBIC ACID<sup>a</sup>**

Group	Hyaluronic acid	Heparan sulfate	Chondroitin 4-sulfate	Chondroitin 6-sulfate	Dermatan sulfate	Heparin
	<i>Basal diet</i>					
Marginal deficiency (0.1 mg ascorbate/100 g b.w.)	1,287 ± 26 <sup>b</sup>	1,280 ± 26	1,050 ± 21	1,459 ± 29	833 ± 17	788 ± 15
Adequate dose of vitamin C (1 mg ascorbate/100 g b.w.)	1,450 ± 28	1,321 ± 27	1,110 ± 22	1,562 ± 30	930 ± 19	926 ± 19
High dose of vitamin C (25 mg ascorbate/100 g b.w.)	1,491 ± 29	1,476 ± 29	1,388 ± 27	1,610 ± 32	956 ± 19	986 ± 20
	<i>Atherogenic cholesterol diet</i>					
Marginal deficiency (0.1 mg ascorbate/100 g b.w.)	1,001 ± 25	990 ± 20	695 ± 15	1,036 ± 26	520 ± 12	610 ± 13
Adequate dose of vitamin C (1 mg ascorbate/100 g b.w.)	965 ± 21	1,100 ± 22	880 ± 18	1,180 ± 31	600 ± 14	730 ± 15
High dose of vitamin C (25 mg ascorbate/100 g b.w.)	989 ± 22	1,124 ± 22	956 ± 20	1,260 ± 31	675 ± 14	824 ± 17

<sup>a</sup> Data from Nambisan and Kurup (1975).

<sup>b</sup> Micrograms uronic acid per gram dry defatted tissue ± SEM.

**Table IX**  
INFLUENCE OF CHRONIC MARGINAL VITAMIN C DEFICIENCY ON ENZYME ACTIVITY  
IN THORACIC AORTA OF GUINEA PIGS

Enzyme	Control	Hypovitaminosis C
$\beta$ -Galactosidase (EC 3.2.1.2.3)	0.046 $\pm$ 0.008 <sup>a</sup>	0.070 $\pm$ 0.008
$\beta$ -N-Acetylglucosaminidase (EC 3.2.1.30)	1.75 $\pm$ 0.20	2.00 $\pm$ 0.21
$\beta$ -Glucuronidase (EC 3.2.1.31)	0.98 $\pm$ 0.12	1.30 $\pm$ 0.09
Carboxylic esterases (EC 3.1.1)	6.0 $\pm$ 0.4	5.2 $\pm$ 0.8
Alkaline phosphatase (EC 3.1.3.1)	0.10 $\pm$ 0.01	0.09 $\pm$ 0.01
Acid phosphatase (EC 3.1.3.2)	1.40 $\pm$ 0.02	1.12 $\pm$ 0.09
Succinic dehydrogenase (EC 1.3.99.1)	23.0 $\pm$ 1.4	32.0 $\pm$ 2.7
Malic dehydrogenase (EC 1.1.1.37)	102 $\pm$ 5	100 $\pm$ 4

<sup>a</sup> Micromoles per gram wet tissue per 60 minutes  $\pm$  SEM.

C-deficient guinea pigs may thus be a sign of the presence of presclerotic changes.

Conversely, large doses of ascorbic acid raise the total glycosaminoglycan level in the guinea pig and rat aorta (Nambisan and Kurup, 1974, 1975). The increase in sulfated glycosaminoglycans (heparan sulfate, chondroitin-4- and 6-sulfate, and dermatan sulfate) is especially marked (Table VIII). At the same time, large doses of ascorbate raise the activity of enzymes concerned with biological sulfation in guinea pig liver (Nambisan and Kurup, 1975). Whether ascorbic acid-2-sulfate plays a role in activation of these processes (Hatanaka *et al.*, 1974; Hornig, 1975; Shapiro and Poon, 1975) is still an open question.

Metabolic disorders and changes in the composition of the intima very probably play an important role in the pathogenesis of atherosclerosis (Robinson *et al.*, 1975). Plasma  $\beta$ -lipoproteins are bound to different mucopolysaccharides to varying degrees. Hyaluronic acid influences the selective permeability of the intima, while heparan sulfates have an anti-lipemic and antithrombotic action. Alteration of these functions can favor atherogenesis through different pathogenetic mechanisms.

#### B. VITAMIN C AND EXPERIMENTAL ATHEROSCLEROSIS

Menten and King observed diffuse hyperplastic arteriosclerosis in the lungs, liver, spleen, and kidneys of vitamin C-deficient guinea pigs injected with diphtheria toxin as early as 1935. Willis (1953) found subendothelial lipid deposits in the aorta of scorbutic guinea pigs. The administration of ascorbic acid to scorbutic guinea pigs led to rapid resorption of early atherosclerotic lesions (Willis, 1957). Fujinami *et al.* (1971), in guinea

pigs fed on a diet to which coconut oil was added, described atheromatous lesions after only 2 weeks of a scorbutogenic regimen. Other authors (Gore *et al.*, 1965a,b; Ginter *et al.*, 1969a) failed to find explicit atherosclerotic lesions in guinea pigs with acute vitamin C deficiency, but described in them reduced silver stainability of the cement lines and increased incidence of nuclear abnormalities in the aortic endothelium. Electron microscopy revealed separation of the endothelial cells and reduction of the cytoplasmic organelles in the aortic endothelium of scorbutic guinea pigs (Gore *et al.*, 1965b; Kishikawa *et al.*, 1971). The mast cell count fell in the *tunica adventitia* of the aortic wall in scorbutic guinea pigs (Pettersson, 1959). In ovalbumin-sensitized subscorbutic guinea pigs, the administration of cholesterol caused an increase in aortic endothelial turnover and destruction of cells (Wright *et al.*, 1975).

The administration of a 0.3% cholesterol diet to guinea pigs with latent vitamin C deficiency produced marked atheromatous changes in the coronary arteries in a few months (Fig. 4, Section IV). Large doses of ascorbic acid (50–100 mg/animal/day) slowed down atheromatous reconstruction of the vascular system but did not prevent fully the formation of atheromatous lesions in the coronary arteries (Ginter *et al.*, 1969b, Ginter, 1975b). The accumulation of cholesterol in the thoracic aorta of hypovitaminous guinea pigs fed on a cholesterol diet was greater than in animals given a 100-fold larger dose of ascorbate, but not even this dose of vitamin C prevented an increase in the cholesterol concentration in the aorta compared with the low cholesterol diet controls (Ginter *et al.*, 1969b) (Fig. 19). Similar results were obtained by Nambisan and Kurup (1975).

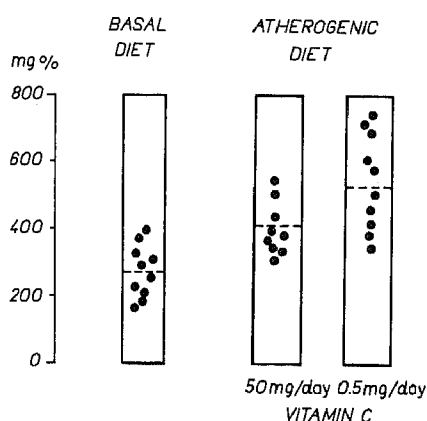


FIG. 19. Total cholesterol concentration in thoracic aorta of control guinea pigs and animals fed 12 weeks on cholesterol diet. The difference between cholesterol-fed animals given 50 or 0.5 mg ascorbic acid daily is statistically significant. From Ginter (1975b).

The continued long-term intake of large amounts of exogenous cholesterol may mask the protective effect of essential fatty acids as well as of vitamin C. Discrepancies between the results of different authors who studied the influence of ascorbic acid on cholesterol atherosclerosis (Section IV) are evidently due partly to this factor. In given circumstances, e.g., in copper deficiency, ascorbic acid actually potentiates an angiopathy (Simpson *et al.*, 1971). On the other hand, in animals synthesizing ascorbate, vitamin C can be shown to have a positive effect if the animals are put in a situation which raises the ascorbate requirements. Vitamin C was shown to have a hypocholesterolemic and/or antiatherosclerotic effect in weanling rats (Nambisan and Kurup, 1974), in hypothyroid rats (Scholz, 1973), in rats with experimental cholestasis and aminonucleoside nephrosis (Froese *et al.*, 1975), in rats with experimental fatty degeneration of the liver (Cajola, 1968), in starved rabbits (Kolmakov, 1957), in rabbits with epinephrine-induced atherosclerosis (Davis and Oester, 1952), and in golden hamsters fed on a fat-free, ascorbate-free, high-glucose diet (Section V,C).

The most important experiments for comprehending the potential role of ascorbic acid deficiency in the pathogenesis of human atherosclerosis, however, are those in which the influence of chronic latent vitamin C deficiency on atherogenesis was studied under conditions of a nutritionally balanced,



FIG. 20. Lipophage layer in intima of thoracic aorta of guinea pig fed on diet with no added cholesterol and kept over 6 months in state of latent vitamin C deficiency. Hematoxylin and eosin;  $\times 400$ .

low-cholesterol diet. In guinea pigs kept in a state of marginal vitamin C deficiency for over 6 months, we found (Ginter, 1974, 1975b) that edema of the vessel wall, vacuolization of the endothelial cells and parietal adhesion of blood plasma occurred in their aorta even when they were given a diet to which no cholesterol was added. In some deficient animals, the formation of lipophages (Figs. 20 and 21) and even of fresh homogeneous atheromatous material was found in the intima of the aorta and the coronary arteries and their branches. It should be emphasized that these findings were made in seemingly healthy animals fed on a standard diet without additional cholesterol, so that the only cause of these changes must have been chronic latent vitamin C deficiency.

After 8 weeks of latent vitamin C deficiency, Fujinami *et al.* (1975) found white patchy plaques in the arch and proximal part of the aorta of two-thirds of their guinea pigs fed on a diet without added cholesterol. The lesions consisted of fibrous thickening of the intima, with lipid accumulation and degenerative changes in the media accompanied by mucopolysaccharide, lipid and calcium deposits. The most marked accumulation of cholesterol and triglycerides was found in the arch of the aorta of deficient animals. In this part of the aorta there was also a significant increase in esterase and lipase activity (Fig. 22). In guinea pigs fed on a diet without

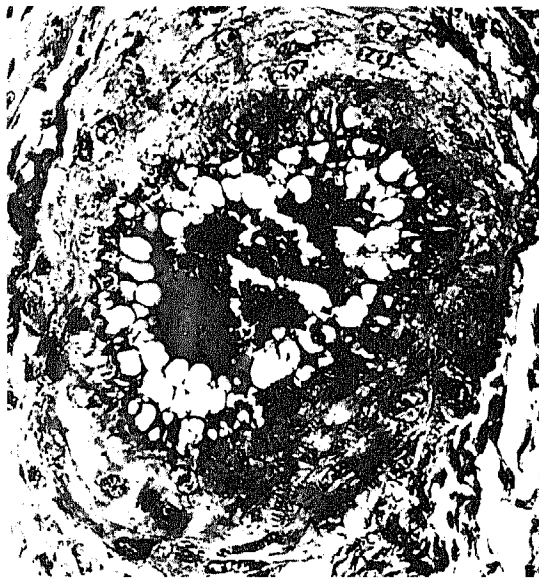


FIG. 21. Intimal foam cells in edematous branch of coronary artery of guinea pig fed on diet with no addition of cholesterol and kept over 6 months in state of latent vitamin C deficiency. Hematoxylin and eosin;  $\times 400$ .

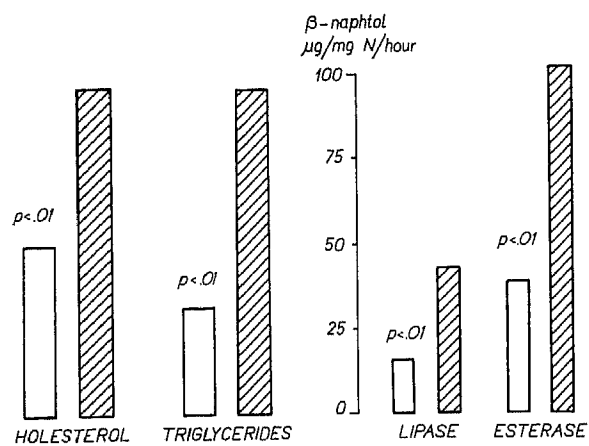


FIG. 22. Significant increase in cholesterol and triglyceride concentration and in lipase and esterase activity in arch of aorta of vitamin C-deficient guinea pigs fed on diet with no added cholesterol: (unshaded columns) controls; (shaded columns) vitamin C deficiency. The figure was constructed from the data of Fujinami *et al.* (1975).



FIG. 23. A section of an aortic arteriosclerotic plaque from a guinea pig that had been on a marginal vitamin C-deficient diet with no added cholesterol for a period of 109 days. Stained with Van-Gieson-Verhoff stain to demonstrate the fibrotic nature of the lesion. From Sulkin and Sulkin (1975).



added cholesterol and kept 100–150 days in a state of marginal vitamin C deficiency, Sulkin and Sulkin (1975) found numerous alterations in the aortic wall; these were large intimal plaques (Fig. 23), which appeared to be of a musculofibrotic type, marked endothelial proliferation, a high degree of metachromasia in the ground substance, and the presence of a fibrous, amorphous material of varying thickness underlying the endothelium (Fig. 24).

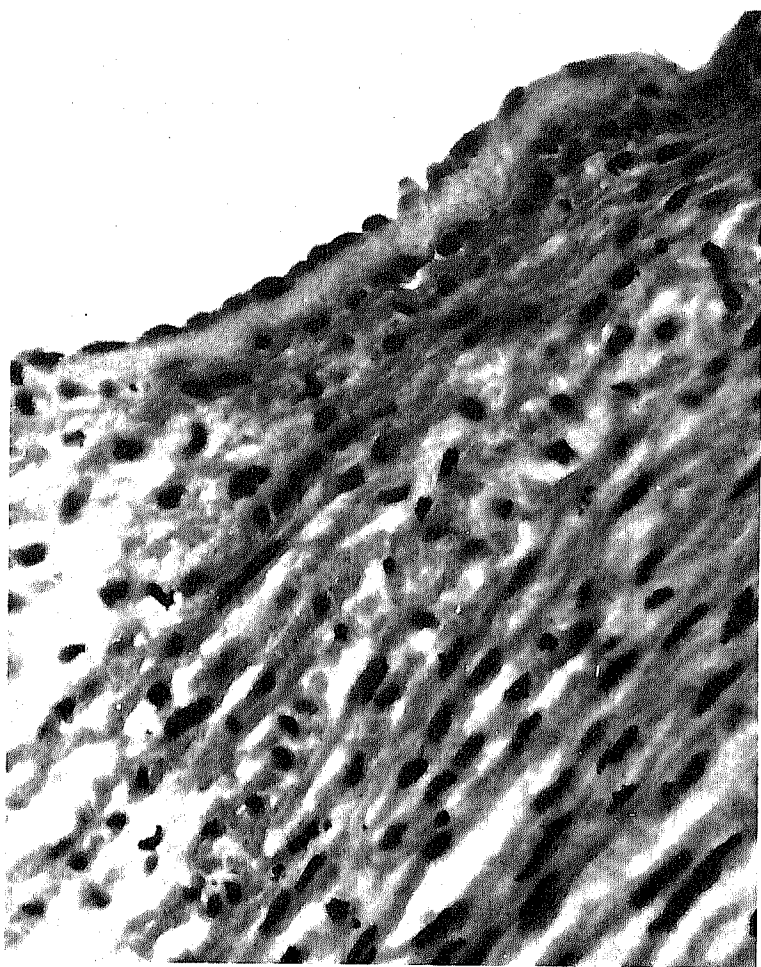
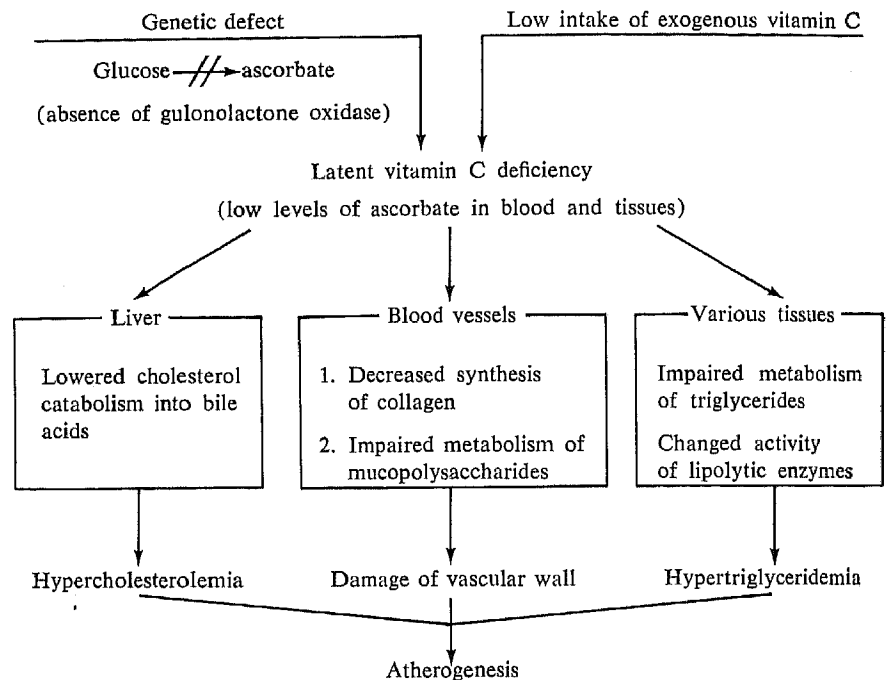


FIG. 24. A section of the aorta of a guinea pig that had been on a marginal vitamin C-deficient diet with no added cholesterol for 105 days. Note the thick band of amorphous substance underlying the epithelium. From Sulkin and Sulkin (1975).

These results demonstrate that chronic latent ascorbic acid deficiency is *per se* capable of producing atheromatous changes in the guinea pig vascular system. The mechanism of this phenomenon is complex and, as shown by the scheme below, it is based on interference with the integrity of the blood vessel wall (impaired collagen and glycosaminoglycan metabolism), on slower catabolism of cholesterol in the liver and resultant hypercholesterolemia and on the development of still incompletely studied disorders of triglyceride metabolism leading to hypertriglyceridemia:



### VIII. Vitamin C, Hyperlipemia, and Atherosclerosis in Man

The literature on the effect of ascorbic acid on the blood lipids and on atherosclerosis in man is very extensive and very contradictory. The majority of clinical studies on these problems are less exact than animal experiments. Basic information is often missing, e.g., on the experimental subjects' vitamin C status at the time of starting the experiment and on changes in the composition of their diet during the experiment. Most authors (Bukovskaya, 1957; Anderson *et al.*, 1958, 1972; Hrubá and

Mašek, 1962; Bronte-Stewart *et al.*, 1963; Crawford *et al.*, 1975), though not all (Spittle, 1971; Hanck, 1973), found that ascorbic acid had no effect in subjects with initial low cholesterol levels. Many authors (Myasnikova, 1947; Sedov, 1956; Bukovskaya, 1957; Myasnikov, 1960; Fedorova, 1960; Sokoloff *et al.*, 1967; Kishikawa *et al.*, 1971) stated that vitamin C led to diminution of hypercholesterolemia and hypertriglyceridemia, to a decrease in the  $\beta$ -lipoprotein level, and to improvement of the state of health of at least some of their subjects with hyperlipemia and atherosclerosis. It should be stressed that, in most of these studies, a hypocholesterolemic effect was achieved in conjunction with other factors, e.g., a therapeutic diet. According to other authors (Krivoruchenko, 1963; Samuel and Salchi, 1964; Peterson *et al.*, 1975), vitamin C did not affect the plasma-cholesterol level in hypercholesterolemic subjects, or actually raised it (Spittle, 1971).

A number of authors described seasonal variations of the blood-cholesterol level in man (Tochowicz *et al.*, 1962; Fyfe *et al.*, 1968). The highest level was usually observed at the time of minimum vitamin C consumption, but whether there is a causal relationship is still an open question. The correlation of ascorbate and cholesterol levels in a single blood sample (Ziffer *et al.*, 1967; Elwood *et al.*, 1970; Bradley *et al.*, 1973) is likewise not very helpful, since the actual vitamin C concentration in the blood does not furnish exact information on the subject's nutritional history. A few authors (Mašek, 1960; Kajaba and Bučko, 1968; Cheraskin and Ringsdorf, 1968) nevertheless found a negative correlation between the vitamin C supply and the blood-cholesterol level, i.e., the lower the former, the higher the latter. On the other hand, in nomadic tribes in Kenya known to have a very low cholesterolemia, raised plasma- or leucocyte-ascorbate concentrations were associated with higher cholesterol levels (Gatenby Davies and Newson, 1974).

Cardiomegaly and pronounced electrocardiographic abnormalities, which returned to normal after ascorbate therapy, have been described in patients with scurvy (Shafar, 1967; Singh and Chan, 1974). Hume *et al.* (1972), a few hours after acute myocardial infarction, found an abrupt decrease in the ascorbate concentration in the leucocytes in association with the migration of ascorbate-rich leucocytes to the site of the lesions; ascorbic acid may stimulate protein synthesis in myocardium (Gudbjarnason *et al.*, 1966). In an arteriographic study, Willis *et al.* (1954) found that atherosclerotic changes receded in some of their patients given vitamin C. Spittle (1973) described the therapeutic use of ascorbic acid for vascular thrombosis and concluded that vitamin C mobilized the cholesterol localized in the wall of blood vessels, so that it was released into the blood stream (Spittle, 1971). Lewin (1974) assumed that high serum ascorbate

concentrations would tend to redissolve the arterial deposits, partly through reduction of the surface tension of the serum and partly through the formation of soluble ascorbate anion complexes with calcium localized in the blood vessel wall. These hypotheses have not yet been verified experimentally.

Although we cannot underestimate the therapeutic value of ascorbic acid for diseases of the vascular system, the question of how to prevent hyperlipemia and atherogenesis in man is far more important. The results obtained in experimental animals rank chronic latent ascorbate deficiency among the risk factors in the pathogenesis of atherosclerosis. A widespread incidence of marginal vitamin C deficiency among humans was demonstrated by an analysis of human tissues (Yavorsky *et al.*, 1934) and human arteries (Willis and Fishman, 1955). Permanent chronic vitamin C deficiency is especially frequent among the aged (Hejda, 1969; Wilson and Nolan, 1970; Booth and Todd, 1972; Silink *et al.*, 1972; Burr *et al.*, 1974), but seasonal vitamin C deficiency occurs in all age categories. As far as results obtained in experimental animals can be applied to the human organism, it is probable that chronic latent vitamin C deficiency also slows down the transformation of cholesterol to bile acids in the human liver, with resultant hypercholesterolemia and slower removal of cholesterol from the blood stream. The administration of large doses of ascorbic acid to such subjects ought to stimulate cholesterol catabolism and lead, in time, to diminution of hypercholesterolemia. A daily intake of 300 mg of ascorbic acid during the period of seasonal vitamin C deficiency did, in fact, produce a mild, but statistically significant, decrease in the blood serum-cholesterol level in 7 weeks (Ginter *et al.*, 1970b). In subjects who had an initial plasma-cholesterol and plasma-triglyceride level of over 230 mg% and 200 mg%, respectively, and to whom the administration of two daily doses of 500 mg of ascorbic acid was started during the period of seasonal vitamin C deficiency, a significant decrease was found in the blood-cholesterol level and an exceptionally pronounced decrease in the plasma-triglyceride level (Ginter *et al.*, 1976, 1977). On keeping up the same dose of ascorbic acid, the distinct hypolipemic effect of vitamin C persisted for a whole year (Table X).

The organism of patients with disorders of the cardiovascular system has repeatedly been found to have a poor vitamin C supply (Kishikawa *et al.*, 1971; Samsonov *et al.*, 1972). Knox (1973) carried out a correlation analysis between the standardized mortality ratios for ischemic heart disease and cerebrovascular disease in different parts of England and dietary intakes of a number of nutrients. Vitamin C intakes showed a strong negative correlation, i.e., mortality from cerebrovascular disease was high in regions with low vitamin C intakes, and vice versa (Fig. 25). The

**Table X**  
**INFLUENCE OF ASCORBIC ACID ON BLOOD PLASMA LIPIDS IN HYPERLIPIDEMIC PERSONS WITH SEASONAL VITAMIN C DEFICIENCY**

Parameter	Number of persons observed	Before treatment	Intake of 1000 mg ascorbic acid daily			
			3 months	6 months	9 months	12 months
Plasma cholesterol (mg/100 ml)	19	263 ± 6	237 ± 8	218 ± 9	238 ± 8	229 ± 8
Plasma triglycerides (mg/100 ml)	24	331 ± 22	205 ± 19	173 ± 19	195 ± 9	188 ± 10
Vitamin C in whole blood (mg/100 ml)	19	0.61 ± 0.08	1.67 ± 0.10	1.39 ± 0.10	1.40 ± 0.12	1.42 ± 0.11

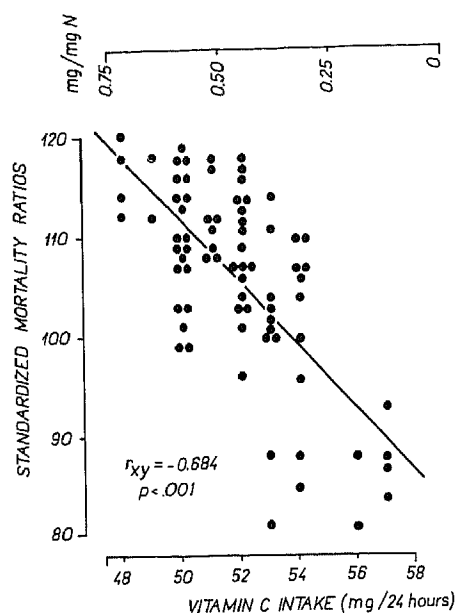


FIG. 25. Negative correlation between vitamin C intakes and cerebrovascular disease standardized mortality ratios in different regions of Great Britain. The figure was constructed from the data of Knox (1973).

relationship between vitamin C intakes and mortality from ischemic heart disease was not so close, but it was likewise statistically highly significant. This type of statistical research does not definitely answer the question of whether vitamin C helps to prevent atherosclerosis from developing in humans. The only way to obtain a full answer to this question would be to conduct an extensive field investigation among a large number of subjects given ascorbic acid over a long period, carrying out a longitudinal study of their blood lipid levels, signs of atherosclerosis and total mortality, which is a more meaningful end point when assessing the health of a whole community (West and Redgrave, 1975). The need for such a study is made all the more urgent by the complete failure of clofibrate and niacin in the secondary prevention of ischemic heart disease (Coronary Drug Project Research Group, 1975).

One obstacle to this study is that we still do not know the optimum dose of vitamin C for the human organism. Pauling (1970) and Stone (1972) regard a daily intake up to two orders higher than the officially recommended dietary allowances for vitamin C in different countries as the optimum. If they were proved to be right, then the dose of 30 mg/adult/day recommended by the FAO/WHO would amount to marginal vitamin

C deficiency. A whole series of laboratories are at present studying the problems of the optimum dose of ascorbic acid (Veen-Baigent *et al.*, 1975; Chatterjee *et al.*, 1975; Yew, 1975; Harper, 1975). Although some authors consider the intake of megadoses of vitamin C to be unnecessary or even harmful (Mašek and Hrubá, 1974; Barness, 1975; Norkus and Rosso, 1975; Schrauzer *et al.*, 1975), well-defined animal experiments indicate that ascorbate requirements are higher than was originally supposed (De Klerk *et al.*, 1973; Rokosova and Chvapil, 1974; Thaete and Grim, 1974; Zannoni and Sato, 1975; Ginter, 1975a; Street and Chadwick, 1975; Kamm *et al.*, 1975; Rivers and Devine, 1975). This applies in a specific manner to different stress situations (pregnancy, the intake of a physiologically unbalanced diet with high sugar and animal fat content, smoking, an excess intake of alcohol and various drugs, the influence of environmental chemical and probably even mental stress) characteristic of our modern civilization, whose future influence can be expected to be still greater.

### IX. Conclusions

Use of the model of chronic marginal vitamin C deficiency, which resembles seasonal latent ascorbic acid deficiency in man, made it possible to study, in experimental animals, the consequences of prolonged ascorbate deficiency for lipid metabolism and for the metabolism of the connective tissue of the blood vessel wall. In guinea pigs with latent ascorbic acid deficiency, the rate of the key reaction of the transformation of cholesterol to bile acids in the liver, i.e., microsomal  $7\alpha$ -hydroxylation of the cholesterol nucleus, is slowed down. The slowing down of cholesterol catabolism leads, in hypovitaminous guinea pigs, to hypercholesterolemia, accumulation of cholesterol in the liver, prolongation of the biological half time of plasma cholesterol, slower total cholesterol turnover, and a decrease in the bile acid pool. Conversely, large doses of ascorbic acid stimulate cholesterol catabolism and total turnover. The plasma-triglyceride concentration rises in vitamin C-deficient guinea pigs. The mechanism of this phenomenon is not altogether clear, but it is probably associated with changes in lipolytic enzyme activities in the blood and tissues of vitamin C-deficient animals. Large doses of ascorbate reduce the plasma triglyceride levels in different laboratory animals. In guinea pigs with marginal vitamin C deficiency, the metabolism of the blood vessel wall is impaired: collagen synthesis is disturbed, changes occur in the mucopolysaccharide composition of the ground substance of the connective tissue in the wall of the aorta.

Vitamin C deficiency in guinea pigs is accompanied by elevated ac-

cumulation of cholesterol and triglycerides in the arch of the aorta, by a raised incidence of nuclear abnormalities in the aortic endothelium, by separation of the endothelial cells, by marked endothelial proliferation, by foam cell formation, by the appearance of large intimal plaques of musculo-fibrotic type, and lastly, by atheromatous reconstruction of the vascular system. The pathogenetic mechanism of these changes is probably complex and is based on impairment of the metabolism of cholesterol, triglycerides, and various components of the blood vessel wall. These changes are induced by latent vitamin C deficiency *per se*, even without the addition of cholesterol to the diet, but the simultaneous intake of cholesterol potentiates them still further.

In most human subjects with elevated plasma-cholesterol and plasma-triglyceride levels and latent vitamin C deficiency, resaturation of their tissues with ascorbic acid can significantly lower their blood plasma-cholesterol and triglyceride levels. The prevention of latent vitamin C deficiency means, for at least part of the population, the hope of physiological control of hyperlipemia. Since chronic latent vitamin C deficiency is probably one of the risk factors in the pathogenesis of human atherosclerosis, it would be both useful and necessary to carry out long-term field investigations with the aim of verifying the possibility of utilizing ascorbic acid in the prevention of vascular diseases.

#### ACKNOWLEDGMENTS

I wish to express my sincere thanks to Prof. Zdeněk Lojda (Charles University, Prague) and to Dr. Jozef Babala (Comenius University, Bratislava) for their excellent cooperation in the histological, histochemical, and enzymological studies and to Ing. Rudolf Nemeč, Mr. Lubomír Ozdín, and Mr. Ladislav Mikuš (Institute for Human Nutrition Research, Bratislava) for their collaboration in the radioisotope studies and for the computer computations. I should further like to thank Mrs. Anna Javorská and Miss Lydia Marková for their invaluable assistance in the preparation of this manuscript.

I am deeply indebted to Prof. Eva Degkwitz (University of Giessen), Mrs. Dorothy F. Sulkin (Wake Forest University, North Carolina), Dr. Ingemar Björkhem and Dr. Anders Kallner (Karolinska Institutet, Stockholm), Dr. Takao Fujinami (Nagoya City University Medical School), Dr. Dietrich Hornig (Hoffmann-La Roche Ltd., Basel), Prof. E. G. Knox (University of Birmingham), Dr. David Kritchevsky (Wistar Institute of Anatomy and Biology, Philadelphia), and Prof. Giorgio Weber (University of Siena) for kindly having allowed me to use the results of their research in this study.

I express my sincere thanks to the Cambridge University Press, ASP Biological and Medical Press (Elsevier Division), Springer-Verlag, New York Academy of Sciences, *American Journal of Clinical Nutrition*, *Lipids*, *Physiologia Bohemoslovaca*, and to Slovak Academy of Sciences for granting us permission to reproduce tabulated data and illustrations.



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