Ann. Rev. Nutr. 1986. 6:365–406

THE BIOCHEMICAL FUNCTIONS OF ASCORBIC ACID

Sasha Englard and Sam Seifter

Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

CONTENTS

SCOPE OF THIS REVIEW

This review is concerned primarily with functions of ascorbate that have been studied at the level of specific enzymatic reactions using in vitro systems. This approach excludes detailed consideration of many functions that become disturbed in the scorbutic animal if they have not also been studied in cell or organ culture systems or using isolated enzymes. In our final discussion we consider

0199-9885/86/0715-0365$02.00

whether, after all, the most important functions of ascorbate reside in other kinds of metabolism, as yet nondescript, for which none of the enzymatic reactions reviewed may be critical. In this article we also list other possible functions of ascorbate, referring only to reviews and a few primary articles. Several more general books and reviews on ascorbate are included in the bibliography (33, 39, 111, 120, 150, 152, 155, 180, 249, 284).

Although this article does not review the literature on the biosynthesis and metabolism of ascorbate per se, these subjects are discussed briefly to provide some background and perspective.

BIOSYNTHESIS AND REQUIREMENTS OF ASCORBIC ACID

Among vertebrates from reptiles through mammals ascorbic acid is probably synthesized by this pathway:

α-D-glucose → → → → UDP-D-glucuronate ⟶ D-glucuronate ⟶ L-gulonate ⟶ L-gulono-γ-lactone ⟶ 2-keto-L-gulonolactone ⟶ L-ascorbic acid.

The first phase of ascorbate synthesis is part of a common pathway for converting glucose to UDP-glucuronate (34). The latter is also used for synthesis of certain glycosaminoglycans and for conjugation reactions in which glucuronides are formed. Diversion of the pathway toward specific synthesis of ascorbate would seem to be at the point where UDP-glucuronate is converted to D-glucuronate. The nature of that conversion is not known in animals, although liver UDP-glucuronyl transferases that catalyze the formation of glucuronides in the presence of a suitable glucuronyl acceptor may possibly hydrolyze UDP-glucuronate in the absence of an acceptor.

The C-1 aldehyde function of D-glucuronate is then reduced enzymatically with NADPH to a C-1 primary alcohol group giving L-gulonate. L-Gulonate lactonizes to form L-gulono-γ-lactone, which is then oxidized by L-gulono-γ-lactone oxidase to form ascorbate.

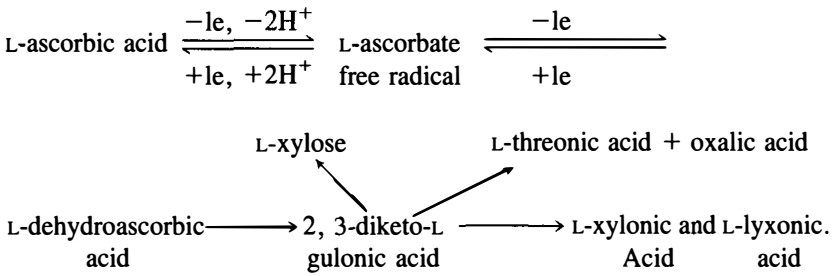
Humans and other primates, flying mammals, guinea pigs, and passeriformes birds do not synthesize ascorbate because they do not express a gene (if they have one) for synthesis of the last enzyme in the pathway, L-gulono-γ-lactone oxidase (39, 241). Although rats are known to synthesize ascorbate, a mutant strain of Wistar rats has been established that does not contain L-gulono-γ-lactone oxidase and must be administered ascorbate in order to develop and grow (184). The actual requirement for ascorbate (vitamin C) in a given species unable to synthesize it is almost impossible to determine, especially since the need may change with physiological status, daily variations, stress, and disease. Indeed some evidence exists that animals capable of synthesizing ascor-

bate can undergo induction of synthesis resulting in increased production (40). Thus, some drugs and anesthetics can induce increased synthesis. That circumstance strongly suggests that species requiring ascorbate in the diet also have a variable need for the vitamin, and that must be considered in the setting of recommended daily allowances. Depending on the margin of safety adopted, different recommendations have been made. In Great Britain the recommended daily allowance for ascorbate in the diets of adult humans is 30 mg, while in the United States it is 60 (227). Recently, despite the recommendation of a panel that the allowance be lowered, the National Academy of Sciences reaffirmed the amount at 60 mg of ascorbate (176).

A recent development that should be noted for its obvious interest and potential is the production by recombinant DNA technology of 2-keto-L-gulonic acid (6), which could be a key intermediate in the chemical synthesis of L-ascorbic acid.

METABOLISM OF ASCORBATE

The main features of metabolism of ascorbate, particularly in vertebrates, are briefly summarized as follows:



Ascorbate can be converted to L-dehydroascorbate by removing two electrons and two protons. Evidence suggests that this occurs in two stages, with a free radical formed intermediately by removal of one electron and two protons (18, 270). Semidehydroascorbate can then give up a second electron to yield L-dehydroascorbate. The free radical can also undergo disproportionation, in which two molecules form one of ascorbate and one of dehydroascorbate (18, 240).

In some plants, ascorbate is converted to L-dehydroascorbate irreversibly by an ascorbic acid oxidase that is a copper-requiring enzyme. In many cells of different species, including mammals, the conversion is reversible to some degree, and is catalyzed by a different enzyme, dehydroascorbate reductase. That enzyme uses reduced glutathione as a cosubstrate, and the products are ascorbate and oxidized glutathione. Should the reaction operate in reverse,

oxidized glutathione would be reduced. In some of these cells another enzyme, glutathione reductase, utilizes NADPH for reduction of oxidized glutathione; one can then construct a cycle in which glutathione and NADPH are used in the overall reconversion of dehydroascorbate to ascorbate, allowing the last two to exist in equilibrium. If NADPH is then syphoned off for use in other reductions (for instance, in the reduction of folate to tetrahydrofolate or biopterin to tetrahydrobiopterin), one can visualize how ascorbate can be used as a source of electrons and protons.

The existence of the intermediate L-ascorbate free radical is incorporated into mechanisms proposed for certain hydroxylation reactions that appear to depend on ascorbate; for instance, such a mechanism has been proposed for the hydroxylation of dopamine to norepinephrine.

Other aspects of the above scheme apply to species that are able to open the lactone ring of L-dehydroascorbate to form 2,3-diketo-L-gulonate. This compound then can go through several possible degradation pathways, as shown above (34, 155, 170, 171, 270). The enzyme involved is a lactonase. If a species were to lack that enzyme, dehydroascorbate could either be reconverted to ascorbate, if mechanisms exist to perform that reaction, or be excreted in the urine. Species that apparently lack the lactonase are humans, other primates, and fishes (270). Significantly, the lactonase is present in the livers of guinea pigs. In theory, the presence or absence of lactonase in species such as the human and guinea pig, both of which cannot synthesize ascorbate, could influence the dietary requirement for the vitamin.

In considering the metabolism of ascorbate, one must recognize that it must be transported into cells and subcellular compartments where it performs its functions. Under physiological conditions ascorbate exists as a monoanion that cannot traverse most membranes readily. However, the subject of transport of ascorbate is only beginning to be studied. We refer to this matter further under our discussion of chromaffin cells of the adrenal glands in synthesis of norepinephrine.

ACTIONS AND FUNCTIONS OF ASCORBATE

Actions and functions of a vitamin can be studied at two levels. First, using a dependent organism one can produce a deficiency disease and examine the disaffected functions and then determine whether these are restored to normal by administration of the vitamin. Second, one can identify specific biochemical reactions in which the vitamin or a cofactor form may act, isolate the enzymes involved, and determine the functions of the vitamin/cofactor in the reactions catalyzed by the enzymes. Reconciling the first and second approaches is frequently difficult. In vivo, observed functional changes may be secondary or

even remote to the specific vitamin deficiency. The complexity of pellagra due to niacin deficiency is a case in point. In vitro, participation of the vitamin in a given reaction does not rule out that another factor operates in vivo. That is a possibility in some of the activities of ascorbate in isolated enzymatic reactions.

Although many physiological and biochemical processes appear to be influenced by ascorbate, the discrete biochemical reactions shown in Figure 1 are chief among those that have been studied sufficiently to warrant consideration here. Even in these reactions, one cannot say that the function of ascorbate is uniquely specific at the in vitro level, because other reductants such as glutathione, cysteine, tetrahydrofolate, tetrahydrobiopterin, dithiothreitol, and 2-mercaptoethanol frequently can be used in place of ascorbate. Still, ascorbate is most effective. If in the whole organism ascorbate is needed to produce another reductant that actually is used in an enzyme reaction, a decline in enzyme activity in scurvy would not be proof of direct participation of ascorbate in the reaction. Furthermore, in vivo, ascorbate could serve other functions to keep the enzyme in its optimal state of activity without directly participating in the mechanism of the reaction. We illustrate this possibility in our discussion of ascorbate and the activity of the prolyl hydroxylases.

Figure 1 is organized to present the known enzymatic reactions influenced by ascorbate according to the following conceptual division. First, the figure shows two monooxygenase reactions (reactions 1 and 2). Both require copper, molecular oxygen, and a reductant such as ascorbate. The ascorbate probably acts at the level of the metal to activate the oxygen and not directly on the substrate (dopamine in one case and a glycine-terminating peptide in the other). Second, a single reaction governed by a dioxygenase (reaction 3) is presented in which both atoms of a dioxygen molecule are incorporated into a single product, homogentisate. In that respect the enzyme involved, 4-hydroxyphenylpyruvate dioxygenase, differs from the other dioxygenases listed subsequently. Those dioxygenases (reactions 4–11) use α-ketoglutarate as a cosubstrate and incorporate one atom of oxygen into succinate and one into the product of oxidation of the specific substrate. All of the dioxygenases appear to be similar in requiring iron in the ferrous state. As we consider below, ascorbate may be required for maintaining iron in the ferrous state should it become adventitiously oxidized; in that case ascorbate would not participate directly in the mechanism of oxidation of the substrates.

A general mechanism for the α-ketoglutarate-dependent dioxygenases can be written as follows:

$$O_2 + \alpha\text{-ketoglutarate} + \text{specific substrate} \xrightarrow[\text{reductant}]{Fe^{2+}} \text{succinate} + CO_2 + \text{hydroxylated substrate.}$$

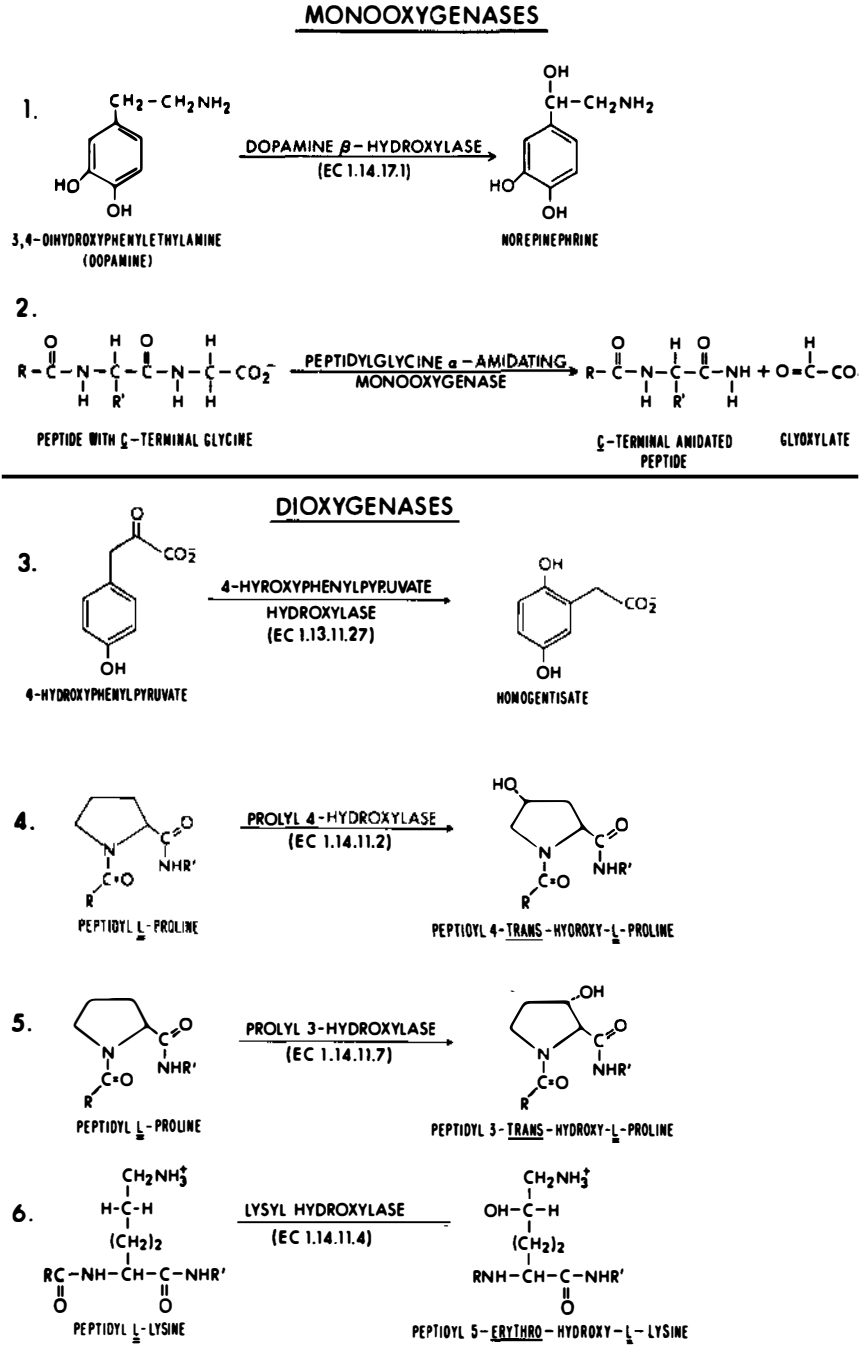
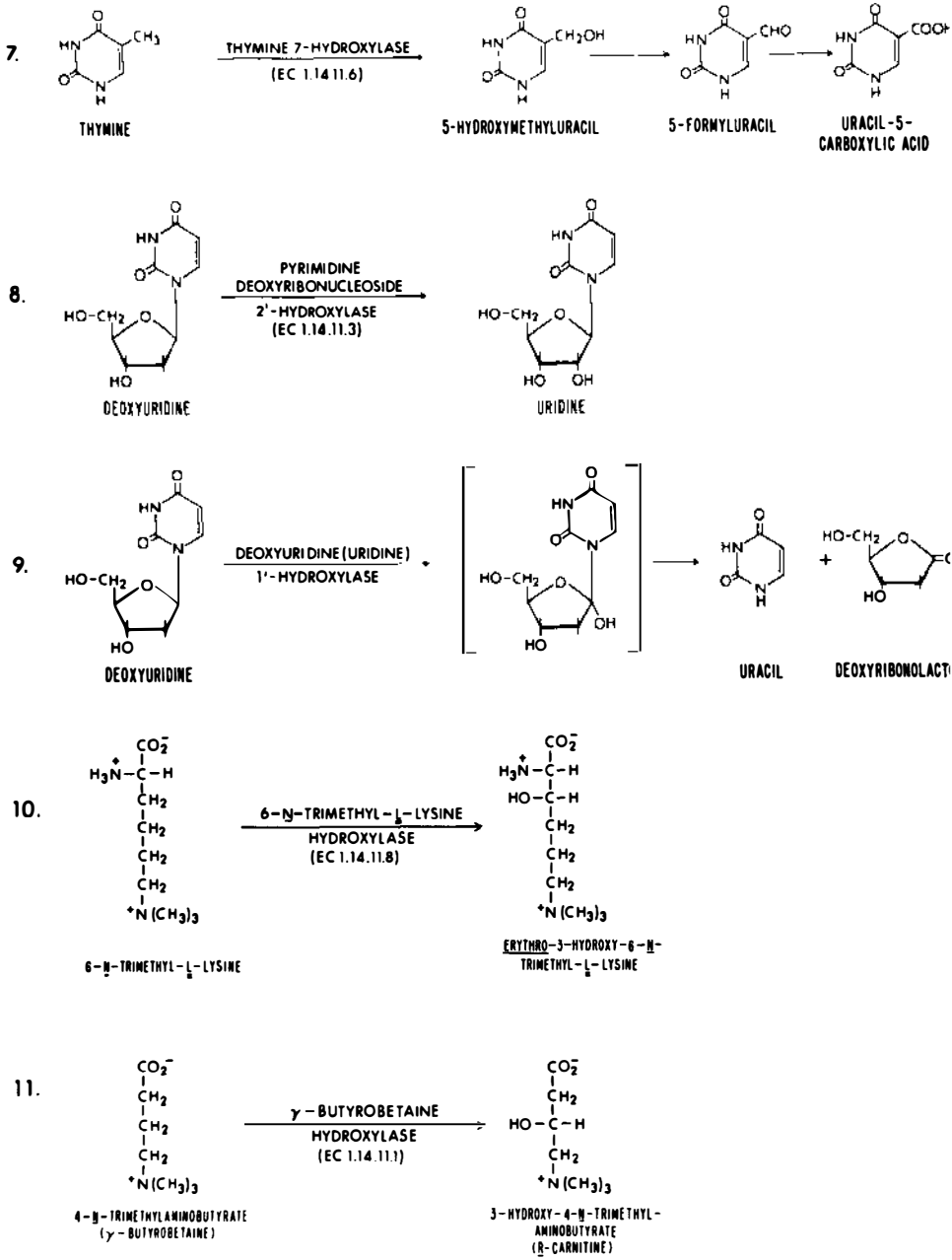


Figure 1 Classification of specific enzymatic reactions in which ascorbate has been implicated. Reaction 3 is a dioxygenase, different from those shown in reactions 4–11 in that α-ketoglutarate is not required as a cosubstrate. Reactions 4–11 do require α-ketoglutarate. Reactions 4–6 are

DIOXYGENASES (Continued)



involved in posttranslational hydroxylation of procollagen chains in animal species. Reactions 7–9 are involved in pyrimidine and pyrimidine nucleoside metabolism in fungi. Reactions 10 and 11 are involved in the biosynthetic pathway of carnitine.

DOPAMINE β-HYDROXYLASE

This enzyme catalyzes the final and probably a rate-determining reaction in the conversion of tyrosine to norepinephrine (reaction 1 in Figure 1). It occurs both as soluble and membrane-bound forms in catecholamine storage vesicles in nervous tissue, and specifically in granules of the chromaffin cells of the adrenal medulla. The enzyme was first purified from bovine adrenal medulla and its action studied (72, 148, 149). It is now known to consist of four identical subunits arranged as dimers joined by disulfide bonds. It has been reported to contain variously from 2 to 12 atoms of copper as Cu^{2+} per tetramer (9, 119, 129, 232, 274), with 8 most probable. All of the above features of the enzyme are reviewed elsewhere in great detail (119, 152, 232, 255).

Ascorbate is considered to be a reductant in the dopamine β-hydroxylase reaction, and indeed the most effective agent when the enzyme is studied in vitro (72, 119, 148, 149). Belief that it may be the physiological reductant is strengthened by the fact that the adrenal medulla contains a very high concentration of ascorbate, exceeded only by adrenal cortex and pituitary in mammalian tissues (100, 121, 152, 177). Furthermore, in the enzymatic assay ascorbate is used stoichiometrically in relation to consumption of oxygen and formation of norepinephrine (72, 119):

$$\text{dopamine} + \text{ascorbate} + O_2 \longrightarrow \text{norepinephrine} + \text{dehydroascorbate} + H_2O.$$

The overall conversion of ascorbate to dehydroascorbate occurs with transfer of two electrons to a suitable acceptor. However, in the dopamine β-hydroxylase reaction some evidence has been obtained showing that the two electrons are donated one at a time in discrete steps, which requires that the ascorbate free radical (semidehydroascorbate) be an intermediate (52, 53, 255, 256, 274). It has been suggested that semidehydroascorbate in this mechanism could be acted on by a reductase to regenerate ascorbate (54), but a problem exists since that reductase occurs in the mitochondria and the hydroxylase is located in the chromaffin granules. Recent studies using mechanism-based inhibitors (44) and analysis of kinetic isotope effects (182) have probed the chemical mechanism of dopamine β-hydroxylase, but the function of ascorbate in reduction of the copper was not considered.

In the adrenal medulla, a further complication exists because the hydroxylase is inside the chromaffin granules (17, 122, 139, 143, 212) and most, but not all, of the ascorbate is found outside in the cytoplasm of the chromaffin cells (55, 108, 151, 266). This could be very important for understanding the function of ascorbate in the physiological reaction. The matter was discussed in great detail by Levine & Morita (152), who summarize several possibilities. For instance, ascorbate could be transferred from the extragranular to the intragranular space

to replenish the ascorbate being consumed in the hydroxylation reaction or, alternatively, the granule membrane could contain an electron carrier system that specifically transports reducing equivalents from ascorbate outside the granules to an electron acceptor in the granules. In the latter case, ascorbate need not participate in the hydroxylation reaction per se, but could serve instead to generate another reductant for the reaction. Thus far no evidence exists for a system of active transport of ascorbate into the granules (269). However, some studies appear to support the concept that the action of ascorbate is related to the transfer of reducing equivalents into the granules, and Levine and his colleagues favor the idea (152, 153).

PEPTIDYL GLYCINE α-AMIDATING MONOOXYGENASE

If one examines the structures of a number of peptides active as hormones, hormone-releasing factors, and neurotransmitters, one notes the frequency with which the carboxyl-terminal residue is amidated. Recently the nature of the amidation reaction was discovered (30, 31, 107, 128, 142). The process is catalyzed by a copper-requiring enzyme that oxidatively cleaves the carboxyl-terminal residue using molecular oxygen (62, 79, 80, 82, 173). This is not a simple hydrolytic cleavage of a peptide bond, because the amino group coming from the terminal residue is retained in the penultimate residue as a terminal amide group, while the remainder of the oxidized terminal residue leaves as an aldehyde (30). Most of the substrates studied in this class of reactions contain a carboxyl-terminal glycine residue, but other amino acid residues have been used in that position (128, 142). When a glycine-extended peptide is the substrate, one can write the reaction as shown in reaction 2 of Figure 1.

The peptides that may be amidated by this enzyme include bombesin (human gastrin-releasing peptide) (259), calcitonin (110), cholecystokinin (octapeptide) (51, 87), corticotropin-releasing factor (273), gastrin (25, 264, 286), growth-hormone-releasing factor (88), α- and γ-melanotropin (81, 195), metorphamide (278), neuropeptide Y (183), oxytocin (140), pancreatic polypeptide (24, 265), substance P (196), vasoactive intestinal peptide (109), and vasopressin (141). The parent precursor proteins of some of those peptides have been studied by molecular biological methods and, as noted in the listed references, their mRNA's reveal a sequence identical to that of the non-amidated peptide extended by a glycine residue.

Bradbury et al (30) proposed a mechanism in which two hydrogen atoms are abstracted from the peptide imino group and the α carbon of the glycine residue so that a double bond forms between the nitrogen and carbon atoms. They considered that the bond then was cleaved hydrolytically with formation of the carboxamide of the penultimate residue and release of glyoxylate.

With studies using a preparation from pituitary glands, Eipper et al (62) then

showed that addition of ascorbate to the incubation medium caused a five-fold increase in amidation over the intrinsic activity. That was compared with a two-fold increase obtained with the best of other reductants used in place of ascorbate. That finding was of special significance since many of the peptides amidated are formed in tissues containing high concentrations of ascorbate, notably the pituitary glands, adrenal glands, and perhaps other nervous tissues.

A partially purified peptidyl amidating enzyme has been prepared from pituitary glands, and requirements for its in vitro assay have been determined (80). The intrinsic amidating activity, that is the activity without added ascorbate, can be diminished by treatment with plant ascorbate oxidase, which suggests that residual ascorbate is present in the preparation and that ascorbate is somehow required (82). On the other hand it has been found necessary to add catalase to the system, which indicates that hydrogen peroxide is formed that can inhibit the enzymatic action (62, 82). The function of catalase, as in the case of other oxygenases, would appear to be largely to protect the enzyme from oxidation by destroying hydrogen peroxide (20).

A nonenzymatic model system for amidation of peptides has been studied (13). This uses molecular oxygen, Cu^{2+} ions and ascorbate. However, catalase added to the system *inhibits* the chemical reaction, which suggests that hydrogen peroxide is a requirement. The investigators proposed a mechanism for the model chemical reaction in which ascorbate is a direct reactant. Comparison of the enzymatic and nonenzymatic reactions therefore has at least one significant difference, the generation and use of hydrogen peroxide. This is a matter yet to be resolved.

Thus in addition to its importance in the formation of catecholamines, ascorbate is given considerably broader significance in the nervous and endocrine systems.

4-HYDROXYPHENYLPYRUVATE DIOXYGENASE

This enzyme catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate as part of the pathway for completely oxidizing tyrosine to carbon dioxide and water. It has been purified from the livers of birds (275) and mammals (144, 194, 230) including humans (157). It has also been prepared from *Pseudomonas* sp. P.J. 874 (166). In its action the enzyme uses both atoms of dioxygen to catalyze the coupled oxidations within the same molecule as depicted in reaction 3 of Figure 1. One atom of oxygen is used for the oxidative decarboxylation of the sidechain pyruvate residue, thus leaving a sidechain acetate residue (156). The second oxygen atom of molecular oxygen is incorporated into a para hydroxy group as the acetate group moves to the ortho position; this occurs within a framework of the so-called NIH shift (156). The enzyme is truly a dioxygenase (156), and the similarity of its action with those

of the α-ketoglutarate dioxygenases has been noted (3, 91), with a significant difference. In the latter group of enzymes the α-keto acid that becomes oxidatively decarboxylated is a separate external molecular substrate, whereas with the 4-hydroxyphenylpyruvate dioxygenase the α-keto acid is attached to the same molecule that becomes hydroxylated. All of the dioxygenases listed here share a requirement for ferrous iron (3, 91). Mechanistic studies have been performed with the 4-hydroxyphenylpyruvate dioxygenase (144, 235, 236). These have not advanced sufficiently to assign a specific function for the iron, although metal chelating agents inhibit the reaction (3, 91). No confirmatory evidence has yet been obtained for the proposed chemical mechanisms (3, 89, 91, 179, 238, 280).

Several reducing substances have been studied as activators for this enzyme, as in the case of the other dioxygenases (3, 83, 91, 288). However, in this reaction the clear superiority of ascorbate over all other reducing agents tried is not as evident. Reduced 2,6-dichlorophenolindophenol is almost as effective as ascorbate in assays done in vitro. The enzyme can be induced in *Pseudomonas* sp. P.J. 874 by inclusion of tyrosine in the medium, and then can be assayed in vitro using ascorbate (166, 167, 236). If the organism uses the induced enzyme in its metabolism of tyrosine, one wonders what the reductant is since it probably is not ascorbate.

That a relationship does exist between ascorbate and metabolism of tyrosine in mammals appears to be firmly established from experimental studies with scorbutic guinea pigs and from clinically related studies with children (83, 103, 130, 133, 137, 138). Scorbutic guinea pigs who are given either free or protein-bound tyrosine in their diets exhibit tyrosinemia and excrete metabolites of tyrosine in the urine, especially 4-hydroxyphenylpyruvate and 4-hydroxyphenyllactate (246–248). Human premature infants exhibit the same pattern: tyrosinemia and appearance of tyrosine metabolites in the urine (154). In both cases administration of ascorbate ameliorates the difficulty. As a matter of fact, since the work of Levine et al (154) and Nitowsky et al (199), premature infants in many nurseries are yet treated routinely with pharmacological doses of 100 to 200 mg ascorbate per day, and the results are clear. Kretchmer et al (134) studied the livers of premature infants for the enzymatic activity of 4-hydroxyphenylpyruvate dioxygenase, and found it to be greatly diminished. However, the activity was not elevated by including ascorbate in the incubation medium. The enzyme is now considered to be a "developmental" enzyme along with tyrosine aminotransferase (83, 133). That is to say, full development of adult enzymatic levels is not achieved until some time after birth. If, in premature infants, the dioxygenase is not yet expressed, one wonders why ascorbate has any effect since the aminotransferase does not require it, and furthermore it too is not fully expressed. There is some evidence that the 4-hydroxyphenylpyruvate that forms in the aminotransferase reaction is in itself

an inhibitor for the dioxygenase, and that the ascorbate as a reductant overcomes the inhibition (3, 91, 167). If that indeed is the case, the action of ascorbate in the dioxygenase reaction again would be indirect and not critical to the mechanism per se.

PROLYL AND LYSYL HYDROXYLASES FOR COLLAGENS

For general background on the subject of collagen structure and metabolism we have listed some representative books and reviews (23, 27, 69, 74, 75, 104, 202, 211, 217).

The known hydroxylases of collagen metabolism, prolyl 4-hydroxylase, prolyl 3-hydroxylase, and lysyl hydroxylase, are all α-ketoglutarate-dependent dioxygenases that require ferrous iron. They catalyze reactions 4, 5, and 6 shown in Figure 1. In their in vitro assay the enzymes require the presence of a reductant, the most effective being ascorbate. Because of the historical association of scurvy and recognized abnormalities of collagen metabolism, the finding that ascorbate is involved in those hydroxylations appeared to provide a logical basis for explaining the scurvy-collagen relationship. However, the subsequent detailed studies of effects of ascorbate on collagen metabolism are fraught with both apparent and real contradictions; and at present the evidence does not permit the conclusion that the main function of ascorbate is in the hydroxylation of prolyl and lysyl residues. Rather it would seem to be in the more nondescript but nevertheless very important effects of ascorbate on protein biosynthesis. Here we briefly trace the history of investigations that have led to this way of thinking.

Early studies in humans and guinea pigs demonstrated conclusively that scurvy is characterized by poor healing of wounds that can be rectified by administration of ascorbate. That, and the diminished patency of blood vessels resulting in bleeding, indicated that major pathology in scurvy is in the realm of the biochemistry of collagen (e.g. 147). About 25 years ago, when it was established that prolyl and lysyl residues in collagen are hydroxylated post-translationally (e.g. 250), the nature of the hydroxylating enzymes came under examination. A substrate called protocollagen was prepared by incubating collagen-forming tissues either in the presence of a metal chelator such as α,α'-bipyridyl or in the absence of oxygen (113, 216). Protocollagen is an underhydroxylated, operational, not biological form of collagen that can be used to study the hydroxylation reactions and to determine their requirements. Later, synthetic polypeptides were introduced as substrates. [Parenthetically, the instability of the underhydroxylated form of collagen led to the now-accepted conclusion that formation of a stable triple-helical procollagen molecule depends on the occurrence of hydroxyproline residues, thus for the first

time giving a function for prolyl hydroxylation. Functions for lysyl hydroxylation are not as well established, except that hydroxylysyl residues, like those of lysine, take part in crosslinking of collagen, and that they make possible subsequent posttranslational modifications, namely glycosylation and the recently described phosphorylation (272c).]

The availability of protocollagen and peptide substrates allowed the preparation and characterization of the hydroxylases. Indeed, then, homogeneous preparations of prolyl 4-hydroxylase were made from chick embryo (17a, 197a, 271b), newborn rat tissues (41a, 228a), human tissues (135a), and cultured L-929 fibroblasts (116a). Prolyl 3-hydroxylase has not been isolated as a homogeneous protein but has been purified extensively (271a). Homogeneous preparations of lysyl hydroxylase have been made from chick embryos (272a) and from human placenta (272b). When the homogeneous preparations were studied, results obtained earlier with less pure preparations were confirmed, and it was established that the hydroxylases require ferrous iron and ascorbate, among other reductants, as activators. The requirement for ascorbate was increasingly more specific in relation to degree of purity of the enzyme.

From the results with ascorbate an impression emerged and seemed to flourish that the collagen "abnormality" in scurvy must be related in some way to poor hydroxylation of collagen. That idea has been expressed in some textbooks and scholarly review articles despite a lack of real supporting evidence. Several investigators, working before hydroxylation was discovered to be posttranslational, had failed to find the collagen of scorbutic guinea pigs to be relatively proline-rich and hydroxyproline-poor, so that no defect in hydroxylation was apparent (84, 86, 229). The seeming contradiction bothered workers in the field, if only subliminally, and studies continued to be made both in scorbutic animals and in cell culture systems to try to define the effect of ascorbate deficiency on collagen metabolism.

Barnes et al (10) found no difference in urinary hydroxyproline excretion in scorbutic as compared to normal guinea pigs. They concluded that even after collagen synthesis was impaired in scurvy no evidence could be found for occurrence of an underhydroxylated collagen. Thus no protein corresponding to protocollagen has been found to occur in scurvy.

In another study, Barnes et al (11) analyzed skin collagen from ascorbate-deficient guinea pigs that had been injected with tritiated proline. They studied a fraction of collagen that was soluble in hot trichloroacetic acid, nondiffusible, and degradable by bacterial collagenase. At several stages of ascorbate deficiency, measured ratios of proline:hydroxyproline indicated that the degree of proline hydroxylation was diminished from normal by only about 10%. The authors were aware that if underhydroxylated collagen formed and turned over more rapidly than normally hydroxylated collagen, the value of 10% decrease could be an underestimation; indeed they did find some diffusible peptide-

bound hydroxyproline. In summary, studies from that laboratory indicated that scorbutic guinea pigs synthesized less collagen but that this was not seriously underhydroxylated.

We now summarize selectively the extensive literature on the effects of the presence or absence of ascorbate in culture media in which various cell lines and strains were grown. Collagen synthesis per se was *increased* in human skin fibroblasts (188, 190) and primary avian tendon cells (244, 245). An *increase* also was found in insoluble collagen in the extracellular matrix of neonatal rat aorta smooth muscle cells and in calf aorta smooth muscle cells (12, 242). *No effect* on collagen synthesis in presence of ascorbate was noted in other studies with human skin fibroblasts (26), human synovial cells (135), chick embryo tendon fibroblasts (207), L-929 cells (200, 208), mouse 3T6 cells (15, 200), and mouse 3T3 cells (207). *Decreased* collagen synthesis was found in human fetal lung fibroblasts (206).

With respect to the activity of prolyl hydroxylase in presence of ascorbate, the following variable results have been reported. *Decreased* activity was observed in cultured human skin fibroblasts (188, 190) and in human skin fibroblasts obtained from persons with Ehler-Danlos syndrome, in which the collagen is hydroxylysine-deficient (220). Hydroxylation was *unchanged* in either the presence or absence of ascorbate in cultured fibroblasts from chick embryo tendons (117). *Increased* activity was found in human skin fibroblasts (189), WI-38 human fetal lung fibroblasts (41), human synovial cells (135), 3T6 fibroblasts (146) and 3T3 fibroblasts (67), and L-929 fibroblasts (260).

With respect to lysyl hydroxylase activity in presence of ascorbate, the following has been reported. *Increased* activity was found in cultured human skin fibroblasts (188, 190). *No change* in activity was reported for human skin fibroblasts from persons with Ehler-Danlos syndrome in which the collagen is hydroxylysine-deficient (220).

Levels of hydroxyproline in collagens formed by cells in the *absence* of added ascorbate were found to range as follows. *Mildly decreased* levels were found in human skin fibroblasts (188), aging human fetal lung fibroblasts (206), primary cultures of chick tendon fibroblasts (118), and virally transformed BALB 3T3 cells (67). *Severely decreased* levels were observed in human skin fibroblasts (26), human fetal lung fibroblasts (68), skin fibroblasts from persons with Ehlers-Danlos syndrome in which collagen is hydroxylysine-deficient (221), chick embryo tendon fibroblasts (67, 207), rat smooth muscle cells (47), 3T6 fibroblasts (8, 14), and 3T3 fibroblasts (207).

Levels of hydroxylysine in collagens formed by cells in the *absence* of ascorbate showed the following. There was *no change* from when ascorbate was present in human skin fibroblasts (188) and in skin fibroblasts from persons with Ehlers-Danlos syndrome in which collagen is hydroxylysine-deficient (221). A *decreased* level was observed in 3T6 fibroblasts (8), the decrease

being less than that observed for the hydroxyproline level in the same experiment.

In order to account for this wide variability, some investigators have searched for the presence of factors that could substitute for ascorbate in collagen metabolism. A reductant has been obtained from L-929 cells that could function in place of ascorbate in the prolyl hydroxylation reaction (178, 209). A different factor stimulating prolyl hydroxylation in cultured muscle cells was obtained from rat embryonic brain (114).

The variability and contradictions evident in the above recital of results are remarkable. Sometimes even in the same experiment measurement of two parameters that should be related gave puzzling results; for example, in some experiments the level of hydroxylation and the activity of hydroxylating enzymes are discordant. Before concluding that our understanding of the function of ascorbate is utterly confused, some probable reasons for the variability should be noted. Murad et al (188) and Schwarz & Bissell (244) and others have considered this matter. Thus one can point to the differences between human cell strains and animal cell lines. The former have finite life span in culture, the latter behave like transformed cells and show no limit in population doublings. The former are mostly diploid, the latter show considerable polyploidy. The human cell strains show density-dependent inhibition of growth in culture, while the animal cell lines do not. The two have different requirements for fetal calf serum. Primary cultures of chick tendon cells show inhibition of collagen synthesis when the serum concentration is greater than 0.5%, a concentration usually exceeded in many of the studies reported for cell lines. In most cases the precise origin of the cell being used is not known; thus even fetal lung fibroblasts or skin fibroblast cultures are grown out from explants of tissue that contain many types of cells. Even the cells that grow out may be of several kinds, making the culture a mixture. This is important because cells of different origin have variable capacity to express one or another collagen genes from the complete repertoire that occurs in most cells of an animal. Cell lines have largely lost the ability to synthesize collagen; whereas, for example, human skin fibroblasts and chick tendon cells in culture devote about 15 and 23% respectively of total protein synthesis to collagen, animal cell lines are much less devoted.

In addition to the above, studies with various cell populations have often employed different methodological approaches that have important influence on the interpretations. Some studies have used methods that do not distinguish between collagen synthesis and change in activity of prolyl hydroxylase. Even when methods are used to permit that distinction, time scales of experiments often differ, and no assurance is given that metabolic states of cells are comparable. For collagen synthesis to occur, an optimal pool of amino acids must be present, and some kinds of cells interconvert amino acids differently

from other cells. Some cells have greater capacity than others to synthesize proline from glutamate and glutamine, and that could become a limiting factor in collagen synthesis (116, 254). Another factor is that cells in culture may not elaborate the peptidases required to process procollagen to extracellular collagen. Related to that is the fact that when procollagen is cleaved by those peptidases, extension propeptides are liberated to the medium. The latter feed information back to the cell, possibly at the transcription level, to regulate the synthesis of more procollagen (99, 131, 203, 279). In the studies of cell culture systems that we have enumerated, many of these factors are largely unmonitored and uncontrolled.

Using calf and rat embryo aortic smooth muscle cells, several studies have been performed to demonstrate that ascorbate greatly influences the synthesis and *deposition* of extracellular matrix conponents, including collagen (12, 242). The idea has been advanced that once the matrix is formed it can, in a kind of "dynamic reciprocity," cause the cells to modulate their protein synthesis in regard to components of the matrix, and the need for added ascorbate might diminish (242, 290). That view was tested by growing cells on a preformed matrix; under those conditions no added ascorbate was needed to maintain the level of collagen synthesis. The stimulation of protein synthesis in fetal calf smooth muscle cells by ascorbate was associated with an increase in the proportion of poly (A^+) RNA in the total RNA pool, perhaps in response to the matrix formed (290). Ascorbate was found not only to affect protein synthesis, but to have a measured effect on growth characteristics and morphology of the cells.

Recently two groups of investigators have sought to develop ways to eliminate some of these many problems. They chose two model systems to study. Chojkier et al (42) returned to the study of the scorbutic guinea pig: they removed calvaria and maintained them in culture with and without ascorbate. After guinea pigs were on an ascorbate-free diet for two weeks, their calvaria showed a preferential decrease in rate of collagen synthesis dissociated in time from decreased hydroxylation of proline residues. Ascorbate added to cultures of calvaria from scorbutic animals restored the rate of prolyl hydroxylation to normal but did not affect the rate of collagen synthesis. Thus the decrease in collagen synthesis was not due to decrease in hydroxylation of prolyl residues in collagen, but must have been related to something else that happens to animals deprived of ascorbate. Chojkier et al (42) then were able to show that the decrease in collagen synthesis was linearly related to the decrease of body weight of the animals as they were continued on the ascorbate-free diet. The experimental protocol was then turned around, and animals were made to lose weight by food restriction while receiving ascorbate in the diet. Such animals showed an apparently specific decrease in collagen synthesis. In other experiments, collagen synthesis was found to decline in a number of different

tissues when guinea pigs were starved for several days while being given ascorbate (258). Again the rate of decline of collagen synthesis was greater than that for other protein synthesis. All of those experiments point to the dissociation of hydroxylation from collagen synthesis, and the control of collagen synthesis by other metabolic events set into operation when an animal is deprived of ascorbate. The investigators thought that collagen mRNA levels were modulated downward in ascorbate-deficient states.

Schwarz and coworkers (244, 245) used cultures of primary avian tendon cells as a model system for studying the effects of ascorbate on collagen metabolism. In that model, maintenance of a large pool of procollagen within the cell would appear critical for regulating the synthesis of more procollagen. Addition of ascorbate to cultures at high cell density increased secretion rate and reduced the procollagen pool within the cell (243). The decreased pool size of procollagen was considered to trigger an increase in its additional synthesis. In other studies that laboratory found that the effect was at both the level of transcription and stability of the mRNA (172, 234).

In order to be able to relate all of the above findings obtained with scorbutic animals and cultured cells with what is known at the molecular level about ascorbate and hydroxylation reactions, we now consider the mechanisms of hydroxylation. Detailed reviews on that subject have appeared (38, 90, 123–126, 215). We refer also to selected primary articles pertinent to the discussion that follows (45, 48, 49, 174, 175, 191–193, 198, 218, 219, 222, 272).

A widely accepted mechanism for prolyl hydroxylation catalyzed by prolyl 4-hydroxylase (127), presumably operative in lysyl hydroxylation as well, is shown in Figure 2. The following can be stated concerning that mechanism.

1. In the overall hydroxylation reaction in vitro, ascorbate is used non-stoichiometrically. Thus, with all substrates and cofactors present, when one α-ketoglutarate is oxidatively decarboxylated concertedly with the hydroxylation of one proline residue of the substrate, the consumption of ascorbate is not stoichiometric.

2. The isolated purified prolyl 4-hydroxylase can go through a number of cycles of activity without added ascorbate before it loses activity (191, 198).

3. Kinetic evidence suggests a proposed mechanism in which the only action of ascorbate is to maintain the iron in the ferrous state (193, 272).

4. The enzyme can be made to carry out an uncoupled reaction: that is, α-ketoglutarate can be oxidized by molecular oxygen to produce succinate and carbon dioxide, without concomitant hydroxylation of prolyl residues in a suitable substrate (38, 45, 222). Under these conditions the ascorbate utilized is stoichiometric with the decarboxylation reaction (49, 192).

5. In the uncoupled reaction, the reactive ferryl-oxo complex decomposes to Fe^{3+} + O·, and ascorbate reduces the enzyme-bound Fe^{3+} to Fe^{2+} (48, 49, 192).

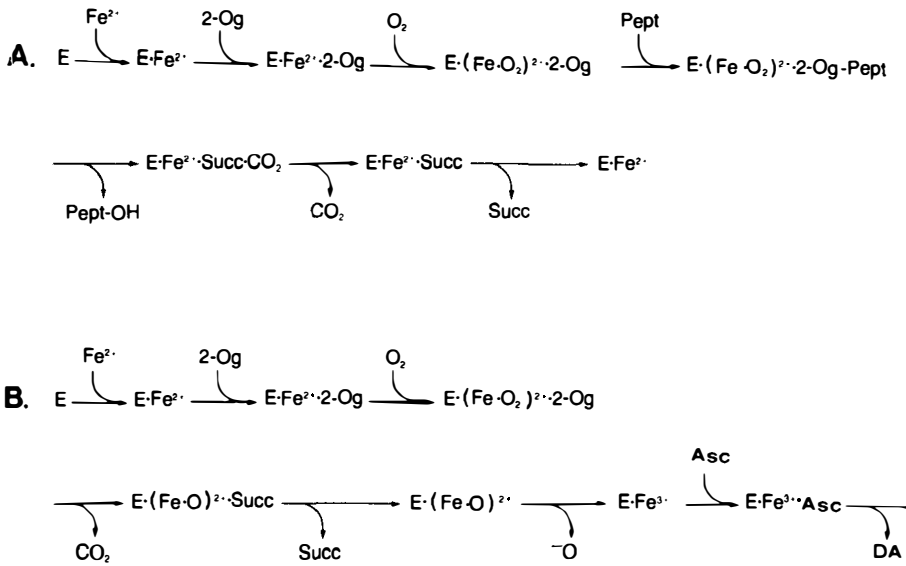


Figure 2 Schematic representation of the mechanism for the prolyl 4-hydroxylase and lysyl hydrox reactions. The complete hydroxylation reaction is thought to proceed according to scheme *A*, in which the orc binding of O_2 and the peptide substrate and the order of release of the hydroxylated peptide and CO_2 are uncer In the absence of the peptide the enzymes catalyze an uncoupled decarboxylation of 2-oxoglutarate ketoglutarate) (scheme *B*). Certain peptides that do not become hydroxylated are known to increase the rate c uncoupled decarboxylation. In the uncoupled reaction the reactive iron-oxo complex is probably converted to and O·, and ascorbate is needed to reactivate the enzyme by reducing the Fe^{3+} to Fe^{2+} (*B*). E = enzyme; 2-(oxoglutarate; Pept-OH = hydroxylated peptide; Succ = succinate; Asc = ascorbate; DA = dehydroascor [This figure and legend are reproduced with permission of the authors from an article in press by K. I. Kivi and R. Myllylä (127)].

Myllylä et al (192) state that since, even in the presence of a hydroxylatable substrate a number of unproductive decarboxylations may occur, inevitably the enzyme-bound iron becomes oxidized and requires a reductant, ascorbate being most effective.

A novel and fascinating series of experiments has been performed with ascorbate and its analogs to determine whether prolyl hydroxylase has a binding center for the reductant (K. I. Kivirikko, personal communication). Ascorbate was found to bind to the enzyme, its ring atoms but not its sidechain being required for that purpose. Thus D-isoascorbate and 5,6-*O*-isopropylidene ascorbate yielded V_{max} and K_m values almost identical to those of ascorbate. The investigators concluded that the binding site consists of two *cis*-positioned coordinated sites of the enzyme-bound iron, and is partially shared by the α-ketoglutarate site of the enzyme, i.e. the site at which succinate and carbon dioxide are formed. In the uncoupled reaction, if that site is not occupied by ascorbate, the ferryl ion intermediate decomposes and the iron is oxidized to the

trivalent state. This is consistent with the stoichiometric requirement of ascorbate in the uncoupled reaction.

The evidence that ascorbate is not required in the hydroxylation reaction per se may then explain much of the work with scorbutic guinea pigs showing that ascorbate deficiency does not produce severe underhydroxylation of collagen.

A different kind of presumptive evidence that ascorbate is concerned in collagen synthesis comes from studies with chick embryos (285). The chicken egg is a closed system. Until time of hatching, the chick egg-embryo system devotes much of its metabolism to the synthesis of collagenous structures. The fertile chick egg contains no detectable ascorbate until it is incubated; then synthesis of ascorbate increases rapidly, and simultaneously there is an increase of L-gulono-γ-lactone oxidase in the mesonephros, metanephros, and the yolk sac membrane. The yolk sac membrane synthesizes and accumulates the largest amount of ascorbate, and this increases throughout the incubation period as collagen synthesis also proceeds actively. (Of interest also is that the embryonic brain tissues contain high levels of ascorbate but do not appear to synthesize it. That might be of importance in relation to the neuroendocrine functions of ascorbate that were considered earlier in this review.)

In considering whether ascorbate is specific as a reductant in all collagen hydroxylations, it would be useful to know whether invertebrate species that manufacture collagens either synthesize ascorbate or concentrate it from their environment. For example, the gross composition of the collagen made by the sponge *Trematomus leonbergii* reveals large numbers of residues of 3-hydroxyproline, 4-hydroxyproline, and hydroxylysine (27). *Ascaris* cuticle collagen contains about 2% of its amino acid residues as those of 4-hydroxyproline, and earthworm cuticle collagen about 17% of its total residues as those of hydroxyproline (250). Neither of the cuticular collagens appears to contain hydroxylysine residues. The physiological reductant for those hydroxylations is not known.

HYDROXYLATION OF OTHER PROTEINS

Elastin

Barnes et al (11) studied the hydroxylation of proline residues in elastin of aortas of scorbutic animals. Elastins have about 1% of their proline residues as hydroxyproline. The investigators found no decrease in elastin from normal, but determined that the protein was underhydroxylated. Much later, Barone et al (12), studying cultured neonatal rat aortic smooth muscle cells with ascorbate present in the growth medium, found that the precursor tropoelastin appeared to be *overhydroxylated,* and the amount of insoluble elastin in the extracellular matrix was decreased from that found when no ascorbate was present in the medium. They considered the possibility that overhydroxylation interfered

with subsequent posttranslational cross-linking reactions required to convert soluble tropoelastin to insoluble elastin. Taken together the studies on elastin seemed to indicate that in vivo ascorbate deficiency did not decrease elastin synthesis but did cause its underhydroxylation; whereas the *presence* of ascorbate in the in vitro experiment decreased the deposition of insoluble elastin, presumably because of the overhydroxylation of tropoelastin. Schwartz et al (242) did not find elastin in the extracellular matrix deposited by cultured calf aortic smooth muscle cells in either the presence or absence of ascorbate.

C1q of Complement

At the beginning of the century, not long after discovery of the complement system, guinea pig serum (among many sera tested) was found to exhibit the highest titer of complement as assayed in a sheep erythrocyte hemolytic system. With the use of complement fixation tests for syphilis, many laboratories housed guinea pigs for bleeding as a source of serum complement. Several investigators found that complement activity was reduced during the winter months, and attributed that to a seasonal lack of leafy green vegetables in the diet and accordingly to a lack of vitamin C (60). Later a correlation was shown to exist between complement levels and ascorbate concentrations of guinea pig and human sera (59, 61). The practice was then adopted of feeding laboratory guinea pigs a diet containing sufficient lettuce, cabbage, or ascorbate per se. The effect of ascorbate on complement activity was considered to be related to its capacity as a reductant, and an interplay with another reductant, glutathione, was studied (58).

In the early 1970s, C1q, one of the subcomponents of the complement system, was shown to contain an assembly of polypeptide chains collagen-like in nature (213, 228). Those chains contain residues of hydroxyproline and hydroxylysine. Müller et al (187), studying C1q synthesis in guinea pig macrophages, noted that α,α'-bipyridyl reduced the secretion of C1q, and they inferred that the mechanism of hydroxylation of its prolyl and lysyl residues was the same as for collagen per se, particularly in sharing a requirement for Fe^{2+}. Bates et al (16) noted that scurvy in guinea pigs did not reduce their total C1 component as measured in a functional assay. That would imply that no change had occurred in C1q since it is a subcomponent of C1.

Those authors then tried to address the question why, in scurvy, collagen synthesis is affected whereas C1 synthesis (i.e. C1q) apparently is not. One consideration was that, even though both systems share collagen or collagen-like features, their individual synthesis might take place in different tissues or cells containing different concentrations of ascorbate. However, a cellular system that makes both collagen and C1q has been studied with and without addition of ascorbate to the culture medium. Morris & Paz (186) studied human

fetal lung fibroblasts for the effect of ascorbate on C1q hydroxylation. They isolated C1q from the culture medium of cells grown with tritiated proline and analyzed collagenase-derived peptides for their contents of radiolabeled proline and hydroxyproline. They found that cells grown with ascorbate produced C1q with more hydroxyproline than those that had been grown without added ascorbate. Furthermore, in a functional test for C1q in which it was combined with $C1\bar{r}$ and $C1\bar{s}$, they found that the C1q produced in the absence of ascorbate, i.e. the underhydroxylated component, was considerably diminished in function; it did not bind as well to antigen-antibody-C4 complex.

The fact remains that overall complement activity in the animal may decrease in ascorbate-deficient states (16) even if C1q activity is unchanged. A similarity with the collagen situation in scurvy is possible. The synthesis of total collagen appears to decrease but the hydroxylation of the collagen is not seriously affected. One may infer that hydroxylation of C1q in ascorbate deficiency in animals is not affected since C1 is functionally unchanged, but perhaps synthesis of some other protein component of the complement system is diminished.

Note that another protein, acetylcholinesterase, also has a covalently attached assembly of collagen-like polypeptide chains; these anchor into basement membranes in certain kinds of neurons (233). That kind of acetylcholinesterase contains both hydroxyprolyl and hydroxylysyl residues. To our knowledge no studies have been made in scorbutic animals concerning the activity of acetylcholinesterase. That other proteins may be found to contain hydroxylysine is presaged by reports noting its occurrence in the precursor peptide of somatostatin of the anglerfish (7) and in bovine conglutinin (46a).

DIOXYGENASE REACTIONS OF PYRIMIDINES AND NUCLEOSIDES

The reactions catalyzed by these dioxygenases are shown in Figure 1 as reactions 7, 8, and 9. They occur, as far as has been studied, in fungi. Reaction 9, the last to be described, occurs in *Rhodotorula glutinis* (263) and reactions 7 and 8 in *Neurospora crassa* (1, 2, 21, 22, 168, 169, 252, 253, 277) and *Rhodotorula glutinis* (263, 282). They are considered to be involved in so-called salvage or reutilization pathways for both the pyrimidines and the deoxyribose moiety of deoxynucleosides (251).

In the three reactions shown, the requirement for Fe^{2+}, molecular oxygen, and α-ketoglutarate appear to be absolute. The incorporation of $^{18}O_2$ has only been studied for the first and third steps in the sequence for the thymine 7-hydroxylase. In that case one atom of $^{18}O_2$ went into succinate and the other into 5-hydroxymethyluracil or into uracil 5-carboxylic acid (97). In reaction 9, the postulated intermediate shown then becomes cleaved to form uracil and a lactone (263).

The mechanisms of these reactions have not been explored in detail, but the purified enzymes are enhanced in their activities by the presence of one of several reducing agents, ascorbate being best. In reaction sequence 7, the thymine 7-hydroxylase catalyzes each of the three reactions, so that a total of three dioxygen molecules and three α-ketoglutarate molecules are used. Each of the three steps has been studied independently and show markedly different requirements for ascorbate (276). A puzzling aspect of the 7-hydroxylase reaction as studied with the purified enzyme is that nucleoside di- and triphosphates partially substitute for ascorbate (283).

Finally, the thymine 7-hydroxylase has been shown to catalyze the uncoupled decarboxylation of α-ketoglutarate in the absence of one of the hydroxylatable substrates and in the presence of a high concentration of uracil (101) or 5-fluorouracil (96). In the latter case, five times more ascorbate was required than when a hydroxylatable substrate was present. Those results appear to be somewhat comparable and strongly related to what was observed in the uncoupling of the prolyl and lysyl hydroxylases referred to earlier in this review. Thus, in both cases the requirement for ascorbate may be accessory to hydroxylation, the ascorbate perhaps being necessary for reduction of the iron atom if it gets oxidized in reactions not scheduled by the mechanism.

One should also add that the physiological reductant for these reactions is not known. Whether the species of fungi that contain these enzymes are able to synthesize ascorbate or at least concentrate it from their environment is not known. It is known that some yeasts are able to synthesize ascorbate from L-gulono-1,4-lactone and L-galactono-1,4-lactone (145). In addition, steady-state kinetic analysis and determination of intra- and intermolecular isotope effects in the reaction do not resolve the question of whether ascorbate is a direct participant (94, 95). Thus, as in the other dioxygenases requiring α-ketoglutarate, two possibilities exist concerning the need for ascorbate: either uncoupling of the reaction hardly occurs in vivo as it does in vitro, or it can use a reductant different from ascorbate if some unscheduled oxidation of the iron occurs.

CARNITINE PATHWAY HYDROXYLASES

The metabolism of carnitine and its functions in humans are considered in another chapter of this volume (226) and its general aspects were reviewed in an earlier volume of this series (28). Our purpose here is to describe the effects of ascorbate on the two hydroxylation steps in the biosynthesis of carnitine (reactions 10 and 11 in Figure 1). However, for purposes of this discussion, we present a few broad statements about carnitine.

Carnitine is required in fatty acid metabolism for formation of acetyl and other acyl carnitine derivatives that can cross mitochondrial and peroxisomal

membranes (32, 71, 73, 271). In particular, acyl carnitines are required for transport of fatty acids into mitochondria, where they can be oxidized. In carnitine deficiency states it is possible that fatty acids not oxidized are then converted to triacylglycerols, which would increase the amount of these.

The biosynthesis of carnitine is discussed in several reviews (e.g. 32, 71). Carnitine is derived ultimately from lysine, with an early step being the conversion of lysine to 6-*N*-trimethyl-L-lysine; the methyl donor is S-adenosyl methionine. Among a great many species that can make carnitine, there are two general ways in which 6-*N*-trimethyl-L-lysine can form. In *Neurospora crassa* the pathway from free lysine is direct (29, 223). However, in mammals this simple direct conversion does not occur. Rather, lysine must be incorporated into certain proteins, not yet adequately identified, in which specific lysine residues are then converted to 6-*N*-trimethyl-L-lysine residues (56, 136). Proteins in which 6-*N*-trimethyl-L-lysine has been found include histones, myosin, calmodulin, and cytochrome c. [Protein methylation is reviewed by Paik & Kim (204).] The residues of 6-*N*-trimethyl-L-lysine are then released by proteolytic cleavage. In either case, the free 6-*N*-trimethyl-L-lysine is converted stepwise to carnitine by processes including the separate hydroxylation steps. The first of these (reaction 10 in Figure 1) is the hydroxylation of 6-*N*-trimethyl-L-lysine to 3-hydroxy-6-*N*-trimethyl-L-lysine, which is of the *erythro* configuration (201). Cleavage between C-2 and C-3 of the latter compound forms glycine and 4-*N*-trimethylaminobutyraldehyde (92, 105). That compound is then oxidized to 4-*N*-trimethylaminobutyrate, known also as γ-butyrobetaine (106). Finally, γ-butyrobetaine is hydroxylated to yield carnitine (reaction 11). The first hydroxylation (reaction 10) has been studied in humans and rats, and found to occur in kidney, liver, heart, and skeletal muscle (105, 224, 237, 261, 262). In several species studied, the second hydroxylation (reaction 11) occurs almost exclusively in the liver (dog, guinea pig, mouse, and rat); however, in other species (cat, hamster, rabbit, monkey, and human) it occurs in both liver and kidney (64, 66, 162, 224).

The two hydroxylation reactions in this pathway are catalyzed by separate Fe^{2+}-requiring dioxygenases that also need α-ketoglutarate as a cosubstrate. Both enzymes have been studied in vitro for dependence upon a reductant; indeed a reductant is required, the most effective being ascorbate (105, 159–161, 164, 237, 261, 262). Of the two enzymes, only the γ-butyrobetaine hydroxylase has been isolated and studied extensively. That enzyme has been purified to homogeneity from calf liver (132) and from *Pseudomonas* sp. AK1 (161). It has also been prepared in highly purified but not homogeneous form from human kidney (163); in that tissue it exists in three isozymic species (165). The γ-butyrobetaine hydroxylase has also been partially purified from rat liver (159). A preparation of the bacterial enzyme was used to study ^{18}O incorporation from molecular oxygen (158). It was found that one of the oxygen atoms of

dioxygen was incorporated into succinate formed by decarboxylation of α-ketoglutarate, but the incorporation of the second oxygen atom into carnitine was not studied. Holme et al (98) reported that during the in vitro hydroxylation of γ-butyrobetaine by the human kidney hydroxylase, uncoupling occurred to a significant extent, meaning that more α-ketoglutarate was converted to succinate than γ-butyrobetaine to carnitine. A similar event appeared to occur when the hydroxylase from *Pseudomonas* was used, but the degree of uncoupling was considerably less (98). In other experiments, when the human hydroxylase was incubated with α-ketoglutarate in the absence of γ-butyrobetaine but in the presence of added D-carnitine, the extent of uncoupling almost doubled, going from 20% in the first experiments to 36% in the last (98). Concerning those findings, Holme et al stated that the loosely coupled reaction observed for the human kidney enzyme has no obvious explanation.

The remainder of our discussion deals principally with the evidence concerning whether ascorbate is directly or indirectly involved in the hydroxylation steps of carnitine biosynthesis. On a historical note, Hughes et al (102) quote Woodall in 1639 and Lind in 1753 to the effect that lassitude is a central feature of scurvy in humans. In a classical study in which a human volunteer was kept for a considerable time on a diet deficient in ascorbate, the subject was found to experience similar lassitude and tiredness even before the clearly defined markers of frank scurvy were evident (46). The overall reasoning to explain those features of scurvy would be as follows. The biosynthesis of carnitine requires two hydroxylation reactions that are stimulated, at least in vitro, by ascorbate. Thus a deficiency of ascorbate could lead to decreased synthesis of carnitine, decreased oxidation of fatty acids in muscle, liver, and other tissues, and consequently fatigue and lassitude.

Bearing on that matter, Hughes et al (102) studied the levels of carnitine in muscle in normal and scorbutic guinea pigs. They found that a reduction of carnitine occurred in skeletal muscle, going from 1.15 to 0.59 μg per gram of tissue. Subsequently it was shown that administration of carnitine to scorbutic guinea pigs increased their survival time by about 10% (112).

In scurvy, decreased carnitine levels have been observed in skeletal muscle (57, 102, 197, 267), heart muscle (43, 57, 197), liver (57, 239, 267), and kidney (57). The carnitine concentration measured in other studies showed no differences between normal and scorbutic guinea pigs with respect to brain and serum (57) and liver (197). Yet another study showed an elevated level of carnitine in plasma of scorbutic guinea pigs (197).

Nelson et al (197) injected radiolabeled precursors of carnitine into normal and scorbutic guinea pigs to study the influence of ascorbate on the two hydroxylation reactions. In one series of experiments they injected 6-*N*-trimethyl-L-lysine, labeled in its methyl groups with ^{14}C, into the inferior vena cavae of anesthetized guinea pigs. The amount of [^{14}C] γ-butyrobetaine pro-

duced by kidneys of either pair-fed or *ad libitum*–fed control guinea pigs was from 8 to 10 times greater than the amount produced by kidneys of scorbutic animals. Those results suggested that the activity of 6-*N*-trimethyl-L-lysine hydroxylase of the guinea pig is influenced by ascorbate concentration. In another series of experiments (197) they injected methyl-labeled γ-butyrobetaine and measured the appearance of methyl-labeled carnitine in the liver. The results appeared to indicate that formation of carnitine from its immediate precursor was no different in scorbutic guinea pigs from its formation in controls. The interpretation offered was that the concentration of ascorbate had no significant influence on hydroxylation of γ-butyrobetaine by the liver in vivo. It should be noted that in those experiments the livers of scorbutic animals showed no decreased carnitine levels when compared to controls. In contrast, Sandor et al (239) obtained results indicating that ascorbate concentration was important for maintaining normal carnitine levels in the liver; and later Dunn et al (57) obtained a similar result.

In a subsequent study (267) from the same laboratory of Nelson et al (197), guinea pigs were maintained on a scorbutic diet different in some respects from that used in the previous experiments. In addition, samples for analysis were obtained much earlier after injection of the radiolabeled precursors. Under those conditions, Thoma & Henderson (267) reported that liver carnitine levels were indeed significantly decreased in scorbutic guinea pigs as compared to controls, as was the in vivo conversion of γ-butyrobetaine to carnitine.

Dunn et al (57) isolated livers from scorbutic and normal animals and perfused them with asialofetuin that had been treated to convert some of its lysine residues to 6-*N*-trimethyl-L-lysine residues. Asialofetuin can be taken up by liver cells since these contain receptors that can combine with desialylated proteins and cause their endocytosis. Free 6-*N*-trimethyl-L-lysine is not taken up significantly by liver cells, and the use of trimethylated asialofetuin was a device, not unlike the physiological one, to introduce a precursor of carnitine into the liver cells. Dunn et al (57) also perfused the livers with suitably labeled γ-butyrobetaine. Furthermore, simultaneous perfusion of the livers with both of the labeled substrates allowed the investigators to determine the effects of ascorbate depletion and replenishment on both of the hydroxylases operating in concert within the same organ. They found that of the recovered radioactivity administered as [1,2,3,4–^{14}C] γ-butyrobetaine, 16% was present as γ-butyrobetaine and 84% appeared as carnitine for the control animals, while the scorbutic animals gave values of 51 and 49% respectively. Ascorbate-deficient livers treated to a prior perfusion with ascorbate subsequently showed that their capacity to convert γ-butyrobetaine to carnitine was restored completely.

For the same livers of scorbutic animals perfused with asialofetuin containing 6-*N*-trimethyl-L-lysine labeled in its methyl groups with ^{3}H, no decrease was observed in production of metabolites beyond the step of hydroxylation of

6-*N*-trimethyl-L-lysine, that is, in the sum of γ-butyrobetaine plus carnitine plus acetylcarnitine. However, in that pool of metabolites, [^{3}H] carnitine constituted only 53% of the total radioactivity as compared to 83% obtained with livers of control animals and 85% obtained with livers of scorbutic animals first perfused with ascorbate. Thus livers of the ascorbate-deficient animals perfused either with methylated asialofetuin or with γ-butyrobetaine showed a decrease in production of carnitine as compared with controls, and that decrease was accompanied by an accumulation of γ-butyrobetaine in the liver.

Results obtained with livers perfused simultaneously with labeled methylated asialofetuin and γ-butyrobetaine strongly supported the conclusion that ascorbate deficiency in guinea pigs affects their livers in such a way that the rate of carnitine synthesis is limited by the activity of γ-butyrobetaine hydroxylase but not by the activity of 6-*N*-trimethyl-L-lysine hydroxylase. That implies a differential effect of ascorbate on the two hydroxylases involved in carnitine synthesis. Yet, in a personal communication to one of us (S.E.), Dr. Charles J. Rebouche has pointed out that some of these results are difficult to reconcile with other information. For example, rats fed 6-*N*-trimethyl-L-lysine but no carnitine, were found by Rebouche & Lehman (225) to excrete 100 times more carnitine than did rats fed no 6-*N*-trimethyl-L-lysine and no carnitine. That was interpreted to mean that the rat liver has an approximate 100-fold excess of γ-butyrobetaine hydroxylase activity, and this enzyme would not likely be the rate-limiting enzyme in carnitine biosynthesis perhaps even in scorbutic guinea pigs. The comparison of rat and guinea pig in this case would seem to be appropriate in view of a previous finding (66) that guinea pig liver has five times greater γ-butyrobetaine hydroxylase activity than rat liver.

Our present knowledge concerning the functions of ascorbate in carnitine biosynthesis can be summarized as follows. First, in vivo, ascorbate deficiency in guinea pigs results in a variable decrease in carnitine levels of several tissues studied. Second, most investigators agree that ascorbate-deficient guinea pigs show decreased activity of liver γ-butyrobetaine hydroxylase, which can be restored rapidly by injection of ascorbate. Third, in vivo, ascorbate deficiency in guinea pigs appears to diminish 6-*N*-trimethyl-L-lysine hydroxylase activity in kidney but not in liver. Fourth, in vitro, addition of ascorbate stimulates the activity of both of the hydroxylases. In addition to the above, based on experiments with γ-butyrobetaine hydroxylase preparations from calf liver and again from *Pseudomonas,* it was found possible to propose chemical mechanisms that do not require direct participation of ascorbate in the hydroxylation reactions (19, 65).

The fact that purified γ-butyrobetaine hydroxylase can catalyze the uncoupled decarboxylation of α-ketoglutarate (98) is reminiscent of the actions of prolyl and lysyl hydroxylases. Thus in this case, as with the collagen hydroxy-

lases, unproductive cycles of activity may possibly occur. Should that happen, the iron atom may become oxidized and require ascorbate for its reduction to the active ferrous state.

OTHER POSSIBLE FUNCTIONS OF ASCORBATE

The main focus of this review has been the possible participation of ascorbate in certain specific enzymatic reactions listed in Figure 1. We want now to categorize uncritically a number of biological and pathological processes that have been studied with relation to the vitamin. Study of these has not yet achieved sufficient scientific status at the enzymatic level to fit them into the scope of this review. That statement is not meant to diminish their possible importance; this may become evident as they receive further experimental investigation. Some of these postulated functions form the basis of controversy into which we are not competent to enter.

Antioxidant functions of ascorbate are reviewed by Johnson (111) and McCay (180). The participation of ascorbate in the metabolism of drugs is considered by Zannoni and his colleagues (287, 289). Some workers have implicated functions for ascorbate in cholesterol and lipid metabolism (70, 76–78, 85, 93, 210). The connection between immune function and ascorbate has received considerable attention over the years; we note only a few of relatively recent articles (4, 5, 50, 214, 268). A relationship appears to exist between iron metabolism and ascorbate (231). Many articles have appeared bearing on the ascorbate-cancer controversy; we call attention to a selected few (35–37, 115, 185, 205, 281).

CONCLUDING REMARKS

Discoveries of the past four decades showing that ascorbate can participate as a reductant in perhaps more than a dozen different reactions performed in vitro generated optimism that its biological functions could be defined specifically, and that the pathophysiology of ascorbate-deficient states, including scurvy, might be explained satisfactorily. To the contrary, further study has raised questions about the unique specificity of ascorbate in those reactions, and the optimism has tended to wane. From investigations such as those reviewed in this article, one comes away with the impression that the vital functions of ascorbate are yet to be identified. A further impression is that some of the answers may yet lie in a closer understanding of the derangements of scurvy, and of how administered ascorbate rescues scorbutic guinea pigs and humans from certain death. From some of the experiments with collagen metabolism, one may infer strongly that ascorbate is concerned generally with

protein biosynthesis and specifically with collagen biosynthesis. Yet only meager strategies for studying that problem are evident.

In relation to mammalian biology, the reactions that we have focused on are important in nervous and endocrine function, the metabolism of tyrosine, the biosynthesis of carnitine required for oxidation of fatty acids for energy, and posttranslational hydroxylations of residues in collagenous proteins (the various types of collagen, basement membranes, C1q, and possibly certain acetylcholinesterases) and elastin. If indeed ascorbate were absolutely and specifically required for those reactions, one should be able to rationalize many of the structural, regulatory, metabolic, and immune disorders associated with scurvy. At least two major difficulties have dampened that hope. First, examination of the in vitro evidence for direct participation of ascorbate in those reactions sometimes leads to the conclusion that, in fact, the participation is probably indirect. Second, the findings in scurvy often appear to contradict expectations coming from a postulated function of ascorbate. For instance, the participation of ascorbate in the in vitro hydroxylation of prolyl residues in collagen does not match the finding that the collagen of scorbutic animals is not substantially underhydroxylated.

Perhaps the strongest case for a direct function of ascorbate can be made for the two monooxygenase reactions that require copper as well. Thus, both the dopamine β-hydroxylase and peptidyl glycine α-amidation reactions occur in tissues in which ascorbate is concentrated to very high levels, providing circumstantial evidence for its requirement. Furthermore, one can write reaction mechanisms for both of these and, in at least one case, conduct model nonenzymatic reactions in which ascorbate has a direct function. Even for those reactions, however, alternative mechanisms, consistent with available experimental data, can be written without direct participation of ascorbate, thus weakening the case.

For 4-hydroxyphenylpyruvate dioxygenase, the in vivo evidence for participation of ascorbate seems at first examination to be quite convincing, but the in vitro evidence seems to be weakest for all of the reactions considered by us. With regard to the former, premature human infants who have not yet expressed fully the gene for the dioxygenase show evidences of diminished tyrosine catabolism as do scorbutic guinea pigs. In both cases administration of ascorbate restores tyrosine metabolism to normal. However, closer examination suggests that part of the effect of administered ascorbate may not be directly in the enzyme mechanism but on removal of substrate inhibition. In vitro studies with the dioxygenase show some other reductants to be almost as effective as ascorbate. Furthermore, even the precise function of the iron constituent of this enzyme is largely unknown, let alone the function of ascorbate.

With regard to prolyl 4-hydroxylase, evidence has been obtained that several cycles of hydroxylation can occur without participation of ascorbate. After that,

some reductant is required to keep the enzyme actively working, ascorbate being best. It has been proposed, with considerable experimental back-up, that the ascorbate is needed in the in vitro reaction in a kind of subsidiary housekeeping function, that is, to bring the iron constituent back to the active ferrous form when it becomes oxidized fortuitously. Thus a chemical mechanism has been proposed for prolyl 4-hydroxylase, seemingly applicable to the other collagen hydroxylases as well, that does not require ascorbate directly. Experimentally, the requirement for ascorbate becomes stoichiometric when the decarboxylation of the α-ketoglutarate substrate is uncoupled from the hydroxylation of prolyl residues. When the enzyme functions in that way the reactive iron-oxo complex is probably converted to Fe^{3+} and $O\cdot$. At that point, presence of a reductant, particularly ascorbate, becomes necessary to keep the enzyme active by reducing the Fe^{3+} to Fe^{2+}. If one considers that such uncoupling is an in vitro phenomenon, these considerations do not contradict the in vivo findings that collagen hydroxylation is not seriously compromised in scorbutic guinea pigs.

The effect of uncoupling of reactions in relation to the requirement for a reducing cofactor is discussed in a recent paper (55a) on the mechanism of phenylalanine hydroxylase action. The authors note that the enzyme can form a complex with Fe^{2+}, O_2, and tetrahydropterin. In the absence of the phenylalanine substrate, the enzyme complex catalyzes the uncoupled oxidation of the tetrahydropterin with formation of an enzyme·Fe^{2+}·OOH intermediate. This is unstable and can be converted to an enzyme·Fe^{3+} intermediate that is no longer active enzymatically. For recovery of enzymatic activity, the Fe^{3+} must be reduced to Fe^{2+}. This requires the investment of additional reduced cofactor (tetrahydropterin). The analogy with the uncoupled prolyl hydroxylase reaction and ascorbate requirement is striking.

In the case of carnitine, evidence has been obtained that its levels in several tissues fall below normal in scorbutic guinea pigs. The levels are restored by administration of ascorbate. Furthermore, perfusion of livers of scorbutic guinea pigs with precursors of carnitine, and examination of products, reveal deficiencies in the hydroxylation reactions involved in carnitine biosynthesis. Despite this, one can write chemical mechanisms for those hydroxylases without invoking direct participation of ascorbate, but assuming that the reductant is needed to restore the iron to its active ferrous form should it be oxidized accidentally—just as in the case of prolyl 4-hydroxylase.

Considering all of the above, one can say that none of the reactions has been shown in vitro to have an absolute requirement for ascorbate as opposed to other reductants, nor does the evidence permit the writing of unique chemical mechanisms involving direct participation of ascorbate. In the most pessimistic case, ascorbate in these reactions could be confined to the housekeeping role of restoring the metal constituents of the respective enzymes to the reduced state

should they become oxidized adventitiously. In the more optimistic case, one can say that ascorbate may yet prove to have a direct function in the action of the monooxygenases.

If indeed these matters become settled in the way just described, one can rejoice that at least some issues will have been resolved, and then get on with the more difficult task of unraveling those as yet mystifying functions of ascorbate that make it necessary for sustaining life. Probably a good place to start is to determine how ascorbate functions at the molecular level in protein synthesis, specifically in collagen synthesis.

ACKNOWLEDGMENTS

We are grateful to Drs. Hartmut M. Hanauske-Abel, Paul M. Gallop, Norman Kretchmer, Julius Marmur, Eli Seifter, and Mercedes A. Paz for many helpful suggestions. We want also to express appreciation to Dr. Mark A. Levine for allowing us to read preprints of his two current reviews. We owe special thanks to Dr. Kari I. Kivirikko for permitting us to reproduce Figure 2 from a manuscript in press from his laboratory, and for allowing us to read an especially pertinent manuscript before publication. Our own research work reviewed in this article was supported by grants from the National Institutes of Health, 2R01 AM 21197 and 2P01 AG 00374.

Literature Cited

1. Abbott, M. T., Dragila, T. A., McCroskey, R. P. 1968. The formation of 5-formnyluracil by cell-free preparation from *Neurospora crassa*. *Biochim. Biophys. Acta* 169:1–6
2. Abbott, M. T., Kadner, R. J., Fink, R. M. 1964. Conversion of thymine to 5-hydroxymethyluracil in a cell-free system. *J. Biol. Chem.* 239:156–59
3. Abbott, M. T., Udenfriend, S. 1974. α-Ketoglutarate-coupled dioxygenases. In *Molecular Mechanisms of Oxygen Activation*, ed. O. Hayaishi, pp. 167–214. New York/London: Academic
4. Anderson, R. 1981. Ascorbic acid and immune functions. In *Vitamin C: Ascorbic Acid*, ed. J. N. Counsel, D. H. Hornig, pp. 249–72. London: Applied Science
5. Anderson, R. 1984. The immunostimulatory, anti-inflammatory and anti-allergic properties of ascorbate. *Adv. Nutr. Res.* 6:19–45
6. Anderson, S., Bernnan-Marks, C., Lazarus, R., Miller, J., Stafford, K., et al. 1985. Production of 2-keto-L-gulonate, an intermediate in L-ascorbate synthesis, by a genetically modified *Erwinia herbicola*. *Science* 230:144–49
7. Andrews, P. C., Hawks, D., Shively, J. E., Dixon, J. E. 1984. Anglerfish preprosomatostatin II is processed to somatostatin-28 and contains hydroxylysine at residue 23. *J. Biol. Chem.* 259(24): 15021–24
8. Anttinen, H., Puistola, U., Pihlajaniemi, T., Kivirikko, K. I. 1981. Differences between proline and lysine hydroxylations in their inhibition by zinc or by ascorbate deficiency during collagen synthesis in various cell types. *Biochim. Biophys. Acta* 674:336–44
9. Ash, D. E., Papadopoulos, N. J., Colombo, G., Villafranca, J. J. 1984. Kinetic and spectroscopic studies of the interaction of copper with dopamine β-hydroxylase. *J. Biol. Chem.* 259:3395–98
10. Barnes, M. J., Constable, B. J., Kodicek, E. 1969. Excretion of hydroxyproline and other amino acids in scorbutic guinea-pigs. *Biochim. Biophys. Acta* 184:358–65
11. Barnes, M. J., Constable, B. J., Morton, L. F., Kodicek, E. 1970. Studies *in vivo* on the biosynthesis of collagen and elastin in ascorbic acid–deficient guinea pigs. Evidence for the formation and degradation of a partially hydroxylated collagen. *Biochem. J.* 119:575–85

12. Barone, L. M., Faris, B., Chipman, S. D., Toselli, P., Oakes, B. W., Franzblau, C. 1985. Alteration of the extracellular matrix of smooth muscle cells by ascorbate treatment. *Biochim. Biophys. Acta* 840:245–54
13. Bateman, R. C. Jr., Youngblood, W. W., Busby, W. H. Jr., Kizer, J. S. 1985. Nonenzymatic peptide α-amidation. Implication for a novel enzyme mechanism *J. Biol. Chem.* 260:9088–91
14. Bates, C. J., Prynne, C. J., Levene, C. I. 1972. The synthesis of underhydroxylated collagen by 3T6 mouse fibroblasts in culture. *Biochim. Biophys. Acta* 263:397–405
15. Bates, C. J., Bailey, A. J., Prynne, C. J., Levene, C. I. 1972. The effect of ascorbic acid on the synthesis of collagen precursor secreted by 3T6 mouse fibroblasts in culture. *Biochim. Biophys. Acta* 278:372–90
16. Bates, C. J., Levene, C. I., Oldroyd, R. G., Lachmann, P. J. 1978. Complement component C1q is insensitive to acute vitamin C deficiency in guinea pigs. *Biochim. Biophys. Acta* 540:423–30
17. Belpaire, R., Laduron, P. L. 1968. Tissue fractionation and catecholamines. *Biochem. Pharmacol.* 17:411–21
17a. Berg, R. A., Prockop, D. J. 1973. Affinity column purification of protocollagen proline hydroxylase from chick embryos and further characterization of the enzyme. *J. Biol. Chem.* 248:1175–82
18. Bielsky, B. H. J., Richter, H. W., Chan, P. C. 1975. Some properties of the ascorbate free radical. *Ann. NY Acad. Sci.* 258:231–38
19. Blanchard, J. S., Englard, S. 1983. γ-Butyrobetaine hydroxylase: primary and secondary tritium kinetic isotope effects. *Biochemistry* 22:5922–29
20. Blanchard, J. S., Englard, S., Kondo, A. 1982. γ-Butyrobetaine hydroxylase: A unique protective effect of catalase. *Arch. Biochem. Biophys.* 219:327–34
21. Blankel, L., Holme, E., Lindstedt, G., Lindstedt, S. 1972. Oxygenases involved in thymine and thymidine metabolism in *Neurospora crassa*. *FEBS Lett.* 21:135–38
22. Blankel, L., Lindstedt, G., Lindstedt, S. 1972. Thymidine 2'-hydroxylation in *Neurospora crassa*. *J. Biol. Chem.* 247:6128–34
23. Blumenfeld, O. O., Bienkowski, R. S., Schwartz, E., Seifter, S. 1983. Extracellular matrix of smooth muscle cells. In *Biochemistry of Smooth Muscle*, ed. N. L. Stephens, 2:137–87. Boca Raton, Fla: CRC
24. Boel, E., Schwartz, T. W., Norris, K. E., Fiil, N. P. 1984. A cDNA encoding a small common precursor for human pancreatic polypeptide and pancreatic icosapeptide. *EMBO J.* 3:909–12
25. Boel, E., Vuust, J., Norris, F., Norris, K., Wind, A., et al. 1983. Molecular cloning of human gastrin cDNA: evidence for evolution of gastrin by gene duplication. *Proc. Natl. Acad. Sci. USA* 80:2866–69
26. Booth, B. A., Polak, K. L., Uitto, J. 1979. Procollagen synthesis by cultured human skin fibroblasts. *Clin. Res.* 27:623 (Abstr.)
27. Bornstein, P., Traub, W. 1979. The chemistry and biology of collagen. In *The Proteins*, ed. H. Neurath, R. L. Hill, 4:411–632. New York: Academic
28. Borum, P. R. 1983. Carnitine. *Ann. Rev. Nutr.* 3:233–59
29. Borum, P. R., Broquist, H. P. 1977. Purification of *S*-adenosylmethionine: ε-*N*-L-lysine methyltransferase. The first enzyme in carnitine biosynthesis. *J. Biol. Chem.* 252:5651–55
30. Bradbury, A. F., Finnie, M. D. A., Smyth, D. G. 1982. Mechanism of C-terminal amide formation by pituitary enzymes. *Nature* 298:686–88
31. Bradbury, A. F., Smyth, D. G. 1983. Substrate specificity of an amidating enzyme in porcine pituitary. *Biochem. Biophys. Res. Commun.* 112:372–77
32. Bremer, J. 1983. Carnitine-metabolism and functions. *Physiol. Rev.* 63:1420–80
33. Burns, J. J., ed. 1961. Vitamin C. *Ann. NY Acad. Sci.*, Vol. 92. New York: NY Acad. Sci. 332 pp.
34. Burns, J. J. 1975. Overview of ascorbic acid metabolism. *Ann. NY Acad. Sci.* 258:5–6
35. Cameron, E. 1982. Vitamin C and cancer: an overview. *Int. Z. Vitam. Ernahrungsforsch.* 23:115–27
36. Cameron, E., Pauling, L. 1976. Supplemental ascorbate in the supportive treatment of cancer: prolongation of survival times in terminal human cancer. *Proc. Natl. Acad. Sci. USA* 73:3685–89
37. Cameron, E., Pauling, L., Leibovitz, B. 1979. Ascorbic acid and cancer: a review. *Cancer Res.* 39:663–81
38. Cardinale, G. J., Udenfriend, S. 1974. Prolyl hydroxylase. *Adv. Enzymol.* 41: 245–90
39. Chatterjee, I. B. 1978. Ascorbic acid metabolism. *World Rev. Nutr. Diet.* 30:69–87
40. Chatterjee, I. B., Majumder, A. K., Nandi, B. K., Subramanian, N. 1975. Synthesis and some major functions of

vitamin C in animals. *Ann. NY Acad. Sci.* 258:24–47
41. Chen, K. H., Evans, C. A., Gallop, P. M. 1977. Prolyl and lysyl hydroxylase activation and cofactor specificity in young and senescent WI-38 fibroblast cultures. *Biochem. Biophys. Res. Commun.* 74:1631–36
41a. Chen-Kiang, S., Cardinale, G. J., Udenfriend, S. 1977. Homology between a prolyl hydroxylase subunit and a tissue protein that crossreacts immunologically with the enzyme. *Proc. Natl. Acad. Sci. USA* 74:4420–24
42. Chojkier, M., Spanheimer, R., Peterkofsky, B. 1983. Specifically decreased collagen biosynthesis in scurvy dissociated from an effect on proline hydroxylation and correlated with body weight loss. *In vitro* studies in guinea pig calvarial bones. *J. Clin. Invest.* 72:826–35
43. Ciman, M., Rizzoli, V., Siliprandi, N. 1979. Deficiency of carnitine induced by scurvy. *J. Int. Res. Commun.* 7:253
44. Colombo, G., Rajashekhar, B., Giedroc, D. P., Villafranca, J. J. 1984. Mechanism-based inhibitors of dopamine β-hydroxylase: inhibition by 2-bromo-3-(*p*-hydroxyphenyl)-1-propene. *Biochemistry* 23:3590–98
45. Counts, D. F., Cardinale, G. J., Udenfriend, S. 1978. Prolyl hydroxylase half reaction: Peptidyl prolyl-independent decarboxylation of α-ketoglutarate. *Proc. Natl. Acad. Sci. USA* 75:2145–49
46. Crandon, J. H., Lund, C. G., Dill, D. B. 1940. Experimental human scurvy. *N. Engl. J. Med.* 223:353–69
46a. Davis, A. E., Lachmann, P. J. 1984. Bovine conglutinin is a collagen-like protein. *Biochemistry* 23:2139–44
47. DeClerck, Y. A., Jones, P. A. 1980. The effect of ascorbic acid on the nature and production of collagen and elastin by rat smooth-muscle cells. *Biochem. J.* 186: 217–25
48. DeJong, L., Albracht, S. P. J., Kemp, A. 1982. Prolyl 4-hydroxylase activity in relation to the oxidation state of enzyme-bound iron. The role of ascorbate in peptidyl proline hydroxylation. *Biochim. Biophys. Acta* 704:326–32
49. DeJong, L., Kemp, A. 1984. Stoichiometry and kinetics of the prolyl 4-hydroxylase partial reaction. *Biochim. Biophys. Acta* 787:105–11
50. Delafuente, J. C., Panush, R. S. 1980. Modulation of certain immunologic responses by vitamin C. *Int. J. Vitam. Nutr. Res.* 50:44–51
51. DeSchenes, R. J., Lorenz, L. J., Haun, R. S., Roos, B. A., Collier, K. J., Dixon, J. E. 1984. Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. *Proc. Natl. Acad. Sci. USA* 81:726–30
52. Diliberto, E. J. Jr., Allen, P. L. 1980. Semidehydroascorbate as a product of the enzymic conversion of dopamine to norepinephrine: coupling of semidehydroascorbate reductase to dopamine-β-hydroxylase. *Mol. Pharmacol.* 17:421–26
53. Diliberto, E. J. Jr., Allen, P. L. 1981. Mechanism of dopamine β-hydroxylation. *J. Biol. Chem.* 256:3385–93
54. Diliberto, E. J. Jr., Dean, G., Carter, C., Allen, P. L. 1982. Tissue, subcellular and submitochondrial distributions of semidehydroascorbate reductase: possible role of semidehydroascorbate reductase in cofactor regeneration. *J. Neurochem.* 39:563–68
55. Diliberto, E. J. Jr., Heckman, G. D., Daniels, A. J. 1983. Characterization of ascorbic acid transport by adrenomedullary chromaffin cell. *J. Biol. Chem.* 258:12886–994
55a. Dix, T. A., Benkovic, S. J. 1985. Mechanism of "uncoupled" tetrahydropterin oxidation by phenylalanine hydroxylase. *Biochemistry* 24:5839–46
56. Dunn, W. A., Englard, S. 1981. Carnitine biosynthesis by the perfused rat liver from exogenous protein-bound trimethyllysine. Metabolism of methylated lysine derivatives arising from the degradation of 6-*N*-[methyl-^{3}H] lysine-labeled glycoproteins. *J. Biol. Chem.* 256:12437–44
57. Dunn, W. A., Rettura, G., Seifter, E., Englard, S. 1984. Carnitine biosynthesis from γ-butyrobetaine and from exogenous protein-bound 6-*N*-trimethyl-L-lysine by the perfused guinea pig liver. Effect of ascorbate deficiency on the *in situ* activity of γ-butyrobetaine hydroxylase. *J. Biol. Chem.* 259:10764–70
58. Ecker, E. E., Kalina, R., Pillemer, L. 1939. The explanation of an apparent inactivation of complement by alloxan. *Enzymologia* 7:307–9
59. Ecker, E. E., Pillemer, L. 1942. Complement. *Ann. NY Acad. Sci.* 53:63–84
60. Ecker, E. E., Pillemer, L., Wertheimer, D., Gradis, H. 1938. Ascorbic acid and complement function. *J. Immunol.* 34: 19–37
61. Ecker, E. E., Pillemer, L., Wertheimer, D. 1938. Complementing activity and ascorbic acid content of guinea-pig serums following ether anesthesia. *J. Immunol.* 34:39–43
62. Eipper, B. A., Mains, R. E., Glembotski, C. C. 1983. Identification in pituit-

ary tissue of a peptide α-amidation activity that acts on glycine-extended peptides and requires molecular oxygen, copper and ascorbic acid. *Proc. Natl. Acad. Sci. USA* 80:5144–48
63. Deleted in proof
64. Englard, S. 1979. Hydroxylation of γ-butyrobetaine to carnitine in human and monkey tissues. *FEBS Lett.* 102:297–300
65. Englard, S., Blanchard, J. S., Midelfort, C. F. 1985. γ-Butyrobetaine hydroxylase: stereochemical course of the hydroxylation reaction. *Biochemistry* 24:1110–16
66. Englard, S., Carnicero, H. H. 1978. γ-Butyrobetaine hydroxylation to carnitine in mammalian kidney. *Arch. Biochem. Biophys.* 190:361–64
67. Evans, C. A., Peterkofsky, B. 1976. Ascorbate-independent proline hydroxylation resulting from viral transformation of Balb 3T3 cells and unaffected by dibutyryl cAMP treatment. *J. Cell. Physiol.* 89:355–68
68. Faris, B., Snider, R., Levene, A., Moscaritolo, R., Salcedo, L., Franzblau, C. 1978. Effect of ascorbate on collagen synthesis by lung embryonic fibroblasts. *In Vitro* 14:1022–27
69. Fessler, J. H., Fessler, L. I. 1978. Biosynthesis of collagen. *Ann. Rev. Biochem.* 47:129–62
70. Fidanza, A., Audisio, M., Mastriacovo, P. 1982. Vitamin C and cholesterol. *Int. Z. Vitam. Ernahrungsforsch.* 23:153–71
71. Frenkel, R. A., McGarry, J. D., eds. 1980. *Carnitine Biosynthesis, Metabolism, and Functions.* New York/London: Academic. 356 pp.
72. Friedman, S., Kaufman, S. 1965. 3,4-Dihydroxyphenylethylamine β-hydroxylase. Physical properties, copper content, and role of copper in the catalytic activity. *J. Biol. Chem.* 240:4763–73
73. Fritz, I. B. 1963. Carnitine and its role in fatty acid metabolism. *Adv. Lipid Res.* 1:285–334
74. Gallop, P. M., Blumenfeld, O. O., Seifter, S. 1972. Structure and metabolism of connective tissue proteins. *Ann. Rev. Biochem.* 41:617–72
75. Gallop, P. M., Paz, M. A. 1975. Posttranslational protein modifications, with special attention to collagen and elastin. *Physiol. Rev.* 55:418–87
76. Ginter, E. 1975. Ascorbic acid in cholesterol and bile acid metabolism. *Ann. NY Acad. Sci.* 258:410–21
77. Ginter, E., Bobek, P., Jurcovicova, M. 1982. Role of L-ascorbic acid in lipid metabolism. See Ref. 249, pp. 381–93
78. Ginter, E., Cerna, O., Budlovsky, J., Balaz, V., Hruba, F., et al. 1977. Effect of ascorbic acid on plasma cholesterol in humans in long-term experiment. *Int. J. Vitam. Nutr. Res.* 47:123–34
79. Glembotski, C. C. 1984. The α-amidation of α-melanocyte stimulating hormone in intermediate pituitary requires ascorbic acid. *J. Biol. Chem.* 259:13041–48
80. Glembotski, C. C. 1985. Further characterization of the peptidyl α-amidating enzyme in rat anterior pituitary secretory granules. *Arch. Biochem. Biophys.* 241:673–83
81. Glembotski, C. C., Eipper, B. A., Mains, R. E. 1983. Adrenocorticotropin (1–14) OH-related molecules in primary cultures of rat intermediate pituitary cells. Identification and role in the biosynthesis of α-melanotropin. *J. Biol. Chem.* 258:7299–7304
82. Glembotski, C. C., Eipper, B. A., Mains, R. E. 1984. Characterization of a peptide α-amidation activity from rat anterior pituitary. *J. Biol. Chem.* 259: 6385–92
83. Goodwin, B. L. 1972. *Tyrosine Catabolism: The Biological, Physiological, and Clinical Significance of p-Hydroxyphenylpyruvate Oxidase.* Oxford/Clarendon: Oxford Univ. Press. 94 pp.
84. Gould, B. S., Manner, G., Goldman, H. M., Stolman, J. 1960. Some aspects of collagen formation. *Ann. NY Acad. Sci.* 85:385–98
85. Greene, Y., Harwood, H. J. Jr., Stacpoole, P. W. 1985. Ascorbic acid regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol synthesis in guinea pig liver. *Biochim. Biophys. Acta* 834:134–38
86. Gross, J. 1959. Studies on the formation of collagen. IV. Effect of vitamin C deficiency on the neutral salt-extractable collagen of skin. *J. Exp. Med.* 109:557–69
87. Gubler, U., Chua, A. O., Hoffman, B. J., Collier, K. J., Eng, J. 1984. Cloned cDNA to cholecystokinin mRNA predicts an identical preprocholecystokinin in pig brain and gut. *Proc. Natl. Acad. Sci. USA* 81:4307–10
88. Guillemin, R., Brazeau, P., Bohlen, P., Esch, F., Ling, N., Wehrenberg, W. B. 1982. Growth hormone–releasing factor from a human pancreatic tumor that caused acromegaly. *Science* 218:585–87
89. Hamilton, G. A. 1974. Chemical models and mechanisms for oxygenases. See Ref. 3, pp. 405–50
90. Hanauske-Abel, H. M., Günzler, V. 1982. A stereochemical concept for the

catalytic mechanism of prolylhydroxylase. Applicability to classification and design of inhibitors. *J. Theor. Biol.* 94:421–55
91. Hayaishi, O., Nozaki, M., Abbott, M. T. 1975. Oxygenases: dioxygenases. *Enzymes* 12:119–89
92. Hochalter, J. B., Henderson, L. M. 1976. Carnitine biosynthesis: The formation of glycine from carbons 1 and 2 of 6-*N*-trimethyl-L-lysine. *Biochem. Biophys. Res. Commun.* 70:364–66
93. Hodges, R. E., Hood, J., Canham, J. E., Sauberlich, H. E., Baker, E. M. 1971. Clinical manifestations of ascorbic acid deficiency in man. *Am. J. Clin. Nutr.* 24:432–43
94. Holme, E. 1975. A kinetic study of thymine 7-hydroxylase from *Neurospora crassa*. *Biochemistry* 14:4999–5003
95. Holme, E. 1982. Determination of intra- and intermolecular tritium isotope effects in the reaction of thymine 7-hydroxylase. *Biochim. Biophys. Acta* 707:259–66
96. Holme, E., Lindstedt, S. 1982. Studies on the partial reaction of thymine 7-hydroxylase in the presence of 5-fluorouracil. *Biochim. Biophys. Acta* 704:278–83
97. Holme, E., Lindstedt, G., Lindstedt, S., Tofft, M. 1971. ^{18}O Studies of the α-ketoglutarate-dependent sequential oxygenation of thymine to 5-carboxyuracil. *J. Biol. Chem.* 246:3314–19
98. Holme, E., Lindstedt, S., Nordin, I. 1982. Uncoupling in the γ-butyrobetaine hydroxylase reaction by D- and L-carnitine. *Biochem. Biophys. Res. Commun.* 107:518–24
99. Horlein, D., McPherson, J., Goh, S. H., Bornstein, P. 1981. Regulation of protein synthesis: translational control by procollagen-derived fragments. *Proc. Natl. Acad. Sci. USA* 78:6163–67
100. Hornig, D. 1975. Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann. NY Acad. Sci.* 258:103–18
101. Hsu, C.-A., Saewert, M. D., Polsinelli, L. F. Jr., Abbott, M. T. 1981. Uracils uncoupling of the decarboxylation of α-ketoglutarate in the thymine 7-hydroxylase reaction of *Neurospora crassa*. *J. Biol. Chem.* 256:6098–101
102. Hughes, R. E., Hurley, R. J., Jones, E. 1980. Dietary ascorbic acid and muscle carnitine (β-OH-γ-(trimethylamino) butyric acid) in guinea-pigs. *Br. J. Nutr.* 43:385–87
103. Huisman, T. H. J., Jonxis, J. H. P. 1957. Some investigations on the metabolism of phenylalanine and tyrosine in children with vitamin C deficiency. *Arch. Dis. Child.* 32:77–81
104. Hukins, D. W. L., ed. 1984. *Connective Tissue Matrix, Topics in Molecular and Structural Biology*, Vol. 5. Weinheim/Deerfield Beach, Fla/Basel: Verlag Chemie. 245 pp.
105. Hulse, J. D., Ellis, S. R., Henderson, L. M. 1978. Carnitine biosynthesis. β-Hydroxylation of trimethyllysine by an α-ketoglutarate-dependent mitochondrial dioxygenase. *J. Biol. Chem.* 253: 1654–59
106. Hulse, J. D., Henderson, L. V. 1980. Carnitine biosynthesis. Purification of 4-*N*-trimethylaminobutyraldehyde dehydrogenase from beef liver. *J. Biol. Chem.* 255:1146–51
107. Husain, I., Tate, S. S. 1983. Formation of the COOH-terminal amide group of thyrotropin-releasing-factor. *FEBS Lett.* 152:277–81
108. Ingebretsen, O. C., Terland, O., Flatmark, T. 1980. Subcellular distribution of ascorbate in bovine adrenal medulla. *Biochim. Biophys. Acta* 628:182–89
109. Itoh, N., Obata, K. I., Yanaihara, N., Okamoto, H. 1983. Human prepro vasoactive intestinal polypeptide contains a novel PHI-27-like peptide, PHM-27. *Nature* 304:547–49
110. Jacob, J. W., Goodman, R. H., Chin, W. W., Dee, P. C., Habener, J. F., et al. 1981. Calcitonin messenger RNA encodes multiple polypeptides in a single precursor. *Science* 213:457–59
111. Johnson, F. C. 1979. The antioxidant vitamins. *CRC Crit. Rev. Food Sci. Nutr.* 11:217–309
112. Jones, E., Hughes, R. E. 1982. Influence of oral carnitine on the body weight and survival time of avitaminotic-C guinea pigs. *Nutr. Rep. Int.* 25:201–4
113. Juva, K., Prockop, D. J. 1964. Puromycin inhibition of collagen synthesis as evidence for a ribosomal or post-ribosomal site for the hydroxylation of proline. *Biochim. Biophys. Acta* 91:174–76
114. Kalcheim, C., Duksin, D., Bachar, E., Vogel, Z. 1985. Collagen-stimulating factor from embryonic brain has ascorbate-like activity and stimulates prolyl hydroxylation in cultured muscle cells. *Eur. J. Biochem.* 146:227–32
115. Kamm, J. J., Dashman, T., Conney, A. H., Burns, J. J. 1973. Protective effect of ascorbic acid on hepatotoxicity caused by sodium nitrite plus amino pyrine. *Proc. Natl. Acad. Sci. USA* 70:747–49
116. Kao, F.-T., Puck, T. T. 1967. Genetics of somatic mammalian cells IV. Proper-

ties of Chinese hamster cell mutants with respect to requirement for proline. *Genetics* 55:513–24

116a. Kao, W. W. Y., Berg, R. A. 1979. Cell density–dependent increase in prolyl hydroxylase activity in cultured L-929 cells requires *de novo* protein synthesis. *Biochim. Biophys. Acta* 586: 528–36

117. Kao, W. W. Y., Berg, R. A., Prockop, D. J. 1975. Ascorbate increases the synthesis of procollagen hydroxyproline by cultured fibroblasts from chick embryo tendons without activation of prolyl hydroxylase. *Biochim. Biophys. Acta* 411:202–15

118. Kao, W. W. Y., Flaks, J. G., Prockop, D. J. 1976. Primary and secondary effects of ascorbate on procollagen synthesis and protein synthesis by primary cultures of tendon fibroblasts. *Arch. Biochem. Biophys.* 173:638–48

119. Kaufman, S., Friedman, S. 1965. Dopamine β-hydroxylase. *Pharmacol. Rev.* 17:71–100

120. King, C. G., Burns, J. J., eds. 1975. Second Conference on Vitamin C, *Ann. NY Acad. Sci.*, Vol. 258. New York: NY Acad. Sci. 552 pp.

121. Kirk, J. E. 1962. Variations with age in the tissue content of vitamins and hormones. *Vitam. Horm.* 20:67–139

122. Kirshner, N. 1962. Uptake of catecholamines by a particulate fraction of the adrenal medulla. *J. Biol. Chem.* 237:2311–17

123. Kivirikko, K. I., Myllylä, R. 1980. The hydroxylation of prolyl and lysyl residues. In *The Enzymology of Post-translational Modifications of Proteins*, ed. R. B. Freedman, H. C. Hawkins, 1:53–104. London/New York/Toronto: Academic

124. Kivirikko, K. I., Myllylä, R. 1982. Post-translational enzymes in the biosynthesis of collagen: intracellular enzymes. *Methods Enzymol.* 82(PA):245–304

125. Kivirikko, K. I., Myllylä, R. 1982. Post-translational modifications. In *Collagen in Health and Diseases*, ed. M. I. V. Jayson, J. B. Weiss, pp. 101–20. Edinburgh: Churchill Livingstone

126. Kivirikko, K. I., Myllylä, R. 1984. Biosynthesis of the collagens. See Ref. 211, pp. 83–118

127. Kivirikko, K. I., Myllylä, R. 1986. Post-translational processing of procollagens. *Ann. NY Acad. Sci.* In press

128. Kizer, J. S., Buzry, W. H. Jr., Cottle, C., Youngblood, W. W. 1984. Glycine-directed peptide amidation: presence in rat brain of two enzymes that convert *p*-GLU-HIS-PRO-GLY-OH into *p*-Glu-His-Pro-NH_2 (thyrotropin-releasing hormone). *Proc. Natl. Acad. Sci. USA* 81:3228–32

129. Klinman, J. P., Krueger, M., Brenner, M., Edmondson, D. E. 1984. Evidence for two copper atoms/subunit in dopamine β-monooxygenase catalysis. *J. Biol. Chem.* 259:3399–3402

130. Knox, W. E., Goswami, M. N. D. 1961. Ascorbic acid in tyrosine metabolism. *Ann. NY Acad. Sci.* 92:192–94

131. Koda, K., Wu, C. H., Wu, G. Y., Seifter, S. 1986. Inhibitory effect of type I procollagen carboxyterminal extension peptide on the synthesis of type I procollagen in human fibroblast culture. *Proc. Natl. Acad. Sci. USA*. In press

132. Kondo, A., Blanchard, J. S., Englard, S. 1981. Purification and properties of calf liver γ-butyrobetaine hydroxylase. *Arch. Biochem. Biophys.* 212:338–46

133. Kretchmer, N. 1959. Enzymatic patterns during development. *Pediatrics* 23: 606–17

134. Kretchmer, N., Levine, S. Z., McNamara, H., Barnett, H. L. 1956. Certain aspects of tyrosine metabolism in the young. I. The development of the tyrosine oxidizing system in human liver. *J. Clin. Invest.* 35:236–44

135. Kuttan, R., Parrott, D. P., Kaplan, S. R., Fuller, G. C. 1979. Effect of ascorbic acid on prolyl hydroxylase activity, collagen hydroxylation and collagen synthesis in human synovial cells in culture. *Res. Commun. Chem. Pathol. Pharmacol.* 26:337–45

135a. Kuutti, E. R., Tuderman, L., Kivirikko, K. I. 1975. Human prolyl hydroxylase purification, partial characterization and preparation of antiserum to the enzyme. *Eur. J. Biochem.* 57:181–88

136. LaBadie, J., Dunn, W. A., Aronson, N. N. Jr. 1976. Hepatic synthesis of carnitine from protein-bound trimethyl-lysine. Lysosomal digestion of methyl-lysine-labelled asialo-fetuin. *Biochem. J.* 160: 85–95

137. LaDu, B. N., Gjessing, L. R. 1972. Tyrosinosis and tyrosinemia. In *The Metabolic Basis of Inherited Diseases*, ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, pp. 296–307. New York/St. Louis/San Francisco: McGraw-Hill. 3rd ed.

138. LaDu, B. N., Zannoni, V. G. 1961. The role of ascorbic acid in tyrosine metabolism. *Ann. NY Acad. Sci.* 92:175–91

139. Laduron, P. L. 1975. Evidence for a localization of dopamine β-hydroxylase

within the chromaffin granules. *FEBS Lett.* 52:132–34

140. Land, H., Grez, M., Ruppert, S., Schmale, H., Rehbein, M., Richter, D., Schütz, G. 1983. Deduced amino acid sequence from the bovine oxytocin-neurophysin I precursor cDNA. *Nature* 302:342–44
141. Land, H., Schütz, G., Schmale, H., Richter, D. 1982. Nucleotide sequence of cloned cDNA encoding bovine arginine vasopressin—Neurophysin II precursor. *Nature* 295:299–303
142. Landymore-Lim, A. E. N., Bradbury, A. F., Smyth, D. G. 1983. The amidating-enzyme in pituitary will accept a peptide with C-terminal D-alanine as substrate. *Biochem. Biophys. Res. Commun.* 117: 289–93
143. Ledbetter, F. H., Kirshner, N. 1981. Quantitative correlation between secretion and cellular content of catecholamines and dopamine β-hydroxylase in cultures of adrenal medulla cells. *Biochem. Pharmacol.* 30:3246–49
144. Leinberger, R., Hull, W. E., Simon, H., Retey, J. 1981. Steric course of the NIH shift in the enzymic formation of homogentisic acid. *Eur. J. Biochem.* 117:311–18
145. Leung, C. T., Loewus, F. A. 1985. Concerning the presence and formation of ascorbic acid in yeasts. *Plant Sci.* 38:65–69
146. Levene, C. I., Aleo, J. J., Prynne, C. J., Bates, C. J. 1974. The activation of protocollagen proline hydroxylase by ascorbic acid in cultured 3T6 fibroblasts. *Biochem. Biophys. Acta* 388:29–36
147. Levenson, S. M., Seifter, E., Van Winkle, W. Jr. 1977. Nutrition. In *Fundamentals of Wound Management in Surgery,* ed. J. Englebert-Dunphy, T. K. Hunt, pp. 286–363. New York: Appleton-Century-Crofts
148. Levin, E. Y., Kaufman, S. 1961. Studies on the enzyme catalyzing conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. *J. Biol. Chem.* 236:2043–49
149. Levin, E. Y., Levenberg, B., Kaufman, S. 1960. The enzymatic conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. *J. Biol. Chem.* 235:2080–86
150. Levine, M. 1986. New concepts in the biology and biochemistry of ascorbic acid. *N. Engl. J. Med.* In press
151. Levine, M., Asher, A., Pollard, H., Zinder, O. 1983. Ascorbic acid and catecholamine secretion from cultured chromaffin cells. *J. Biol. Chem.* 258:13111–15
152. Levine, M., Morita, K. 1985. Ascorbic acid in endocrine systems. *Vitam. Horm.* 42:1–64
153. Levine, M., Morita, K., Pollard, H. 1986. Enhancement of norepinephrine biosynthesis by ascorbic acid in cultured bovine chromaffin cells. *J. Biol. Chem.* In press
154. Levine, S. Z., Gordon, H. H., Marples, E. 1941. A defect in the metabolism of tyrosine and phenylalanine in premature infants. II. Spontaneous occurrence and eradication by vitamin C. *J. Clin. Invest.* 20:209–19
155. Lewin, S. 1976. *Vitamin C: Its Molecular Biology and Medical Potential.* New York/London: Academic
156. Lindblad, B., Lindstedt, G., Lindstedt, S. 1970. The mechanism of enzymic formation of homogentisate from *p*-hydroxyphenyl pyruvate. *J. Am. Chem. Soc.* 92:7446–49
157. Lindblad, B., Lindstedt, G., Lindstedt, S., Rundgren, M. 1977. Purification and some properties of human 4-hydroxyphenyl pyruvate dioxygenase (I). *J. Biol. Chem.* 252:5073–84
158. Lindblad, B., Lindstedt, G., Tofft, M., Lindstedt, S. 1969. The mechanism of α-ketoglutarate oxidation in coupled enzymatic oxygenations. *J. Am. Chem. Soc.* 91:4604–6
159. Lindstedt, G. 1967. Hydroxylation of γ-butyrobetaine to carnitine in rat liver. *Biochemistry* 6:1271–82
160. Lindstedt, G., Lindstedt, S. 1970. Cofactor requirements of γ-butyrobetaine hydroxylase from rat liver. *J. Biol. Chem.* 245:4178–86
161. Lindstedt, G., Lindstedt, S., Nordin, I. 1977. Purification and properties of γ-butyrobetaine hydroxylase from *Pseudomonas* sp. AK1. *Biochemistry* 16: 2181–88
162. Lindstedt, G., Lindstedt, S., Nordin, I. 1980. Hydroxylation of γ-butyrobetaine. See Ref. 71, pp. 45–56
163. Lindstedt, G., Lindstedt, S., Nordin, I. 1982. γ-Butyrobetaine hydroxylase in human kidney. *Scand. J. Clin. Lab. Invest.* 42:477–85
164. Lindstedt, G., Lindstedt, S., Tofft, M. 1970. γ-Butyrobetaine hydroxylase from *Pseudomonas* sp. AK1. *Biochemistry* 9: 4336–42
165. Lindstedt, S., Nordin, I. 1984. Multiple forms of γ-butyrobetaine hydroxylase (EC 1.14.11.1). *Biochem. J.* 223:119–27
166. Lindstedt, S., Odelhög, B., Rundgren, M. 1977. Purification and some properties of 4-hydroxyphenylpyruvate dioxygenase from *Pseudomonas* sp. P.J. 874. *Biochemistry* 16:3369–77

167. Lindstedt, S., Rundgren, M. 1982. Inhibition of 4-hydroxyphenylpyruvate dioxygenase from *Pseudomonas* sp. strain P.J.874 by the *ENOL* tautomer of the substrate. *Biochim. Biophys. Acta* 704: 66–74
168. Liu, C. K., Hsu, C. A., Abbott, M. T. 1973. Catalysis of three sequential dioxygenase reactions by thymine 7-hydroxylase. *Arch. Biochem. Biophys.* 159:180–87
169. Liu, C. K., Shaffer, P. M., Slaughter, R. S., McKroskey, R. P., Abbott, M. T. 1972. Stoichiometry of the pyrimidine deoxyribonucleoside 2'-hydroxylase reaction and of the conversion of 5-hydroxymethyluracil to 5-formyluracil and of the latter to uracil-5-carboxylic acid. *Biochemistry* 11:2172–76
170. Loewus, F. A., Helsper, J. P. F. G. 1982. Metabolism of ascorbic acid in plants. See Ref. 249, pp. 249–61
171. Loewus, F. A., Wagner, G., Yang, J. C. 1975. Biosynthesis and metabolism of ascorbic acid in plants. *Ann. NY Acad. Sci.* 258:7–23
172. Lyons, B. L., Schwarz, R. I. 1984. Ascorbate stimulation of PAT cells causes an increase in transcription rates and decrease in degradation rates of procollagen mRNA. *Nucleic Acids Res.* 12:2569–79
173. Mains, R. E., Glembotski, C. C., Eipper, B. A. 1984. Peptide α-amidation activity in mouse anterior pituitary ALT-20 cell granules: properties and secretion. *Endocrinology* 114:1522–30
174. Majamaa, K., Hanauske-Abel, H. M., Günzler, V., Kivirikko, K. I. 1984. The 2-oxoglutarate binding site of prolyl 4-hydroxylase. Identification of distinct subsites and evidence for 2-oxoglutarate decarboxylation in a ligand reaction at the enzyme-bound ferrous ion. *Eur. J. Biochem.* 138:239–45
175. Majamaa, K., Turpeenniemi-Hujanen, T. M., Latipää, P., Günzler, V., Hanauske-Abel, H. M., et al. 1985. Differences between collagen hydroxylases and 2-oxoglutarate dehydrogenase in their inhibition by structural analogues of 2-oxoglutarate. *Biochem. J.* 229:127–33
176. Marshall, E. 1985. The academy kills a nutrition report. *Science* 230:420–21
177. Martin, G. R. 1961. Studies on tissue distribution of ascorbic acid. *Ann. NY Acad. Sci.* 92:141–47
178. Mata, J. M., Assad, R., Peterkofsky, B. 1981. An intramembranous reductant which participates in the proline hydroxylation reaction with intracisternal prolyl hydroxylase and underhydroxylated procollagen in isolated microsomes from L-929 cells. *Arch. Biochem. Biophys.* 206:93–104
179. Matsuura, T. 1977. Bio-mimetic oxygenation. *Tetrahedron* 33:2869–905
180. McCay, P. B. 1985. Vitamine E: interaction with free radicals and ascorbate. *Ann. Rev. Nutr.* 5:323–40
181. Deleted in proof
182. Miller, S. M., Klinman, J. P. 1985. Secondary isotope effects and structure-reactivity correlations in the dopamine β-monooxygenase reaction: evidence for a chemical mechanism. *Biochemistry* 24:2114–27
183. Minth, C. D., Bloom, S. R., Polak, J. M., Dixon, J. E. 1984. Cloning characterization, and DNA sequence of a human cDNA encoding neuropeptide. *Proc. Natl. Acad. Sci. USA* 81:4577–81
184. Mizushima, Y., Harauchi, T., Yoshizaki, T., Makinos, S. 1984. A rat mutant unable to synthesize vitamin C. *Experientia* 40:359–61
185. Moertel, C. G., Flemming, T. R., Creagen, E. T., Rubin, J., O'Connell, M. J., Ames, M. M. 1985. High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. *N. Engl. J. Med.* 312:137–41
186. Morris, K. M., Paz, M. A. 1980. Post-translational hydroxylation of C1q: immunochemical and functional evidence for an effect of ascorbic acid. *8th Int. Complement Workshop* (Abstr.)
187. Müller, W., Hanauske-Abel, H., Loos, M. 1978. Reversible inhibition of C1q release from guinea pig macrophages by 2,2'dipyridyl. Evidence for a posttranslational hydroxylation step in the biosynthesis of C1q, a subcomponent of the first component of complement (C1). *FEBS Lett.* 90:218–22
188. Murad, S., Grove, D., Lindberg, K. A., Reynolds, G., Sivarajah, A., Pinnell, S. R. 1981. Regulation of collagen synthesis by ascorbic acid. *Proc. Natl. Acad. Sci.* 78:2879–82
189. Murad, S., Sivarajah, A., Pinnell, S. R. 1980. Prolyl and lysyl hydroxylase activities of human skin fibroblasts: effect of donor age and ascorbate. *J. Invest. Dermatol.* 75:404–7
190. Murad, S., Sivarajah, A., Pinnell, S. R. 1981. Regulation of prolyl and lysyl hydroxylase activities in cultured human skin fibroblasts by ascorbic acid. *Biochem. Biophys. Res. Commun.* 101:868–75
191. Myllylä, R., Kuutti-Savolainen, E. R., Kivirikko, K. I. 1978. The role of ascorbate in the prolyl hydroxylase reaction.

Biochem. Biophys. Res. Commun. 83: 441–48
192. Myllylä, R., Majamaa, K., Günzler, V., Hanauske-Abel, H. M., Kivirikko, K. I. 1984. Ascorbate is consumed stoichiometrically in the uncoupled reaction catalyzed by prolyl 4-hydroxylase and lysyl hydroxylase. *J. Biol. Chem.* 259:5403–5
193. Myllylä, R., Tuderman, L., Kivirikko, K. I. 1977. Mechanism of the prolyl hydroxylase reaction 2. Kinetic analysis of the reaction sequence. *Eur. J. Biochem.* 80:349–57
194. Nakai, C., Nozaki, M., Hayaishi, O., Saito, J., Matsuura, T. 1975. Studies on a possible reaction intermediate of *p*-hydroxyphenylpyruvate dioxygenase. *Biochem. Biophys. Res. Commun.* 67: 590–95
195. Nakanishi, S., Inoue, A., Kita, T., Nakamura, M., Chang, A. C. Y., et al. 1979. Nucleotide sequence of cloned cDNA for bovine corticotropin-β-lipotropin precursor. *Nature* 278:423–27
196. Nawa, H., Hirose, T., Takashima, H., Inayama, S., Nakanishi, S. 1983. Nucleotide sequences of cloned cDNAs for two types of bovine brain substance P precursor. *Nature* 306:32–36
197. Nelson, P. J., Pruitt, R. E., Henderson, L. L., Jenness, R., Henderson, L. M. 1981. Effect of ascorbic acid deficiency on the *in vivo* synthesis of camitine. *Biochim. Biophys. Acta* 672:123–27
197a. Nietfeld, J. J., Kemp, A. 1980. Properties of prolyl 4-hydroxylase containing firmly-bound iron. *Biochim. Biophys. Acta* 613:349–58
198. Nietfeld, J. J., Kemp, A. 1981. The function of ascorbate with respect to prolyl 4-hydroxylase activity. *Biochim. Biophys. Acta* 657:159–67
199. Nitowsky, H. M., Govan, C. D. Jr., Gordon, H. E. 1953. Effect of hemopoietic and other agents on the hydroxyphenyluria of premature infants. *Am. J. Dis. Child.* 85:462–80
200. Nolan, J. C., Cardinale, G. J., Udenfriend, S. 1978. The formation of hydroxyproline in collagen by cells grown in the absence of serum. *Biochim. Biophys. Acta* 543:116–22
201. Novak, R. F., Swift, T. J., Hoppel, C. L. 1980. N^6-trimethyl-lysine metabolism. Structural identification of the metabolite 3-hydroxy-N^6-trimethyl-lysine. *Biochem. J.* 188:521–27
202. Olsen, B., Berg, R. A. 1979. Post-translational processing and secretion of procollagen in fibroblasts. In *Secretory Mechanisms,* ed. C. R. Hopkins, C. J. Duncan, pp. 57–78. Cambridge: Cambridge Univ.
203. Paglia, L., Wilczek, J., Diaz DeLeon, L., Martin, G. R., Horlein, D., Muller, P. 1979. Inhibition of procollagen cell-free synthesis by amino-terminal extension peptides. *Biochemistry* 18:5030–34
204. Paik, W. K., Kim, S. 1980. *Protein Methylation.* New York: Wiley. 282 pp.
205. Pauling, L., et al. 1982. Workshop on vitamin C in immunology and cancer. *Int. Z. Vitam. Ernahrungforsch.* 23: 209–19
206. Paz, M. A., Gallop, P. M. 1975. Collagen synthesized and modified by aging fibroblasts in culture. *In Vitro* 11:302–12
207. Peterkofsky, B. 1972. Regulation of collagen secretion by ascorbic acid in 3T3 and chick embryo fibroblasts. *Biochem. Biophys. Res. Commun.* 49:1343–50
208. Peterkofsky, B. 1972. The effect of ascorbic acid on collagen polypeptide synthesis and proline hydroxylation during the growth cultured fibroblasts. *Arch. Biochem. Biophys.* 152:318–28
209. Peterkofsky, B., Kalwinsky, D., Assad, R. 1980. A substance in L-929 cell extract which replaces the ascorbate requirement in a tritium release assay for reducing cofactor; correlation of its concentration with the extent of ascorbate-independent proline hydroxylation and the level of prolyl hydroxylase activity in these cells. *Arch. Biochem. Biophys.* 199:362–73
210. Peterson, V. E., Crapo, P. A., Weiniger, J., Ginsberg, H., Olefsky, J. 1975. Quantification of plasma cholesterol and triglyceride levels in hypercholesterolemic subject receiving ascorbic acid supplements. *Am. J. Clin. Nutr.* 28:584–87
211. Piez, K. A., Reddi, A. H., eds. 1984. *Extracellular Matrix Biochemistry.* New York/Amsterdam/Oxford: Elsevier. 473 pp.
212. Pollard, H. B., Pazoles, C. J., Cruetz, C. C., Scott, J. H., Zinder, O., Hotchkiss, A. 1984. An osmotic mechanism for exocytosis from dissociated chromaffin cells. *J. Biol. Chem.* 259:1114–21
213. Porter, R. R., Reid, K. B. 1978. The biochemistry of complement. *Nature* 275:699–704
214. Prinz, W., Bortz, R., Bregin, B., Hersch, M. 1977. The effect of ascorbic acid supplementation on some parameters of the human immunological defense system. *Int. J. Vitam. Nutr. Res.* 47:248–57
215. Prockop, D. J., Berg, R. A., Kivirikko, K. I., Uitto, J. 1976. Intracellular steps in the biosynthesis of collagen. In *Biochemistry of Collagen,* ed. G. N. Ramachandran, A. H. Reddi, pp. 163–273. New York: Plenum.

216. Prockop, D. J., Juva, K. 1965. Synthesis of hydroxyproline *in vitro* by the hydroxylation of proline in a precursor of collagen. *Proc. Natl. Acad. Sci. USA* 53:661–68
217. Prockop, D. J., Tuderman, L. 1982. Posttranslational enzymes in the biosynthesis of collagens: extracellular enzymes. *Methods Enzymol.* 82(PA): 305–19
218. Puistola, U., Turpeenniemi-Hujanen, T. M., Myllylä, R., Kivirikko, K. I. 1980. Studies on the lysyl hydroxylase reaction I. Initial velocity kinetics and related aspects. *Biochim. Biophys. Acta* 611:40–50
219. Puistola, U., Turpeenniemi-Hujanen, T. M., Myllylä, R., Kivirikko, K. I. 1980. Studies on the lysyl hydroxylase reaction II. Inhibition kinetics of the reaction mechanism. *Biochim. Biophys. Acta* 611:51–60
220. Quinn, R. S., Krane, S. M. 1976. Abnormal properties of collagen lysyl hydroxylase from skin fibroblasts of siblings with hydroxylysine-deficient collagen. *J. Clin. Invest.* 57:83–93
221. Quinn, R. S., Krane, S. M. 1979. Collagen synthesis by cultured skin fibroblasts from siblings with hydroxylysine-deficient collagen. *Biochim. Biophys. Acta* 585:589–98
222. Rao, N. V., Adams, E. 1978. Partial reaction of prolyl hydroxylase. *J. Biol. Chem.* 253:6327–30
223. Rebouche, C. J., Broquist, H. P. 1976. Carnitine biosynthesis in *Neurospora crassa:* Enzymatic conversion of lysine to ϵ-*N*-trimethyllysine. *J. Bacteriol.* 126:1207–14
224. Rebouche, C. J., Engel, A. G. 1980. Tissue distribution of carnitine biosynthetic enzymes in man. *Biochim. Biophys. Acta* 630:22–29
225. Rebouche, C. J., Lehman, L. J. 1985. Availability of a dietary ϵ-*N*-trimethylysine for carnitine biosynthesis in growing rat. *Fed. Proc.* 44(3):763 (Abstr.)
226. Rebouche, C. J., Paulson, D. J. 1986. Carnitine metabolism and function in humans. *Ann. Rev. Nutr.* 6:41–66
227. *Recommended Daily Allowances*. 1980. Food and nutrition board, National Academy of Sciences, pp. 72–82. Washington, DC: Natl. Res. Council. 9th ed.
228. Reid, K. B. M., Porter, R. R. 1976. Subunit composition and structure of subcomponent C1q of the first component of human complement. *Biochem. J.* 155:19–23
228a. Risteli, J., Tuderman, L., Kivirikko, K. I. 1976. Intracellular enzymes of collagen biosynthesis in rat liver as a function of age and in hepatic injury induced by dimethylnitrosamine. Purification of rat prolyl hydroxylase and comparison of changes in prolyl hydroxylase activity with changes in immunoreactive prolyl hydroxylase. *Biochem. J.* 158:369–76
229. Robertson, W. B., Hewitt, J., Herman, C. 1959. The relation of ascorbic acid to the conversion of proline to hydroxyproline in the synthesis of collagen in the carrageenan granuloma. *J. Biol. Chem.* 234:105–8
230. Roche, P. A., Moorehead, T. J., Hamilton, G. A. 1982. Purification and properties of hog liver 4-hydroxyphenylpyruvate dioxygenase. *Arch. Biochem. Biophys.* 216:62–73
231. Roeser, H. P. 1983. The role of ascorbic acid in the turnover of storage iron. *Semin. Hematol.* 20:91–100
232. Rosenberg, R. C., Lovenberg, W. 1980. Dopamine β-hydroxylase. *Essays Neurochem. Neuropharmacol.* 4:163–209
233. Rosenberry, T. L., Richardson, J. M. 1977. Structure of 18S and 14S acetylcholinesterase. Identification of collagen-like subunits that are linked by disulfide bonds to catalytic subunits. *Biochemistry* 16:3550–58
234. Rowe, L. B., Schwarz, R. I. 1983. Role of procollagen mRNA levels in controlling the rate of procollagen synthesis. *Mol. Cell. Biol.* 3:241–49
235. Rundgren, M. 1982. Tritium isotope effects in the reaction catalyzed by 4-hydroxyphenylpyruvate dioxygenase from *Pseudomonas* sp. strain P.J. 874. *Biochim. Biophys. Acta* 704:59–65
236. Rundgren, M. 1983. Some kinetic properties of 4-hydroxyphenylpyruvate dioxygenase from *Pseudomonas* sp. strain P.J. 874. *Eur. J. Biochem.* 133:657–63
237. Sachan, D. S., Hoppel, C. L. 1980. Carnitine biosynthesis. Hydroxylation of N^6-trimethyl-lysine to 3-hydroxy-N^6-trimethyl-lysine. *Biochem. J.* 188:529–34
238. Saito, I., Yamane, M., Shimazu, H., Matsuura, T., Cahnmann, H. J. 1975. Biogenetic type conversion of *p*-hydroxyphenylpyruvic acid into homogentisic acid. *Tetrahedron Lett.* 1975:641–44
239. Sandor, A., Kispal, G., Kerner, J., Alkonyi, I. 1983. Combined effect of ascorbic acid deficiency and underfeeding on the hepatic camitine level in guinea-pigs. *Experientia* 39:512–13
240. Sapper, H., Kang, S. O., Paul, H. H., Lohmann, W. 1982. The reversibility of the vitamin C redox system: Electrochemical reasons and biological aspects. *Z. Naturforsch. Teil C* 37:942–46

241. Sato, P., Udenfriend, S. 1978. Studies on ascorbic acid related to the genetic basis of scurvy. *Vitamin Horm.* 36:33–52
242. Schwartz, E., Bienkowski, R. S., Coltoff-Schiller, B., Goldfischer, S., Blumenfeld, O. O. 1982. Changes in the components of extracellular matrix and in growth properties of cultured aortic smooth muscle cells upon ascorbate feeding. *J. Cell. Biol.* 92:462–70
243. Schwarz, R. I. 1985. Procollagen secretion meets the minimum requirements for the rate-controlling step in the ascorbate induction of procollagen synthesis. *J. Biol. Chem.* 260:3045–49
244. Schwarz, R. I., Bissell, M. J. 1977. Dependence of the differentiated state on the cellular environment: Modulation of collagen synthesis in tendon cells. *Proc. Natl. Acad. Sci. USA* 74:4453–57
245. Schwarz, R. I., Mandell, R. B., Bissell, M. J. 1981. Ascorbate induction of collagen synthesis as a means for elucidating a mechanism for quantitative control of tissue-specific function. *Mol. Cell. Biol.* 1:843–53
246. Sealock, R. R., Goodland, R. L. 1951. Ascorbic acid, a coenzyme in tyrosine oxidation. *Science* 114:645–46
247. Sealock, R. R., Goodland, R. L., Summerwell, W. N., Brierly, J. M. 1952. The role of ascorbic acid in the oxidation of L-tyrosine by guinea pig liver extract. *J. Biol. Chem.* 196:761–67
248. Sealock, R. R., Silberstein, H. E. 1940. The excretion of homogentisic acid and other tyrosine metabolites by the vitamin C–deficient guinea pig. *J. Biol. Chem.* 135:251–58
249. Seib, P. A., Tolbert, B. M., eds. 1982. *Ascorbic Acid: Chemistry, Metabolism and Uses*, Adv. Chem. Ser. 200. Washington, DC: Am. Chem. Soc. 604 pp.
250. Seifter, S., Gallop, P. M. 1966. The structure of proteins. In *The Proteins*, ed. H. Neurath, 4:153–458. New York/London: Academic. 2nd ed.
251. Shaffer, P. M., Hsu, C. A., Abbott, M. T. 1975. Metabolism of pyrimidine deoxyribonucleosides in *Neurospora crassa*. *J. Bacteriol.* 121:648–55
252. Shaffer, P. M., McCroskey, R. P., Abbott, M. T. 1972. Substrate specificity of the hydroxylase reaction in which thymidine is converted to thymine ribonucleoside. *Biochim. Biophys. Acta* 258: 387–94
253. Shaffer, P. M., McCroskey, R. P., Palmatier, R. D., Midgett, R. J., Abbott, M. T. 1968. The cell-free conversion of a deoxyribonucleoside to a ribonucleoside without detachment of the deoxyribose. *Biochem. Biophys. Res. Commun.* 33: 806–11
254. Shen, T. F., Strecker, H. J. 1975. Synthesis of proline and hydroxyproline in human lung (WI-38) fibroblasts. *Biochem. J.* 150:453–61
255. Skotland, T., LJones, T. 1979. Dopamine β-mono-oxygenase: Structure, mechanism and properties of the enzyme-bound copper. *Inorg. Perspect. Biol. Med.* 2:151–80
256. Skotland, T., LJones, T. 1980. Direct spectrophotometric detection of ascorbate free radical formed by dopamine β-monooxygenase and by ascorbate oxidase. *Biochim. Biophys. Acta* 630: 30–35
257. Deleted in proof
258. Spanheimer, R. G., Peterkofsky, B. 1985. A specific decrease in collagen synthesis in acutely fasted, vitamin C–supplemented guinea pigs. *J. Biol. Chem.* 260:3955–62
259. Spindel, E. R., Chin, W. W., Price, J., Rees, L. H., Besser, G. M., Habener, J. F. 1984. Cloning and characterization of cDNAs encoding human gastrin releasing peptides. *Proc. Natl. Acad. Sci. USA* 81:5699–5703
260. Stassen, F. I. H., Cardinale, G. J., Udenfriend, S. 1973. Activation of prolyl hydroxylase in L-929 fibroblasts by ascorbic acid. *Proc. Natl. Acad. Sci. USA* 70:1090–93
261. Stein, R., Englard, S. 1981. The use of a tritium release assay to measure 6-*N*-trimethyl-L-lysine hydroxylase activity: Synthesis of 6-*N*-[3-^{3}H]trimethyl-D,L-lysine. *Anal. Biochem.* 116:230–36
262. Stein, R., Englard, S. 1982. Properties of rat 6-*N*-trimethyl-L-lysine hydroxylases: Similarities among kidney, liver, heart, and skeletal muscle activities. *Arch. Biochem. Biophys.* 217:324–31
263. Stubbe, J.-A. 1985. Identification of two α-ketoglutarate-dependent dioxygenases in extracts of *Rhodotorula glutinis* catalyzing deoxyuridine hydroxylation. *J. Biol. Chem.* 260:9972–75
264. Sugano, K., Aponte, G. W., Yamada, T. 1985. Identification and characterization of glycine-extended post-translational processing intermediates of prograstin in porcine stomach. *J. Biol. Chem.* 260: 11724–29
265. Takeuchi, T., Yamada, T. 1985. Isolation of a cDNA clone encoding pancreatic polypeptide. *Proc. Natl. Acad. Sci. USA* 82:1536–39
266. Terland, O., Flatmark, T. 1975. Ascorbate as a natural constituent of chromaf-

fin granules from bovine adrenal medulla. *FEBS Lett.* 59:52–56
267. Thoma, W. J., Henderson, L. L. 1984. Effect of vitamin C deficiency on hydroxylation of trimethylaminobutyrate to carnitine in the guinea pig. *Biochim. Biophys. Acta* 797:136–39
268. Thomas, W. R., Holt, P. G. 1978. Vitamin C and immunity: An assessment of the evidence. *Clin. Exp. Immunol.* 32:370–79
269. Tirrell, J. G., Westhead, E. W. 1979. The uptake of ascorbic acid and dehydroascorbic acid by chromaffin granules of the adrenal medulla. *Neuroscience* 4:181–86
270. Tolbert, B. M., Ward, J. B. 1982. Dehydroascorbic acid. See Ref. 249, pp. 101–23
271. Tolbert, N. E. 1981. Metabolic pathways in peroxisomes and glyoxysomes. *Ann. Rev. Biochem.* 50:133–57
271a. Tryggvason, K., Majamaa, K., Risteli, J., Kivirikko, K. I. 1979. Partial purification and characterization of chick-embryo prolyl 3-hydroxylase. *Biochem. J.* 183:303–7
271b. Tuderman, L., Kuutti, E. R., Kivirikko, K. I. 1975. An affinity-column procedure using poly (L-proline) for the purification of prolyl hydroxylase. Purification of the enzyme from chick embryos. *Eur. J. Biochem.* 52:9–16
272. Tuderman, L., Myllylä, R., Kivirikko, K. I. 1977. Mechanism of the prolyl hydroxylase reaction. 1. Role of cosubstrates. *Eur. J. Biochem.* 80:341–48
272a. Turpeenniemi-Hujanen, T. M., Puistola, U., Kivirikko, K. I. 1980. Isolation of lysyl hydroxylase, an enzyme of collagen synthesis, from chick embryos as a homogeneous protein. *Biochem. J.* 189:247–53
272b. Turpeenniemi-Hujanen, T. M., Puistola, U., Kivirikko, K. I. 1981. Human lysyl hydroxylase: purification to homogeneity, partial characterization and comparison of catalytic properties with those of a mutant enzyme from Ehler-Danlos syndrome type VI fibroblasts. *Collagen Rel. Res.* 1:355–66
272c. Urushizaki, Y., Seifter, S. 1985. Phosphorylation of hydroxylysine residues in collagen synthesized by cultured aortic smooth muscle cells. *Proc. Natl. Acad. Sci. USA* 82:3091–95
273. Vale, W., Spiess, J., Rivier, C., Rivier, J. 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. *Science* 213:1394–97
274. Villafranca, J. J., Colombo, G., Ratashekhar, B., Giedroc, D., Baldoni, J. 1982. Dopamine β-hydroxylase: Studies of the Cu^{+2} environment and development of suicide inhibitors. In *Oxygenases and Oxygen Metabolism,* ed. M. Nozaki, S. Yamamoto, Y. Ishimura, M. J. Coon, L. Ernster, R. W. Estabrook, pp. 125–35. New York/London/Paris: Academic
275. Wada, G., Fellman, J. H., Fujita, T. S., Roth, E. S. 1975. Purification and properties of avian liver *p*-hydroxyphenylpyruvate hydroxylase. *J. Biol. Chem.* 250:6720–26
276. Warn-Cramer, B. J., Macrander, L. A., Abbott, M. T. 1983. Markedly different ascorbate dependencies of the sequential α-ketoglutarate dioxygenase reactions catalyzed by an essentially homogeneous tymine 7-hydroxylase from *Rhodotorula glutinis*. *J. Biol. Chem.* 258:10551–57
277. Watanabe, M. S., McCroskey, R. P., Abbott, M. T. 1970. The enzymatic conversion of 5-formyluracil to uracil 5-carboxylic acid. *J. Biol. Chem.* 245: 2023–26
278. Weber, E., Esch, F. S., Bohlen, P., Patterson, S., Corbett, A. D., et al. 1983. Metorphamide: Isolation, structure, and biologic activity of an amidated opioid octapeptide from bovine brain. *Proc. Natl. Acad. Sci. USA* 80:7362–66
279. Wiestner, M., Krieger, T., Horlein, D., Glanville, R. W., Fietzek, P., Muller, P. K. 1979. Inhibiting effect of procollagen peptides on collagen biosynthesis in fibroblast cultures. *J. Biol. Chem.* 254: 7016–23
280. Witkop, B. 1973. In *Current Topics in Biochemistry,* ed. C. B. Anfinsen, A. N. Schechter, pp. 109–33. New York: Academic
281. Wittes, R. E. 1985. Vitamin C and cancer. *N. Engl. J. Med.* 312:178–79
282. Wondrack, L. M., Hsu, C.-A., Abbott, M. T. 1978. Thymine 7-hydroxylase and pyrimidine deoxyribonucleoside 2^1-hydroxylase activities in *Rhodotorula glutinis*. *J. Biol. Chem.* 253:6511–15
283. Wondrack, L. M., Warn, B. J., Saewert, M. D., Abbott, M. T. 1979. Substitution of nucleoside triphosphates for ascorbate in the thymine 7-hydroxylase reaction of *Rhodotorula glutinis*. *J. Biol. Chem.* 254:26–29
284. Woodruff, C. W. 1975. Ascorbic acid—scurvy. *Prog. Food Nutr. Sci.* 1:493–506
285. Yew, M. S. 1985. Biosynthesis of ascorbic acid in chick embryos. *Experientia* 41:943–44
286. Yoo, O. J., Powell, C. T., Agarwal, K. L. 1982. Molecular cloning and nucle-

otide sequence of full-length cDNA coding for porcine gastrin. *Proc. Natl. Acad. Sci. USA* 79:1049–53

287. Zannoni, V. G., Holsztynska, E. J., Lau, S. S. 1982. Biochemical functions of ascorbic acid in drug metabolism. See Ref. 249, pp. 349–68
288. Zannoni, V. G., LaDu, B. N. 1959. The tyrosine oxidation system of liver. IV. Studies on the inhibition of *p*-hydroxyphenylpyruvic acid oxidase by excess substrate. *J. Biol. Chem.* 234:2925–31
289. Zannoni, V. G., Susick, R. L., Smart, R. C. 1984. Ascorbic acid as it relates to the metabolism of drugs and environmental agents. *Curr. Concepts Nutr.* 13:21–35
290. Zern, M. A., Schwartz, E., Giambrone, M.-A., Blumenfeld, O. O. 1985. Ascorbate-generated endogenous extracellular matrix affects cell protein synthesis in calf aortic smooth muscle cells. *Exp. Cell. Res.* 160:307–18