VITAMINS AND HORMONES

VOLUME 42

VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

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Preface

Volume 42 of *Vitamins and Hormones* reflects well our goal to provide informative, current, and stimulating reviews of topics important to researchers and scholars in endocrinology and nutrition. We thank the authors for their timely contributions that include a wealth of knowledge, provocative concepts, and healthy controversy.

A new look, long overdue for readers of *Vitamins and Hormones*, at ascorbic acid and its role in endocrine processes, nutrition, and enzymology is presented by M. A. Levine and K. Morita. The interactions of ascorbic acid with the endocrine system are fascinating, as is the function of the vitamin in biochemical mechanisms. The precise functions of ascorbate in endocrine systems remain elusive, but once fully understood may provide better indices than those currently used to determine nutritional figure for nutritional requirements for the vitamin.

Bone GLA protein or osteocalcin, discussed by P. A. Price, has been structurally characterized and established as a major noncollagenous protein of bone. It is known to be under the control of two vitamins, D and K, yet its actual function in bone physiology is still to be elucidated.

H. B. Pollard and coauthors have organized for us the complex array of information on secretory mechanisms in the adrenal medulla. They provide a theory on mechanisms of exocytosis, progressing from biosynthesis of catecholamines to packaging in the secretory granule, transport toward the cell periphery, fusion of the granule with the cell membrane, and lysis with release of contents to the exterior.

P. F. Hall reviews the functions of cytochrome *P*-450 in the biosynthesis of steroid hormones. This mixed function oxygenase(s) utilizes molecular oxygen in catalyzing such reactions as side-chain cleavage and hydroxylation at the 11 and 21 positions of the steroid nucleus.

J. F. Harper and colleagues discuss compartmentalization of intracellular messengers. Cyclic AMP, cyclic GMP, and calcium show distinct subcellular localizations, and such compartmentalization may constitute an important regulatory mechanism in the control of cellular function by intracellular messengers.

J. B. Buse and G. S. Eisenbarth have reviewed the fascinating clinical syndromes of autoimmune endocrine disease. In these disturbances an-

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PETER F. HALL

Discovery consists in seeing what everybody else has seen and thinking what nobody has thought.

ALBERT SZENT-GYORGI

I. PROLOGUE

A. INTRODUCTION

Ascorbic acid[1] is found in highest concentration in certain endocrine tissues of almost all mammals (Svirbely and Szent-Gyorgi, 1932; Harris and Ray, 1933; Glick and Biskind, 1935; Yavorsky *et al.*, 1934; Hornig, 1975; Table I). Yet we have only begun to appreciate the importance of ascorbic acid physiologically, functionally, and dynamically in these tissues. In this review we emphasize emerging knowledge and the perhaps critical importance of ascorbic acid in some endocrine tissues. We will review briefly the chemistry of ascorbic acid as well as what is currently known about ascorbic acid in specific endocrine systems. We wish not to simply annotate the existing literature on ascorbate, but to pinpoint areas of controversy, propose new hypotheses, and emphasize what we believe are vital investigations for the future. Most importantly, we propose that study of ascorbic acid in endocrine systems may awaken scientists to the need to find the optimal requirements of cofactors in cells and organisms, and perhaps change medical practice accordingly.

B. STATEMENT OF THE PROBLEM

Ascorbic acid is required by human beings (Lind, 1753; Svirbely and Szent-Gyorgi, 1932). Ingestion of ~0.9 mg/kg/day affords adequate protection against scurvy (Abt *et al.*, 1963; Hodges *et al.*, 1969, 1971; Baker *et al.*, 1971; Recommended Daily Allowances, 1980). Yet ascorbic acid is synthesized by most other mammals at a rate of ~40–275 mg/kg/day (Chatterjee, 1973; Table II). These synthetic rates imply that optimal requirements for ascorbic acid in humans may exceed those required merely to prevent scurvy. Ascorbate requirements may further be affected by the milieu of the cell, tissue, or animal. Therefore, it is possible ascorbic acid requirements that afford adequate protection from scurvy are not at all equivalent to optimal require-

[1] Ascorbic acid and ascorbate are used interchangeably. Likewise, dehydroascorbic acid and dehydroascorbate are used interchangeably.

TABLE I
TISSUE CONCENTRATIONS OF ASCORBIC ACID

Tissue	Ascorbic acid (mg/100 g tissue)
Rats[a]	
Adrenal glands	280–400
Pituitary gland	100–130
Liver	25–40
Spleen	40–50
Lungs	20–40
Kidneys	15–20
Testes	25–30
Thyroid	22
Thymus	40
Brain	35–50
Eye lens	8–10
Skeletal muscle	5
Heart muscle	5–10
Bone marrow	12
Plasma	1.6
Blood	0.9
Adult human tissues[b]	
Adrenal glands	30–40
Pituitary gland	40–50
Liver	10–16
Spleen	10–15
Lungs	7
Kidneys	5–15
Testes	3
Thyroid	2
Heart muscle	5–15
Skeletal muscle	3–4
Brain	13–15
Pancreas	10–15
Eye lens	25–31
Plasma	0.4–1.0
Saliva	0.07–0.09

[a] Tissue concentrations of ascorbic acid in rats. Data are compiled from many investigators (modified from Hornig, 1975).

[b] Tissue concentrations of ascorbic acid in humans, from multiple autopsy studies (modified from Hornig, 1975).

ments; furthermore, optimal requirements may be dependent on homeostasis.

Until now study of optimal ascorbic acid requirements has been extremely difficult (Baker, 1967). It has not been clear what constitutes an appropriate measure of ascorbic acid need other than preven-

TABLE II
ASCORBIC ACID SYNTHETIC RATES IN MAMMALS[a]

Mammal	Rate (mg/kg/day)
Mouse	275
Rabbit	226
Goat	190
Rat	150
Dog	40
Cat	40
Human RDA	0.9

[a] Data were calculated from synthetic rates in liver homogenates. Each synthetic rate (milligram of synthesized ascorbate/gram of tissue/hour) was multiplied by the weight of the liver and then by 24 hours to obtain an estimate of daily synthetic capacity. The estimated human requirement was determined by dividing the Recommended Daily Allowance by 70 kg (modified from Chatterjee, 1973).

tion of scurvy. We believe that an ideal model system for investigation of these problems has been ascorbic acid in endocrine systems. Ascorbic acid concentration is highest in several mammalian endocrine tissues (Table I). Study of ascorbic acid function in these tissues is still in its infancy. It is first important to learn why ascorbate is present at all in these tissues, particularly adrenal medulla, cortex, and pituitary. Since the behavior of the adrenal and pituitary glands is intricately intertwined with homeostasis, further study of these tissues may provide unique models to determine optimal ascorbic acid requirements as a function of cellular milieu.

II. ASCORBIC ACID: THE SUBSTANCE

Overviews of the chemistry of ascorbic acid and of methods for its detection are essential for understanding ascorbic acid in biological systems. Indeed, appreciation of basic ascorbate chemistry will permit us to suggest functions for ascorbate in endocrine tissues. Knowledge of the difficulties with older ascorbate assays may help to explain why formulations for ascorbate function are just emerging now. Therefore, we will review ascorbic acid chemistry and assay techniques before considering ascorbic acid in biological systems. We will first highlight historical aspects of ascorbic acid, since these aspects are important for a basic appreciation of this field.

A. History

The earliest physicians did not know what ascorbic acid was, but they clearly were familiar with the end results of lack of ascorbate in the diet. A disease remarkably similar to scurvy was described by the ancient Egyptians in the Papyrus Ebers (see Hodges, 1980). The ancient Greeks were likewise ravaged by a disease described in nearly identical terms (Major, 1945; Mettler, 1947). Explorers of the New World such as Jacques Cartiers were aware of a pestilence that could be cured by ingestion of the bark and leaves of the "Ameda tree" (sassafras or possibly spruce tree: see Major, 1945; Mettler, 1947). Two hundred years later, in the mid-eighteenth century, the Scottish physician James Lind described, in his "Treatise on the Scurvy" (Lind, 1753; Major, 1945, Mettler, 1947), how this disease could be prevented by consumption of citrus fruits. Physicians then were just as remarkably recalcitrant as in later ages to accept the potential value of cofactors in disease prevention. It was not for another two generations that citrus fruits were included in the rations of British sailors, or "limeys."

Although scurvy could be prevented, the substance responsible was not isolated for another 150 years. In the late 1920s Szent-Gyorgi isolated hexuronic acid (Szent-Gyorgi 1927, 1928), which was simultaneously found to be the specific antiscorbutic factor by Svirbely and Szent-Gyorgi (1932) and by Waugh and King (1932a,b). It is more than coincidence that Szent-Gyorgi used bovine adrenal glands to isolate hexuronic or ascorbic acid (Szent-Gyorgi, 1928; Svirbely and Szent-Gyorgi, 1932). We now know that the highest concentration of ascorbic acid is found in the adrenal (Table I), but we have only begun to recognize the link between ascorbate and its biological function.

B. Chemistry of Ascorbic Acid and Dehydroascorbic Acid

Ascorbic acid is a ketolactone, formula $C_6H_6O_8$, with a molecular weight of 176.1 (see Fig. 1). Ascorbic acid ionizes in two stages. The first pK value is ~4.2 at 37°C, and the second is ~11.6. Thus, at physiological pH ascorbate is nearly totally in its anionic form. The two ionizations are thought to occur at C-2 and C-3 (Lewin, 1976; Fig. 1).

The critical function of ascorbate in biological systems may derive from its ability to donate electrons while itself undergoing reversible oxidation to dehydroascorbic acid (Bielski *et al.*, 1975; Lewin, 1976). The oxidation pathway is shown in Fig. 2. An intermediate oxidation product is thought to exist between ascorbate and dehydroascorbic acid and has variously been called the ascorbate free radical or semidehydroascorbate (see Fig. 2).

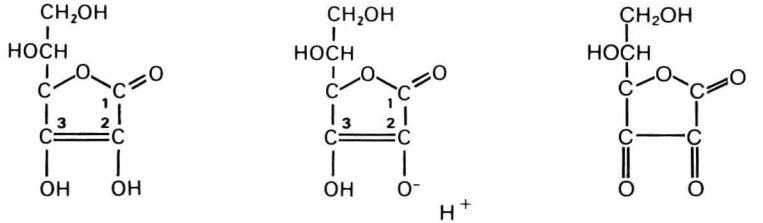

FIG. 1. Ascorbic acid, the ascorbate anion, and dehydroascorbic acid are shown. Ionization occurs at C-2 or C-3.

Expressed in conventional terms, where oxidant + ne^- = reductant, the standard oxidation–reduction potential of dehydroascorbate to ascorbate is +0.058 V (Lewin, 1976).[2] The standard redox potential of ascorbate free radical to ascorbate is +0.34 V (Everling *et al.*, 1969). The Nernst equation expresses the relationship between the standard redox potential of a chosen conjugate pair, the observed potential, and the concentration of the oxidant and reductant as follows:

$$E_h = E'_0 + (2.303RT/nF) \log[\text{oxidant}/\text{reductant}]$$

At standard conditions, the concentrations of oxidant and reductant are equal, so that $E_h = E'_0$. Under physiological conditions the concentrations of oxidant and reductant may not at all be equal. This may be of great importance to the function of ascorbate as an electron donor in living systems.

The first chemically stable product in the oxidation of ascorbic acid is dehydroascorbate. For a basic understanding of the dehydroascorbate/ascorbate redox pair, it is easiest to conceptualize the two species as existing under some equilibruim. The conditions of the equilibrium are influenced not only by the original concentrations of the two species but by light, pH, and possibly temperature. At physiologic pH, i.e., pH 7, dehydroascorbic acid is apparently unstable with a half-life as short as a few minutes (Borsook *et al.*, 1937; Tolbert and Ward, 1982), with subsequent hydrolysis to diketogulonic acid and loss of antiscorbutic properties. At pH of 2–3, dehydroascorbate has been reported to be stable in aqueous solutions for at least 24 hours (Tolbert and Ward, 1982), but the concentration necessary for this stability is not clear.

[2] At standard conditions, where pH 7.0 and temperature = 25°C or 298 K, all concentrations are 1.0 *M*.

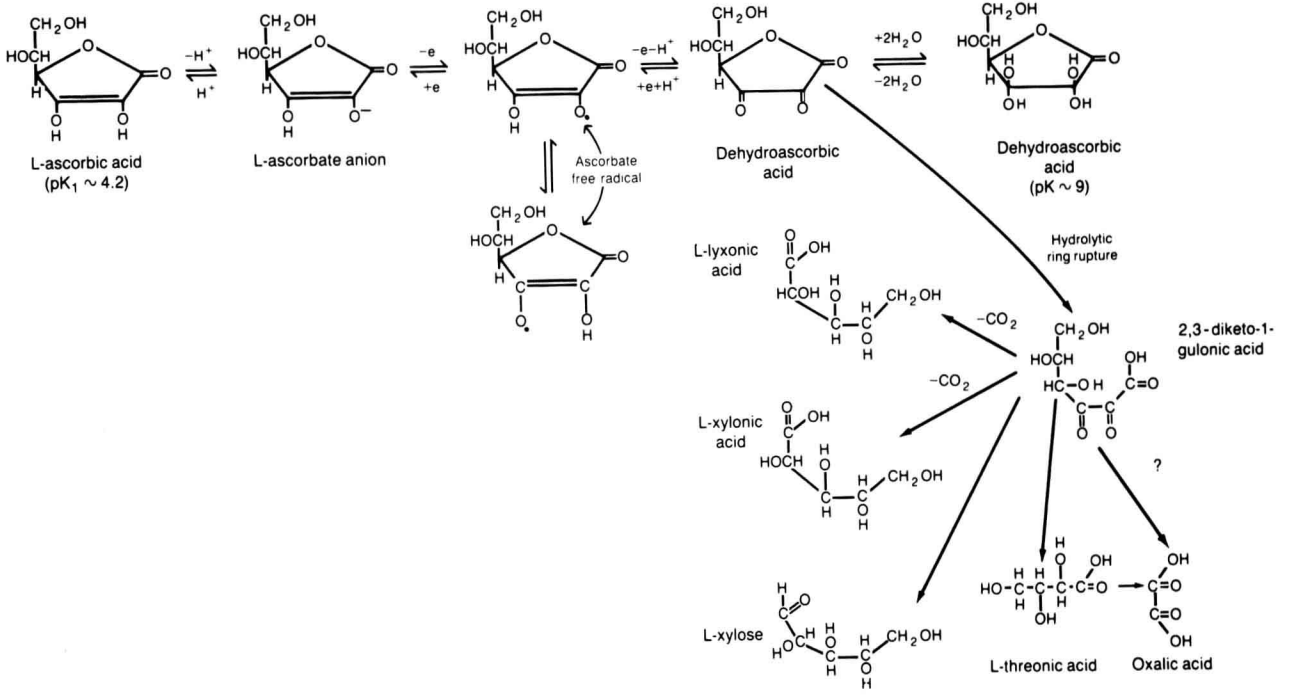

Fig. 2. Oxidation products of ascorbic acid and dehydroascorbic acid. (Modified from Lewin, 1976.)