CHAPTER - I

11) General review of ascorbic acid.
INTRODUCTION

Vitamin C, or ascorbic acid is an essential nutrient. Because of its availability in abundance in natural fresh foods, neither was it felt nor there were any manifestations of signs and symptoms of lack of it in a primitive society. But as man's life-style became more complicated so did his dependence on stored foods. As these stored foods were deficient in ascorbic acid, man began to suffer from a dread disease called scurvy. Scurvy is a disease due to a disturbance of metabolism, resulting from the deficiency or absence of ascorbic acid from the diet.

Scurvy was formerly very prevalent among prisoners, armies in the field, and sailors on long voyages, all unable to obtain fresh food. Cures for the scourge were devised through the ages and forgotten when the danger passed, the subject of cures of scurvy in practice has been well reviewed by Chick (1953), Dudley (1953), Lorens (1953).

Hawkins in 1593 found that oranges and lemons were effective in treating scurvy, but it was James Lind (1753) who studied the disease on a scientific basis and recommended the use of lemon and orange juice
in eradicating scurvy. Despite these discoveries, scurvy was prevalent during the world wars, and cases are known to occur even today.

The first significant step in elucidating the nature of the compound, the absence of which from the diet caused scurvy, was taken by Holst and Frolich (1907, 1912), who produced experimental scurvy in guinea pigs. Szent-Gyorgi (1928) isolated a crystalline substance from various sources such as adrenal cortex, cabbage and citrus juices, and found that it had anti-scorbutic properties. Szent-Gyorgi called this compound hexuronic acid, which was later shown to be identical with ascorbic acid (Haworth and Szent-Gyorgi, 1933; Harris and Ray, 1932). The isolation of crystalline vitamin C from lemon juice was announced by King and Waugh, and by Svirezky and Szent-Gyorgi in 1932.

The structure of ascorbic acid was established and its synthesis was studied by many workers (Hirst et al., 1933; Reichstein et al., 1933; Michael and Kraft, 1933).
CHEMISTRY OF ASCORBIC ACID:

Ascorbic acid is a white crystalline solid with a melting point of 192° and [\(\alpha\)]\(D\) of +24°. The molecular formula of the compound is established as \(\text{C}_6\text{H}_8\text{O}_6\). Ascorbic acid is very closely related to the monosaccharides, and is a \(\gamma\)-lactone of hexonic acid. Chemical evidence obtained by various workers, and X-ray analyses of ascorbic acid have shown it to be an almost flat molecule with the structure shown below:

\[
\begin{align*}
\text{O} & \quad \text{C}^1 \\
\text{HO} & \quad \text{C}^2 \\
\text{HO} & \quad \text{C}^3 \\
\text{H} & \quad \text{C}^4 \\
\text{HO} & \quad \text{C}^5 \quad \text{H} \\
\text{CH}_2\text{OH} & \\
\end{align*}
\]

Ascorbic acid has a \(\lambda_{\text{max}}\) at 265 millimicrons in neutral solution, and an intense band at 245 millimicrons in acid solution. There is a weak band between 350 and 400 millimicrons. The absorption is lost on oxidation. Pullman and Pullman (1963) calculated the energies of the molecular orbitals of the ascorbic acid molecule to be:

Filled : 0.498 1.826 2.246 2.503 3.098
Empty : -1.049.
The resonance energy of the molecule was calculated to be 0.965.

The electric charges and bond orders were calculated to be of the magnitude shown below:

Ascorbic acid is extremely soluble in water and insoluble in less polar solvents. Ionization at particular positions of the molecule depends upon the reaction of the media. It behaves as a monobasic acid, an aqueous solution having a pH of 3.0. It is the hydrogen of the C-3 enol group which is ionised (pK 4.25). In alkaline solution, however, it is the hydrogen atom of the C-2 enol group that ionises and is replaced by the metal.

Ascorbic acid behaves as an unsaturated compound and as a strong reducing agent. It also forms a phenylhydrazones and gives a violet colour with ferric
chloride. Ascorbic acid is readily and reversibly oxidised to dehydroascorbic acid by mild oxidizing agents. Dehydroascorbic acid is neutral, having lost its dissociable enol groups, and behaves as the lactone of a monobasic hydroxy acid. It is readily hydrolysed in water to the free acid, 2,3-diketo-L-gulonic acid. Once the lactone ring is opened the compound can no longer be reduced to ascorbic acid, but is readily oxidised to L-threonic and oxalic acids.
The oxidation-reduction potential of ascorbic acid ($E_0 = +0.051$ volts at pH 7.24 and 30°C) is higher than that of most biological compounds. The oxidation is catalyzed by trace amounts of heavy metals present in most natural products. It will reduce metal salts, such as those of Cu, Fe, Ag and Au, a variety of organic dyes, and oxygen itself in the presence of a suitable catalyst. It will also reduce cytochrome c, and this is the basis of many cytochrome assays.

In certain reactions the oxidation of ascorbic acid occurs with the loss of an electron resulting in the formation of a free radical, monodehydroascorbic acid.

Grinstead (1960) showed that the rate-limiting step of the oxidation of ascorbic acid by $Fe^{3+}$ and $H_2O_2$ was
this one-electron oxidation reaction. This monodehydro-
ascorbic acid radical has been identified by electron
paramagnetic resonance spectroscopy in solutions of
hydrogen peroxide – ascorbic acid at pH 4.8, where it
was stable for about 15 minutes. Yamasaki and co-workers
(1960) demonstrated that it was formed during the ensy-
matic oxidation of ascorbic acid by peroxidase.

The dye 2,6-dichlorophenolindophenol is revers­
sibly oxidised by ascorbic acid, and this reaction
forms the basis of many titrimetric procedures for the
estimation of the vitamin. Silver nitrate, iodine,
ferricyanide and methylene blue are other compounds
which are also readily oxidised by the vitamin.

Ascorbic acid is very closely related to the
monosaccharides. In common with many sugars, it can be
decomposed into furfural by boiling in acid. Dehydro-
ascorbic acid and its oxidation product, 2,3-diketo-
L-gulonic acid form an osazone which is stable in very
strongly acid solutions.

Ascorbic acid is optically active. There are
two assymmetric centres in carbon atoms 4 and 5, hence
four stereoisomers exist.
The isomers of L-ascorbic acid have much reduced activities as compared to L-ascorbic acid.

A number of analogs of ascorbic acid have been prepared, all of which contain the enediol grouping, e.g., 6-deoxy-L-ascorbic acid, L-glucoascorbic acid, L-fucoascorbic acid, D-glucoheptoascorbic acid. These analogs all have antiscorbutic properties, but in a lesser degree, as compared to L-ascorbic acid. The only naturally occurring forms of ascorbic acid are L-
ascorbic acid and dehydro-L-ascorbic acid, both of which have equal biological activity (Table No. II.).

**TABLE II.**

Relative antiscorbutic activities of ascorbic acid and its analogs (from Knox and Goswami, 1961).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-xyloascorbic</td>
<td>100</td>
</tr>
<tr>
<td>Dehydro-L-ascorbic</td>
<td>100</td>
</tr>
<tr>
<td>6-Deoxy-L-ascorbic</td>
<td>33</td>
</tr>
<tr>
<td>L-Rhamnoascorbic</td>
<td>20</td>
</tr>
<tr>
<td>D-Araboascorbic</td>
<td>5</td>
</tr>
<tr>
<td>L-glucoascorbic</td>
<td>2.5</td>
</tr>
<tr>
<td>L-Fucoascorbic</td>
<td>2</td>
</tr>
<tr>
<td>D+Glucoheptoaascorbic</td>
<td>1</td>
</tr>
<tr>
<td>2,3-diketo-L-gulonic</td>
<td>0</td>
</tr>
</tbody>
</table>

**SYNTHESIS OF ASCORBIC ACID:**

The vitamin was first synthesized by the method of Haworth and Hirst (1933). L-lyxose was converted into L(-)-xylosone, on treatment with phenylhydrazine followed by hydrolysis of the osazone with
hydrochloric acid. L(-)-xylosone, on treatment in an atmosphere of nitrogen with aqueous potassium cyanide containing calcium chloride gave the $\beta$-keto cyanide, which hydrolyzes spontaneously into pseudo-L-ascorbic acid. This, on heating for 26 hours with 8% hydrochloric acid at 45 - 50°, gave L(+) ascorbic acid.

Ascorbic acid is now synthesized commercially by several methods. Large-scale commercial syntheses of L-ascorbic acid usually utilize the oxidation of
L-sorbose to 2-keto-L-gulonic acid followed by the simultaneous lactonization and isomerization of this product to L-ascorbic acid.

**Biosynthesis of Ascorbic Acid:**

Ascorbic acid is present in nearly all tissues in plants and animals. All the higher varieties of plants and most animals are known to be able to synthesize the vitamin. The notable exceptions to this rule are primates and guinea pigs who lack the ability to synthesize ascorbic acid and hence suffer from scurvy. Chatterjee et al. (1961) demonstrated that scurvy can be induced in the Indian fruit-eating bat and the red-vented bulbul. The demonstration by Haworth and Hirst (1933) that ascorbic acid could be synthesized in the laboratory from sugars such as D-galactose led many workers to speculate whether biological synthesis proceeded along similar lines.

The first convincing evidence that ascorbic acid is formed from hexose sugars was provided by the feeding experiments of Ray (1934), who showed that excised pea embryos growing in a synthetic medium, could produce ascorbic acid if supplemented with various hexose sugars. He favoured the view that mannose, more than any other sugar, was the precursor but this viewpoint was subseque-
ntly not supported by Mapson et al. (1949) who observed that mannose actually depressed the synthesis of ascorbic acid when fed to creas seedlings. Ahmad et al. (1946) found glucose to have a stronger response than mannose. In other species of plants, however, mannose stimulates the formation of ascorbic acid (De and Barai, 1949; Takodoro and Misida, 1940). Of the other commonly occurring hexose sugars, glucose and fructose, as well as sucrose, appear to be equivalent as ascorbic acid sources (Butkewitsch, 1938; Ito and Mizuno, 1948; Mapson et al., 1949). Mapson et al. (1949) and Abern (1953) obtained negative results with pentose sugars and with L-sorbose. This last result was interesting in that L-sorbose has a close structural similarity to L-ascorbic acid.

The first convincing evidence that ascorbic acid could be synthesized from a hexose sugar in animals came from the work of Jackel and his collaborators (1950). They fed uniformly-labelled D-glucose to rats treated with chloroquine; the L-ascorbic acid which was excreted was also found to be uniformly labelled.

Earlier experiments had suggested that L-ascorbic acid might be synthesized from three-carbon precursors, but this hypothesis was subsequently rejected.
(Mapson, 1955; Mapson et al., 1954). Studies using the radioactive labelled sugars indicated that the conversion of D-glucose to L-ascorbic acid "appears to proceed without fragmentation of the carbon chain or, if fragmentation takes place, the fragments are recombined without major differential dilution effect" (Mapson, 1967).

Loewus et al. (1956, 1957) and Loewus (1961) fed D-glucose-1-C14 and D-glucose-6-C14 to strawberry fruits and cress seedlings and showed that the L-ascorbic acid formed was labelled on C-1 and C-6, respectively. In isotopic studies on the albino rat, administration of D-glucose labelled on C-1 and C-6, formed ascorbic acid labelled predominantly at C-6 and C-1, respectively, both in chloretone-treated rats (Horowitz et al., 1952; Horowitz and King, 1953) and in normal rats (Burns and Mosbach, 1956). In these experiments it was apparent that the carbon chain was not broken and that the whole carbon chain was inverted during the conversion of D-glucose to L-ascorbic acid.

A number of biochemical pathways were postulated to explain the findings. The scheme which finally found acceptance was initially proposed by Isherwood et
al. (1954). The scheme was advanced as a result of a study in which L-gulono-γ-lactone, L-galactono-γ-lactone and D-glucurono-γ-lactone, fed to cross seedlings and injected into rats, resulted in increasing the ascorbic acid synthesis and excretion respectively.

D-glucose  D-glucuronic acid  L-gulonic acid  L-ascorbic acid

**SCHEME 1.**

D-galactose  D-galacturonic acid  L-galactonic acid  L-ascorbic acid

**SCHEME 2.**
Definite evidence for the conversion of the intact chain of D-glucuronolactone and L-gulonolactone to L-ascorbic acid was provided by the work of Horovitz and King (1953a) and Burns and Evans (1956). The former showed that administration of uniformly labelled D-glucuronolactone to chloroform-treated rats resulted in the excretion of uniformly labelled ascorbic acid; while the latter obtained carboxyl-labelled L-ascorbic acid when carboxyl-labelled L-ascorbic acid was administered to both normal and chloroform-treated rats. In addition, the conversion of D-glucose-1-C\textsuperscript{14} to urinary D-glucuronic acid and L-gulonic acid has been shown in rats (Burns, 1957; Burns et al., 1957).

The evidence available at present suggests that the first scheme is of greater importance in the animal, whereas the second sequence of reactions are more important in the plant (Mapson, 1967). The observations that labelled sugars fed to plants did not always result in an inversion of configuration led workers to postulate the existence of another biosynthetic pathway in the plant. One such alternative biosynthetic pathway proposed by Finkle et al. (1960) suggested that D-glucose is oxidised via gluconic acid to 5-oxo-D-gluconic acid
and 3,5-dioxo-D-gluconic acid, followed by subsequent
enolization and enzymatic reduction to L-ascorbic acid.
However, evidence to support this suggestion is lacking
from both animal and plant studies.

Further evidence for the second reaction comes
from the findings that enzymes exist in liver which
convert D-glucuronic and L-gulonic acids to L-ascorbic
acid (Ul Hassan and Lehninger, 1956; Chatterjee et al.
1958a, 1960a; Shimasono and Mano, 1961). Evans and co-
workers (1960) found galactose-1-C\textsuperscript{14} to be a considera-
bly better precursor of L-ascorbic in rats than is
glucose-1-C\textsuperscript{14}. Their studies also indicated that galac-
tose is converted to L-ascorbic acid without the inter-
mediate formation of glucose, and that rat liver posse-
ses the enzymes required for the conversion through D-
glucuronic acid.

The overall scheme for the biosynthesis of
L-ascorbic acid from glucose or galactose is shown in
Fig. 13.

That this pathway is operative in animals
has been proved by
i) evidence from the feeding of labelled hexose sugars
Fig 13: Scheme for synthesis and breakdown of L-ascorbic acid in animals.
ii) the formation of L-ascorbic acid by injection of labelled glucurono- and gulono-\(\gamma\)-lactones

iii) the isolation of enzymes from tissues of rats and other animals that convert the various intermediates to L-ascorbic acid.

**MNDP-L-hexonate dehydrogenase** (L-gulono-\(\gamma\)-lactone : NADP oxidoreductase E.C. 1.1.1.20) was reported by Ul Hassan and Lehninger (1956) as the enzyme that oxidises L-gulonic acid to form D-glucuronic acid. Mano and his co-workers (1959) reported that this enzyme acts to form L-gulonolactone from D-glucuronolactone. This enzyme has been shown to be present in the soluble fraction of liver (Shimazono and Mano, 1961).

**Lactonase I** (L-gulono-\(\gamma\)-lactone hydrolase E.C. 3.1.1.18) is present in the soluble fraction of liver extract (Ul Hassan and Lehninger, 1956; Yamada et al., 1959) and catalyzes the hydrolysis of L-gulono-\(\gamma\)-lactone. It acts on D-glucuronolactone also, but to a lesser degree.

**Lactonase II** (D-glucurono-\(\delta\)-lactone hydrolase E.C. 3.1.1.19) activity was observed in the microsomal fraction of rat liver by Winkelman and Lehninger.
(1950) and Yamada and co-workers (1959). The latter group of workers observed that the microsomal lactonase acts only on D-glucuronolactone but not on D- or L-gulonolactone.

The enzyme L-gulonolactone oxidase (L-gulono-γ-lactone : O₂ oxidoreductase E.C. 1.1.3.8) oxidizes L-gulonolactone to ascorbic acid in the presence of oxygen. This enzyme is present in the microsomal fraction in animals capable of synthesizing ascorbic acid (Chatterjee, et al., 1960b).

Man, other primates, and the guinea pig are the only mammals known to be unable to synthesize L-ascorbic acid. Ul Hassan and Lehninger (1956) and Grollman and Lehninger (1957) found no net synthesis of L-ascorbic acid from L-gulonolactone in human, monkey and guinea pig liver homogenates, and Burns (1957a) reported similar results in studies with labelled gulonolactone. These species can carry out the various steps required for the biosynthesis of the vitamin except for the oxidation of L-gulonolactone, which is presumably the result of a gene-controlled enzyme deficiency (Burns, 1959).
The diet, age and sex greatly influence the synthesis of ascorbic acid. Male rats have higher tissue values of ascorbic acid than females (Stubbs and McKernan, 1967). Stubbs and Griffin (1973) demonstrated that starvation caused a decrease in the L-gulono-\(\gamma\)-lactone hydrolase, and that dietary protein affects ascorbic acid biosynthesis in rats. The synthesis of ascorbic acid from D-glucurononate is decreased greatly during starvation or short term deprivation of carbohydrate (Stirpe and Comporti, 1965).

**Catabolism of Ascorbic Acid**

The degradation of ascorbic acid in animals occurs to a very significant degree. Studies with label-\(\text{lled}\) ascorbic acid have shown that the vitamin is extensively oxidised to respiratory carbon dioxide in guinea pig (Burns et al., 1951; Dayton et al., 1959), rat (Curtin and King, 1955) and man (Von Schuching and Abt, 1965). Abt and co-workers (1962) demonstrated that in man and the monkey the major portion of ascorbic acid is excreted within the first few hours following ingestion, both through the expired air and the urine. Hallman and Burns (1958) showed the half-life of ascorbic acid in man to be about sixteen days; the corresponding value
for guinea pigs is about three days (Salomon, 1957). Rats injected with L-ascorbic acid-1-C\textsubscript{14} were found to excrete labelled oxalate in the urine (Gambardella and Richardson, 1977). L-ascorbic acid-2-sulphate has been found in rat tissues and human urine (Roy, 1975).

Penny and Zilva (1943) postulated the formation of dehydroascorbic acid and 2,3-diketo-L-gulonic acid, and were proved correct by subsequent workers. The conversion of L-ascorbic acid to dehydroascorbic acid is a reversible process, but the formation of 2,3-diketogulonic acid occurs irreversibly. The enzyme, dehydroascorbatase, responsible for the oxidation of dehydroascorbic acid, has been shown to be present in the soluble fraction of liver and kidney (Kagawa et al., 1961). A portion is then split oxidatively between the two keto groups and accounts for the oxalic acid formed from L-ascorbic acid-1-C\textsubscript{14} (Burns et al., 1951). Another portion enolizes and is decarboxylated by an enzyme, 2,3-diketoaldonate decarboxylase (Shimazono and Mano, 1961). This gives rise to labelled carbon dioxide from C-1 labelled ascorbic acid, and to L-lyxonic and L-xylonic acids.

Dayton et al. (1959) observed that L-ascorbic acid-6-C\textsubscript{14} produced labelling of liver glycogen in carbons
1 and 6 of the glucose molecule. This suggests that further degradation of ascorbic acid to the triose intermediates of the glycolytic cycle occurs. The degradation reactions of ascorbic acid are shown in fig. 13.

**THE GLUCURONIC ACID CYCLE**

The glucuronic acid pathway is of importance for the synthesis of L-ascorbic acid in animals other than primates and guinea pigs. This pathway was proposed after studies on the biosynthesis of L-ascorbic acid and L-xylulose (Burns et al., 1957; Burns and Kanfer, 1957), and represents a new oxidative pathway of glucose metabolism in animals. It differs from the hexose monophosphate shunt in that glucose is oxidized at C-6 instead of at C-1. However, both pathways channel glucose into the same pentose pool (Burns, 1960). The reactions of the glucuronic acid cycle have been defined by studies in intact men and animals and by identification of the individual cell-free enzymes. The reactions of the cycle are shown in fig. 14. Ashwell and his collaborators (1959) provided support for the participation of 3-keto-L-gulonic acid as an intermediate in the formation of L-xylulose by a NAD-dependent enzyme in the soluble fraction of kidney. Hollmann and Touster (1957) demonstrated
Fig. 14: Glucuronic acid pathway of glucose metabolism.
the presence of an enzyme system in liver capable of the interconversion of D- and L-xylulose, through xylitol. With the discovery of a liver kinase capable of forming D-xylulose-5-phosphate (Hickman and Ashwell, 1956), it was established that mammals could convert L-xylulose to D-glucose via the pentose cycle. Evidence for the occurrence of this cyclic pathway in the intact rat and guinea pig has come from the findings of Burns et al. (1957a) and Eisenberg et al. (1959) that D-glucuronolactone and L-gulonolactone are converted to liver glycogen according to this scheme.

The glucuronic acid cycle of reactions has a number of clinically significant importances. At least four hereditary metabolic diseases result from the inactivity of particular enzymes associated with this pathway, galactosemia, congenital hyperbilirubinemia, scurvy and pentosuria. Various drugs markedly increase the rate at which glucose is metabolized via the glucuronic acid pathway (Enklewitz and Lasker, 1935; Conney and Burns, 1959).

**EFFECT OF DRUGS ON ASCORBIC ACID METABOLISM**

Many drugs, such as chloretone, barbital, phenobarbital, aminopyrine, phenylbutazone, orphenadrine,
and the carcinogenic hydrocarbons are known to stimulate the synthesis of L-ascorbic acid in animals capable of synthesizing ascorbic acid (Longenecker et al., 1939; Smythe and King, 1942; Conney and Burns, 1959). Evidence for this has come from the observations that these drugs markedly increase the urinary excretion of L-ascorbic acid (Conney and Burns, 1959; Boyland and Grover, 1961; Touster and Hollmann, 1961), and that they stimulate the conversion of glucose-1-\textsuperscript{14}C to labelled D-glucuronic, L-gulonic, and L-ascorbic acids (Burns, 1957; Burns et al., 1957). Evans et al. (1960) demonstrated that the enhancement of ascorbic acid excretion involves an increased rate of formation of the vitamin and its precursors, D-glucuronate and L-gulonate. This increase is abolished by hypophysectomy but not by adrenalectomy (Conney et al., 1959b) or nephrectomy (Burns et al., 1960) in rats. The latter workers suggest that the raised biosynthesis of ascorbic acid that takes place after an injection of a wide variety of drugs represents an adaptive response to them.

The precise mechanism by which foreign compounds affect ascorbic acid biosynthesis is not known. Both synthesis and degradation (to carbon dioxide) of
ascorbic acid were increased by these drugs (Knox and Goswami, 1961). The increased degradation may be due to mass action or increased activity of the degradative enzymes.

**INTERRELATIONS OF ASCORBIC ACID AND OTHER VITAMINS:**

**B. Vitamins:** Stimulatory interaction between ascorbic acid and other water-soluble vitamins belonging to the B group have been observed by different groups of workers (Banhidi, 1961; Roy et al., 1946; Ray et al., 1935; Sure et al., 1939; Schwartz and Williams, 1952a and 1952b). Deficiency of B vitamins is noted to diminish the urinary excretion as well as the tissue reserve of ascorbic acid. Chatterjee and Kar (1960) noted, in studies on rats deficient in thiamine, riboflavine, pyridoxine, pantothenic acid or folic acid, that the synthesis of L-ascorbic acid in vitro by the microsomal enzymes of liver was diminished. Addition of the vitamins or their coenzyme forms to the enzyme system in vitro, did not restore the synthesis. Aloisi and Polanyi (1939, 1940) found that the neurological symptoms which developed in thiamine-deficient rats, regressed on administration of ascorbic acid. The synergistic action of ascorbic acid and thiamine was also observed by
Kasahara et al. (1939). They injected ascorbic acid and thiamine simultaneously into young rats and observed an increase in growth. When thiamine was given alone, the animals lost weight. The growth rate with thiamine alone equalled that of ascorbic acid and thiamine together only when five times higher concentrations of thiamine was used.

Reid (1954) offered a twofold explanation for such behaviour. She concluded that ascorbic acid augments the biosynthesis of some vitamins. On the other hand, thiamine, riboflavin and pantothenic acid were believed to stimulate independently the synthesis of ascorbic acid in some tissues.

Roy et al., (1946) observed that increased synthesis of ascorbic acid following chloretone administration was not observed in thiamine and riboflavin-deficient rats. Chatterjee et al. (1961) have shown that the microsomal activity can be restored by feeding the respective vitamins. However, they found that addition of a number of other substances, other than thiamine or riboflavin, also restores the activity of liver microsomes from the deficient rats.

**Vitamin A:** Reports of lowered concentrations of ascorbic acid during avitaminosis A (Sure et al., 1939;
Mapson and Walter (1948; Sutton et al., 1942; Mayer and Krehl, 1948) led Morehouse et al. (1952) to suggest the possibility that the biosynthesis of ascorbic acid in the rat may be influenced by vitamin A intake. Morehouse et al. (1952) failed to demonstrate any appreciable lowering of ascorbic acid concentration in the rat liver during avitaminosis A. Single or repeated depletion of vitamin A causes a fall in the ascorbic acid content of plasma and other tissues (Sure et al., 1939; Sutton et al., 1942; Jonsson et al., 1942). Mapson and Walker (1948) however, doubted that the decrease in synthesis was due to a lack of vitamin per se. By measurement of the ascorbic acid values in vitamin A-deficient and pair-fed rats evidence is presented that the synthesis of ascorbic acid is impaired in the vitamin A deficiency state.

Mayer and Krehl (1948) noted that under conditions of hypovitaminosis A symptoms, symptoms of scurvy appear that cannot be cured by the administration of ascorbic acid alone. Mapson and Walker (1948) noted that the enhanced ascorbic acid excretion in chloretone-treated rats was not much affected in vitamin A deficiency when compared with pair-fed controls.
Rodahl (1949) confirmed the similarity between the clinical symptoms and post-mortem findings associated with hypervitaminosis A and those observed in human and experimental scurvy. Vedder and Rosenberg (1938) reported that the toxicity of large doses of vitamin A concentrate was counteracted by feeding ascorbic acid, but Moore and Wang (1945) were unable to confirm this observation. The latter workers could demonstrate no significant differences between the ascorbic acid content of the liver, adrenals and urine of normal and hypervitaminotic rats. Morehouse et al. (1952) obtained evidence that massive doses of vitamin A (60,000 - 80,000 U.S.P. units daily) lowers the concentration of ascorbic acid significantly in rats.

Chatterjee et al. (1961) reported the activity of L-gulono oxidase in the microsomes of vitamin A-deficient rats to be somewhat lower than in pair-fed controls. Ghosh et al. (1965) noted that the synthesis of L-ascorbic acid from either D-glucuronolactone or L-gulonolactone is decreased under conditions of hypervitaminosis A, and that the microsomal conversion of L-gulonolactone to L-ascorbic acid is impaired in liver tissues of rats deficient in vitamin A. Chatterjee and Kar (1960) have suggested that structural derangement of
the microsomes occurs at times of vitamin deficiency
and that this brings about impairment of the enzymes
involved.

**Vitamin D**: Lecoq et al. (1944, 1945) suggested that
ascorbic acid is interrelated with vitamin D. Ghosh
et al. (1965) demonstrated a decreased ascorbic acid
synthesis from L-gulonolactone by microsomes of rats
made deficient with respect to vitamin D. They also
noted that synthesis of L-ascorbic acid by rat liver
microsomes, under conditions of hypervitaminosis D,
from D-glucuronolactone is increased and that from L-
gulonolactone is not affected.

**Vitamin E**: Vitamin E deficiency resulted in a reduced
synthesis of L-ascorbic acid (Caputto et al., 1958, 1961;
Ghosh and Kar, 1963; Chatterjee et al., 1960). Kitabchi
et al. (1959) found the impairment to be due to inhibi-
tion of the enzyme, L-gulono oxidase. Carpenter et al.
(1959) showed that the enzyme inhibition was due to
lipid peroxidation, and that the inhibition could be pre-
vented by the addition of \( \alpha \)-tocopherol. Chatterjee et
al. (1961) found that the microsomes of vitamin E defi-
cient animals may be stimulated by addition of other
compounds besides vitamin E. Thus the role of \( \alpha \)-tocopherol
appears to be of a secondary nature in that it prevents lipid peroxidation, rather than a direct action on the enzymes concerned in the synthesis (Mapson, 1967).

TRANSPORT AND TISSUE DISTRIBUTION OF ASCORBIC ACID:

The concentration of ascorbic acid varies markedly in different tissues, the concentration of all tissues being several times more than that of the blood. There is hardly any difference in the pattern of the ascorbic acid concentrations in the tissues of the animals capable of synthesizing ascorbic acid and the animals without this capacity (Lloyd and Sinclair, 1953). Tissues can be roughly divided into three groups on the basis of their ascorbic acid concentrations (Knox and Goswami, 1961):

i) Tissues which have concentrations several hundred times that of the plasma, e.g., adrenal, corpus luteum, hypophysis, retina and thymus.

ii) Tissues with concentrations 50-100 times the plasma levels, e.g., brain, liver, lungs, lymph glands, pancreas, salivary gland, small intestinal mucosa, testicle, thyroid and white blood cells.

iii) Tissues with about 10 times the plasma concentration, e.g., erythrocytes, kidney, skeletal, smooth
and cardiac muscle.

The ascorbic acid concentrations in different tissues in the rat, guinea pig and human are shown in table III.

**TABLE III.**

Tissue distribution of ascorbic acid in different species in mgm/100 gm.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rat²</th>
<th>Guinea³</th>
<th>Human¹</th>
</tr>
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<tbody>
<tr>
<td>Plasma</td>
<td>1.6</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Whole Blood</td>
<td></td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>7.3</td>
<td>3.12</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td></td>
<td>8.90</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>8.98</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>19.8</td>
<td>11.60</td>
<td>10 - 15</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td>20.40</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td>22.80</td>
<td>19 - 46</td>
</tr>
<tr>
<td>Liver</td>
<td>37.8</td>
<td>32.80</td>
<td>15 - 16</td>
</tr>
<tr>
<td>Spleen</td>
<td>63.0</td>
<td>41.80</td>
<td></td>
</tr>
<tr>
<td>Adrenal</td>
<td>449.5</td>
<td>166.0</td>
<td>39 - 58</td>
</tr>
</tbody>
</table>

1. Yavorsky et al. (1934)
2. Stubbs and McKernan (1967).
The extremely high concentrations of the vitamin in some tissues may be due either to a strong active transport into these cells, or the existence of a non-diffusible, bound form of ascorbic acid. Heinemann (1941) demonstrated that when ascorbic acid is added to blood it is preferentially taken up by the leucocytes while dehydroascorbic acid is taken up and reduced to ascorbic acid by the erythrocytes (Lloyd, 1951). However, the ratio of ascorbic acid to dehydroascorbic acid in plasma remains constant on administration of either form (Stewart et al., 1953). Martin (1961) drew attention to the fact that dehydroascorbic acid is un-ionized and so is more diffusible at body pH than the negatively charged ascorbate ion. It was, therefore, suggested that dehydroascorbic acid was the form penetrating brain (Patterson and Mastin, 1951), erythrocytes (Lloyd, 1951) and the placental barrier (Raiha, 1956). Experiments with isotopically labelled forms of the vitamin showed that dehydroascorbic acid was taken up more rapidly by brain, eye, and red blood corpuscles. Administration of the reduced form did not increase the levels of ascorbic acid in the brain (Martin, 1961). These results indicate that dehydroascorbic acid readily penetrates into the brain and is then reduced to ascorbic
It is possible that the high intracellular concentrations of ascorbic acid found are due to the free diffusion of dehydroascorbic acid into the cell and its subsequent reduction into the non-diffusible form (Knox and Goswami, 1961).

Studies carried out by Martin (1961) showed that nephrectomised animals receiving ascorbic acid showed a marked increase of the vitamin in the serum level, while tissue concentrations remained at control levels. Samples of serum obtained from the carotid artery and the jugular and femoral veins were found to contain less oxidised ascorbic acid than the samples of serum collected from the renal and mesenteric veins. This finding is consistent with the theory that the kidney and possibly the intestine facilitate the transport of ascorbic acid by oxidizing the vitamin to an unionized form that readily penetrates cellular barriers.

Animals requiring exogenous supply of ascorbic acid must be able to almost completely absorb the dietary ascorbic acid, in order to maintain health. Since ascorbic acid is soluble at physiological pH, it would not be readily absorbed by the intestine in absence of a specific transport mechanism. Influx in guinea pig
and human ileum follows saturation kinetics, indicating that a carrier mediated process is operative at low concentrations (Mellors et al., 1977). The carrier mechanism appears to have a high specificity for the L-isomer of ascorbic acid. Influx of the vitamin into guinea pig and human ileum was highly dependant on mucosal sodium, but independent of the intracellular sodium concentrations. Mellors et al. (1977) suggest that sodium and ascorbic acid enter the intestinal cell simultaneously, and the energy for the intracellular accumulation of the vitamin against a concentration gradient resides in the existing electrochemical gradient for sodium across the mucosal membrane. Thus influx of ascorbic acid into ileal mucosa appears to proceed by a mechanism similar in nature to that for sugars and amino acids.

**REQUIREMENT OF ASCORBIC ACID IN HEALTH AND DISEASE**

Ascorbic acid is found in greatest concentration in tissues of high metabolic activity, with the plasma having the lowest concentration.

Krebs (1953) stated that the minimum daily requirement for prevention of scurvy is 10 mg, however Goldsmith (1961) suggested that the desirable intake for
the maintainence of optimal health may be considerably greater than this. It has been argued that, since a state of tissue saturation exists in those animal species which synthesize ascorbic acid, a similar state is desirable in those species prone to scurvy. The findings of various workers suggest that an intake of less than 40 mg. of ascorbic acid daily leads to depletion of tissue stores, while an intake of 60 - 100 mg. is needed for saturation of tissues.

Physical stress, such as that following surgery, leads to a fall in the plasma levels and urinary excretion (Lund et al., 1947). Kark (1953) noted that ascorbic acid requirements of man are increased as a result of burns, severe injuries, infections and rheumatic diseases. Early studies reported low values of the vitamin in plasma and urine during the course of a wide variety of infections. Massive doses of the vitamin restore the plasma levels to normal, and this value can be maintained only if high doses of ascorbic acid are continued till the stress is terminated. Deficiency of ascorbic acid interrupts wound healing, often resulting in wound dehiscence (Crandon et al., 1961). Wound dehiscence can be prevented by the daily administration of
100 - 300 mg. of ascorbic acid daily (Crandon et al., 1961).

Gopalan and Balasubramanian (1966) suggest that a well-balanced diet for school children and adults should contain some 30 - 50 mg. of ascorbic acid per day. A dose of 50 - 100 mg. 3 - 5 times a day clears lesions of scurvy, while in acute cases doses upto 1000 mg. can be given (Vilter, 1967).

**SOURCES OF THE VITAMIN:**

Of all the vitamins, ascorbic acid is most easily susceptible to destruction by atmospheric oxidation. The tendency of ascorbic acid to rapidly oxidise in air and its intense reducing action results in loss of the vitamin when vegetables get dry and stale. With the exception of a few animal products, only fruits and vegetables contribute natural vitamin C to the diet.

The distribution of ascorbic acid within an individual fruit or vegetable is often extremely variable. A greater concentration of the vitamin is usually found in the skin as compared with the pulp of the fruit, and significantly different values may exist between leaf and petiole, and between plants of identical variety and adjacent growth. Some seed-containing tissues, such as blackcurrants and peas, show striking changes in
ascorbic acid concentration with the onset of maturation, but the levels of the vitamin remain relatively stable in storage organs, such as leaves and tubers (Olliver, 1967). Pulses and cereal grains in the dry state do not normally contain vitamin C, but the vitamin is formed in the grain and shoot during germination. About 85% of the vitamin is present in the grain and only 15% in the shoot (Gopalan and Balasubramanian, 1966). Sprouted Bengal gram (Cicer arietinum) is known to be highly efficacious in preventing scurvy during famines in India. Sprouted mung (Phaseolus radiatus) is about three times more potent in vitamin C. Amla (Phyllanthus emblica, Linn) is one of the richest natural sources of ascorbic acid. Fresh citrus fruits are rich sources of the vitamin. The ascorbic acid content of various foodstuffs are shown in table IV.

**TABLE IV.**

Ascorbic acid content of various foods.

<table>
<thead>
<tr>
<th>Name of foodstuff</th>
<th>mg. ascorbic acid/100gm</th>
<th>Edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cereals and legumes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rice (raw)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Wheat (whole)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Maize (tender)</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>
**TABLE IV (Contd.)**

<table>
<thead>
<tr>
<th>Name of foodstuff</th>
<th>mg. ascorbic acid/100 gm. edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>*Bengal gram (whole)</td>
<td>3</td>
</tr>
<tr>
<td>*Lentil</td>
<td>0</td>
</tr>
<tr>
<td>*Green gram (whole)</td>
<td>1</td>
</tr>
<tr>
<td>*Peas (dried)</td>
<td>0</td>
</tr>
</tbody>
</table>

*Pulses after sprouting contain 10–15 mg. of ascorbic acid per 100 gm.

2. **Leafy vegetables**:  
   - Agathi: 169  
   - Amaranth (tender): 99  
   - Asparagus: 40  
   - Bamboo (tender shoots): 5  
   - Brussels sprouts: 100  
   - Cabbage: 124  
   - Cauliflower: 75  
   - Celery leaves: 62  
   - Coriander leaves: 135  
   - Drumstick leaves: 220  
   - Knol-khol greens: 157  
   - Lettuce: 15  
   - Mustard and cress: 18
TABLE IV (Contd.)

<table>
<thead>
<tr>
<th>Name of foodstuff</th>
<th>mg. ascorbic acid/100 gm. edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem (mature)</td>
<td>218</td>
</tr>
<tr>
<td>Parsley</td>
<td>281</td>
</tr>
<tr>
<td>Radish tops</td>
<td>103</td>
</tr>
<tr>
<td>Spinach</td>
<td>28</td>
</tr>
<tr>
<td>Turnip greens</td>
<td>120</td>
</tr>
<tr>
<td>Watercress</td>
<td>60</td>
</tr>
</tbody>
</table>

3. Roots and Tubers:
   Beet root               | 88                                      |
   Carrot                  | 3                                       |
   Lotus root              | 22                                      |
   Marrow                  | 5                                       |
   Potato                  | 30                                      |
   Radish                  | 25                                      |
   Sweet Potato            | 20                                      |
   Tapioca                 | 25                                      |
   Turnip                  | 43                                      |
   Yam                     | 9                                       |

4. Other vegetables:
   Beans (scarlet or runner)| 27                                      |
   Broad beans             | 30                                      |
   Bitter gourd            | 88                                      |
   Cucumber                | 9                                       |
<table>
<thead>
<tr>
<th>Name of foodstuff</th>
<th>mg. ascorbic acid/100</th>
<th>gm. edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drumstick</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>Eggplant</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Giant chillies</td>
<td>127</td>
<td></td>
</tr>
<tr>
<td>Nellikai (amla)</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Pumpkin</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><strong>5. Nuts:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coconut (dry)</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Groundnut</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Sunflower seeds</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Walnuts (green)</td>
<td>1,600</td>
<td></td>
</tr>
<tr>
<td><strong>6. Condiments &amp; Spices:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chillies (green)</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>Garlic (dry)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ginger (fresh)</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Onion (mature)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Tamarind pulp</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><strong>7. Fruits:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Banana</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Currant (black)</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Cashew fruit</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>Custard apple</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>
### Table IV. (Contd.)

<table>
<thead>
<tr>
<th>Name of foodstuff</th>
<th>1 mg. ascorbic acid/100</th>
<th>1 gm. edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guava</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>Jam</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Lemon (juice)</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Lichi</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Lime (juice)</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Mango (ripe)</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Orange (loose-jacketed)</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Orange (juice)</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Papaya (ripe)</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Pineapple</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Strawberry</td>
<td>52</td>
<td></td>
</tr>
</tbody>
</table>

### 8. Fishes:

<table>
<thead>
<tr>
<th>Name of fish</th>
<th>mg. ascorbic acid/100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bhangar</td>
<td>12</td>
</tr>
<tr>
<td>Bhetki</td>
<td>10</td>
</tr>
<tr>
<td>Hilsa</td>
<td>24</td>
</tr>
<tr>
<td>Katla</td>
<td>9</td>
</tr>
<tr>
<td>Koi</td>
<td>32</td>
</tr>
<tr>
<td>Magur</td>
<td>11</td>
</tr>
<tr>
<td>Puti</td>
<td>15</td>
</tr>
<tr>
<td>Rohu</td>
<td>20</td>
</tr>
<tr>
<td>Singhi</td>
<td>9</td>
</tr>
<tr>
<td>Sole</td>
<td>9</td>
</tr>
<tr>
<td>Tengra</td>
<td>18</td>
</tr>
</tbody>
</table>
**TABLE IV. (Contd.)**

<table>
<thead>
<tr>
<th>Name of foodstuff</th>
<th>1 mg. ascorbic acid/100</th>
<th>1 gm. edible portion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>9. Other flesh foods:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef muscle</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Egg (hen)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Liver (sheep)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>10. Milk and Milk Products:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk (cow)</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Milk (buffalo)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Milk (human)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Curds</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Channa (Cow's milk)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Skimmed milk powder</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>(Cow's milk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole milk powder</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>(Cow's milk)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The loss of ascorbic acid of fruits and vegetables on storage depends on the temperature and time of storage, extent of tissue damage, structure of the fruit or vegetable, and the presence or absence of ascorbic acid oxidase enzyme. Hard fruits and roots can usually be stored for several days without significant fall in vitamin C content, whereas soft fruits and green vegetables rapidly lose the vitamin after harvesting.
The value of a food also depends on the method of processing. Slicing, cutting or bruising of fruits or vegetables leads to loss of ascorbic acid. Boiling with water does not affect the vitamin concentration, but a significant loss due to oxidation occurs on pressure cooking. When fruit is boiled with sugar, as in jam making, the ascorbic acid is remarkably stable. Freezing has a negligible effect on ascorbic acid content, and no loss occurs if the storage temperature is below -20°F. Rapid dehydration of foods also does not destroy the vitamin.

**ASCORBIC ACID AND ELECTRON TRANSPORT**:  

The ease with which ascorbic acid is oxidised and reduced determines its role in metabolism, and many of the physiological roles of the vitamin are related to this redox system. Ascorbic acid occurs in both the reduced and oxidised forms in plant tissues, as well as in animal tissues (Mapson, 1967a), about 95% of the total usually being present in the reduced form (Mapson, 1953).

It appears that the oxidation of L-ascorbic acid involves an electron transfer. Since it is entirely impossible for L-ascorbic acid to act as an intermediate.
hydrogen acceptor in its reduced form, Kersten et al. (1957, 1958) postulated a "radical-like, semi-oxidized" mono-dehydroascorbic acid that lost only one electron. Working on a plant NADH oxidase system, Nason et al. (1954) and Kern and Racker (1954) also came to a similar conclusion. The existence of this radical has been postulated in microsomes of pig and beef adrenals (Kersten et al., 1958) and rat liver and kidney (Staudinger, 1958). Evidence for the actual existence of this radical has been provided by Kern (1954) and Yamazaki et al. (1959).

Almost all the terminal oxidases in plant and animal tissues are capable of directly or indirectly catalyzing the oxidation of L-ascorbic acid. Some of the enzymes are ascorbic acid oxidase, cytochrome oxidase, laccase, peroxidase and phenolase. Under aerobic conditions, oxidation is readily induced by many haemochromogens, metal ions and quinones (Mapson, 1967a).

It may be of some significance that those plants which possess no polyphenolase enzymes are found to possess the enzyme ascorbic acid oxidase (E.C.1.10.3.3.) (Mapson, 1953). This is the only enzyme known to catalyze a direct reaction between ascorbic acid and oxygen. The primary product of the oxidation is dehydroascorbic
acid, and 1 gram atom of oxygen is absorbed per mole of ascorbic acid oxidised (Lovett-Janison and Nelson, 1940). Ascorbic acid oxidase is a protein containing approximately 0.26% of copper (Dawson & Tokuyama, 1961), or 6–12 copper atoms per molecule of enzyme (Van Leeuwen et al., 1975). Unlike the corresponding oxidation catalyzed by cupric ions, no hydrogen peroxide is formed during the reaction (Hand and Greisen, 1942; Steinman and Dawson, 1942). Joselow and Dawson (1951) confirmed that the copper-protein bond is of a non-dissociable nature.

Ascorbic acid oxidase oxidises L-ascorbic acid and certain of its analogues, e.g., L-glucosascorbic acid and L-galactoascorbic acid (Dodds, 1948; Johnson and Zilva, 1937). The oxidation rate is characteristically linear and falls off only when the substrate is almost completely oxidised (Hopkins and Morgan, 1936). The Michaelis constant is of the order 2 x 10⁻⁶ M (Crook, 1941). It is inhibited by cyanide and copper-chelating agents (Tauber and Kleiner, 1935; Giri and Rao, 1944).

Cytochrome c and cytochrome oxidase are present in plant tissues. The former will oxidise many mild reducing agents, including ascorbic acid (Schneider and
Potter, 1943). If cytochrome oxidase is also present in the plant tissues a cyclic oxidation system is establi­shed, whereby ascorbic acid may be oxidized to comple­tion (Keilin and Hartree, 1938). Cytochrome oxidase will not oxidise ascorbic acid in the absence of cytochrome c (Mapson, 1953). The oxidative action of the enzyme is, therefore, an indirect one.

The existence of enzyme systems capable of reducing both forms of the oxidized vitamin has been demonstrated. Glutathione and ascorbic acid may also function as components of electron-transport systems in plants. NADP-linked glutathione reductase enzymes have been observed in various plant and animal tissues. Many plant enzymes also catalyze the reduction of dehydro­ascorbic acid by reduced glutathione (Kit, 1960). The series of reactions may be summarized as follows:

\[
\begin{align*}
\text{GSSG + NADPH}_2 & \xrightarrow{\text{reductase}} 2\text{GSH + NADP} \quad (1) \\
\text{Dehydroascorbic acid + 2GSH} & \xrightarrow{\text{reductase}} \text{Ascorbic acid + GSSG} \quad (2) \\
\text{Ascorbic acid + H}_2\text{O}_2 & \xrightarrow{\text{oxidase}} \text{dehydroascorbic acid + H}_2\text{O} \quad (3)
\end{align*}
\]
The last reaction (3) could be mediated by any other terminal oxidase. This pathway is not observed in animal tissues as they lack the dehydroascorbic acid reductase. These individual reactions have been demonstrated in plant tissues (Mapson and Moustafa, 1955). The stability of ascorbic acid in these tissues is dependent on the balance between the oxidation and reduction reactions (Mapson, 1961).

Another scheme utilising NADH and monodehydroascorbic acid has been suggested by Staudinger et al. (1961) as described below:

\[ \text{NADH}_2 + 2 \text{ monodehydroascorbate} \rightarrow \text{NAD} + 2 \text{ ascorbic acid.} \]

The reaction is catalyzed by the enzyme NADH-monodehydroascorbic acid transhydrogenase, a flavoprotein, dependent on SH groups for its action (Strittmatter and Velick, 1956). Evidence was obtained that the terminal oxidase was cytochrome b5. This led Staudinger and his co-workers (1961) to propose the following scheme for microsomal electron transport in plant and animal tissues:
ASCORBIC ACID AND IRON METABOLISM:

It is well known that ferrous ion is absorbed from the intestinal tract of animals much more rapidly than ferric ion, and that ascorbic acid can reduce ferric to ferrous ion (Knox and Goswami, 1961). Masur et al. (1960) obtained evidence for the participation of ATP and ascorbic acid in the transfer of plasma-bound iron to the liver and its incorporation into ferritin in this tissue. Plasma-bound iron exists in the form of an iron-protein complex called transferrin. Reduction of the ferric iron, attached to transferrin, to the ferrous state appears to be the first step (Masur, 1961). This reaction is stimulated by ATP with the simultaneous oxidation of ascorbate. Masur (1961) suggests that ATP, ascorbic acid, and iron (ionic or transferrin bound) form an activated complex that greatly enhances the flow of electrons, leading to an active reduction of ferric to ferrous ions.
In the presence of an iron overload in man, large doses of ascorbic acid disappear, without appreciable rise in blood levels or urinary excretion of the end products of ascorbic acid metabolism (Coons, 1964). Vilter et al. (1963) reported that guinea pigs need ascorbic acid for normal red cell maturation and survival. Lochhead and Goldberg (1959) suggest ascorbic acid takes part in the transfer of iron, from ferritin and haemosiderin, for haem biosynthesis.

**ASCORBIC ACID AND TYROSINE METABOLISM**

Sealock and Silberstein (1939, 1940) were the first to demonstrate that ascorbic acid is needed for the complete metabolism of tyrosine. They showed that when L-tyrosine was added to the diet of scorbutic guinea pigs, homogentisic acid (HGA), p-hydroxyphenyl pyruvic acid (p-HPP) and p-hydroxyphenyl lactic acid were excreted in the urine. Levine et al. (1939) observed a defect in tyrosine metabolism in infants which resulted in excretion of HGA and p-HPP in the urine. The defective metabolism in both instances were found to be corrected after administration of ascorbic acid. Painter and Zilva (1947) studied the quantitative relationship between the amount of ascorbic acid required
to prevent the excretion of tyrosyl metabolites as the amount of tyrosine fed to guinea pigs was increased. They noted that the defect occurred only when extra tyrosine was fed to the guinea pigs, and that the amount of vitamin required to cure the defect far exceeded the dose necessary to cure scurvy.

The major pathway for the catabolism of tyrosine is through C-2 and C-5 hydroxylation of the phenyl ring giving rise to the intermediate HGA. The phenyl ring of the HGA is then cleaved to eventually form fumaric acid and acetoacetic acid. The route through hydroxylation at C-3 and C-4 to yield dihydroxyphenylalanine, the adrenalin compounds and melanin compounds is less important quantitatively (Greenberg, 1969). Five enzyme steps have been shown to be required in the catabolism. The pathway of tyrosine metabolism in the mammalian metabolism is shown in fig. 15. The first step is a transamination with \( \alpha \)-ketoglutarate, pyridoxal phosphate acting as coenzyme.

The next step is the conversion of p-HPP acid to HGA. This step is catalysed by the enzyme p-HPP-acid hydroxylase (p-HPP ascorbate : oxygen oxidoreductase (hydroxylating) E.C.1.14.2.2). The enzyme is present in
Fig. 15: Metabolic pathway of tyrosine (La Du and Zannoni, 1961).
the livers of most mammalian species, and has been purified from livers of dog (La Du and Zannoni, 1959), beef and pig (Hager et al., 1957). These authors have suggested that p-HPP hydroxylase is a copper enzyme similar to tyrosinase. The enzyme is relatively insensitive to cyanide and carbon monoxide, and has a very high affinity for oxygen.

Knox and Knox (1951) demonstrated that the presence of ascorbic acid was necessary in the oxidation of p-HPP by liver homogenates. La Du and Zannoni in 1961, proved conclusively that ascorbic acid, instead of acting as cofactor in the enzymatic step, has the rather unusual role of protecting the enzyme from inhibition by its substrate. This protection is only necessary when large amounts of tyrosine are being metabolized, and is not required under ordinary dietary conditions. The p-HPP hydroxylase system is intact in scorbutic guinea pigs, and p-HPP accumulates and is excreted only when sufficient ascorbic acid is not available to prevent inactivation of the enzyme (Knox and Goswami, 1961a). The role of the vitamin, therefore, is not on the initial rate of reaction, but rather on maintaining this rate.
ASCORBIC ACID AND COLLAGEN:

In scurvy, one of the prominent lesions is the presence of abnormalities in the connective tissue. Early work done on this defect led to the following general conclusions (Robertson, 1961):

i) The formation and maintenance of normal collagen require ascorbic acid

ii) A nonfibrous collagen precursor is formed, during an ascorbic acid deficiency, instead of fibrous collagen.

iii) Abnormalities of the mucopolysaccharides of ground substance accompany an ascorbic acid deficiency.

iv) The connective tissue lesions of ascorbic acid deficiency are found preeminently in tissues subject to physical stress.

Synthesis of collagen is a function of the fibroblast; the fibroblast is the cell in which the polypeptide chains which will form the final protein are assembled on polyribosomes in the usual manner. However, there are several unusual features in this process (Schepartz, 1973), one being the peculiar composition of the final protein. Collagen is characterized
by a high content of glycine and proline, and by having in its composition two amino acids, hydroxyproline and hydroxylysine for which there exist no transfer RNAs. The hydroxylated amino acids are not incorporated into the peptide chains at the stage of translation. At this stage the chains contain proline and lysine and are known as protocollagen.

Hydroxylation of protocollagen is believed to be the rate-limiting step in collagen synthesis. It has been demonstrated by Hutton et al. (1967) and Fujimoto and Prockop (1969), that L-ascorbic acid is required for the enzymatic hydroxylation of the proline and lysine residues of protocollagen in collagen-forming cells. Certain prolyl and lysyl residues are attacked by specific hydroxylases, utilizing molecular oxygen, requiring ascorbate and ferrous ion as cofactors and coupled with the oxidation of ketoglutarate to succinate (Schepartz, 1973). The basic collagen protein tropocollagen, is produced intracellularly, but aggregates extracellularly to form fibrils and then fibres (Smith, 1971). Tropocollagen is metabolically very active and is soluble in cold, neutral salt solution, but as fibres are formed it becomes less active and less soluble (Robertson, 1961).
Fig. 16: Pathway of collagen formation. (+++→) denotes acceleration; (---→) denotes inhibition (from Schepartz, 1973).
The collagen pathway of proline and lysine is shown in fig. 16.

Robertson (1950) believed that collagen once laid down is metabolically inert even in acute or chronic scurvy, though recent work seems to contradict this; high collagen turnover rates have been observed in growing children and in patients suffering from bone disease (Bell et al. 1972). The decreased concentration of soluble collagen in the skin of scorbutic guinea pigs suggests that the defect of scurvy precedes fibre formation. Jackson and Bentley (1961) have concluded from isotopic studies that the rate of conversion of soluble collagen to fibres is unaffected in ascorbic acid deficiency. The finding by Woessner and Gould (1957) that collagen was formed by fibroblasts even in the absence of ascorbic acid, led Gould (1961) to postulate that there are two pathways for collagen biosynthesis, an ascorbic acid dependent one and an ascorbic acid independent one. He suggested that the former pathway is operative in the synthesis of "repair" collagen, such as that involved in wound healing or in subcutaneously implanted polyvinyl sponges. The collagen formed in scorbutic guinea pigs was attributed to the second pathway, and also suggested the possibility that ascorbic
acid is involved in the formation of growth collagen but at a level that is extremely small compared with that of repair collagen. Levene and Bates (1976) suggest that ascorbate creates a mildly reducing intracellular environment, which not only favours the hydroxylation reaction, but also provides a milieu which is conducive to long term stability of the enzyme, prolyl hydroxylase.

ASCORBIC ACID AND TRYPTOPHAN:

Isotopic experiments have demonstrated that tryptophan is the precursor of serotonin (Udenfriend et al., 1956). The first step in the formation of serotonin is the hydroxylation of tryptophan to 5-hydroxytryptophan, followed by decarboxylation to yield 5-hydroxytryptamine (serotonin).
5-hydroxytryptophan has been isolated in the urine of a number of mammalian species, and it has been shown to be formed in liver slices (Renson et al., 1962), and in human metastatic carcinoid tumour slices (Grahame-Smith, 1964).

Enzyme preparations that form 5-hydroxytryptophan have been isolated from liver (Cooper and Meler, 1961), intestinal mucosal cells and kidney (Cooper, 1961), neoplastic murine mast cells (Schindler, 1958) Lovenberg et al., 1965), brain stem (Green and Sarver, 1966) and in the cells of chromobacterium violaceum (Mitoma et al., 1956). The tryptophan hydroxylase of brain stem has a very low degree of activity and has not been so well characterized as that of the liver and the mast cell tumour (Greenberg, 1969).

The enzyme, tryptophan-5-hydroxylase, is found in the particulate fraction of the cell, with equal activities being observed for nuclei, mitochondria and microsomes; the soluble fraction appeared to contain an inhibitor of the activity (Cooper, 1961). The activity of the enzyme system depends on the presence of ascorbic acid and cupric ions. Ascorbic acid can be replaced by other members of ascorbate family, but not by other
electron acceptors such as cytochrome c, flavin nucleotides or methylene blue. The need for cupric ions is specific and cannot be replaced by ferric, molybdenum (VI), or manganese ions (Cooper, 1961). Cooper also demonstrated that the reaction proceeded equally well under both aerobic and anaerobic conditions. The mechanism of the reaction is not certain, but Cooper (1961) suggests that a hydride ion is removed from the S-position of tryptophan, and replaced by a hydroxyl ion from water. The two protons and electrons then reduce dehydroascorbic acid to ascorbic acid; the cycle is complete with the oxidation of ascorbic acid by copper.

ASCORBIC ACID AND HORMONES:

ACTH: Szent-Gyorgi (1928) observed that adrenal glands contain ascorbic acid in very high concentrations. This concentration is decreased during scurvy (Harris and Ray, 1932; Waugh and King, 1932). It is present chiefly in the zona fasciculata and the zona reticularis, in association with the mitochondria, mainly as ascorbic acid. Stimulation of the adrenal cortex by ACTH or adrenalin leads to depletion of ascorbic acid (Sayers et al., 1944; Salomon, 1958; Banerjee, 1944-1945). Constable et al. (1956) noted that although a reduction was
observed in ascorbic acid concentration in the adrenal
glands after ACTH administration, the total quantity of
ascorbic acid present was increased due to the enlarge­
ment of the glands. The administration of ACTH to human
subjects resulted in a rise in the concentration of
plasma total ascorbic acid and of plasma ascorbic acid
with a diminution in the concentration of plasma dehy­
droascorbic acid (Stewart et al., 1953). Slusher and
Roberts (1957) and Briggs and Toepel (1958) found that
ascorbic acid lost from the adrenal gland could be
quantitatively recovered from the adrenal vein. Salomon
(1958) showed that ACTH injection caused the release of
the bound form of ascorbic acid from the adrenal glands.
The free form of ascorbic acid in the adrenal glands was
in equilibrium with ascorbic acid in the blood and was
unaffected by ACTH.

Eisenstein and Shank (1951) noted a delay in
onset of scurvy and an increase in mean survival time
in scorbutic guinea pigs treated with ACTH. They inter­
preted this as indicating that ascorbic acid functions
as a non-specific stress and that this vitamin is not
directly involved in mechanisms of elaboration of adre­
nal cortical hormones with an O-atom at the C-11 position.
Studies of the biosynthesis of corticosteroids by adrenal gland homogenates indicated that the rate of 11β-hydroxylation of deoxycorticosterone was increased by ascorbic acid (Kersten et al., 1958a), but could not be substantiated by later studies.

**Adrenalin**:

A high concentration of ascorbic acid is found in the adrenal medulla of mammals. It is present in the form of dehydroascorbic acid, and is one of the factors required in the enzymic catalysis of dihydroxyphenylethylamine to norepinephrine (Friedman and Kaufman, 1966):

\[
\begin{align*}
3,4\text{-dihydroxyphenylethylamine} & \rightarrow \text{fumarate} \rightarrow \text{norepinephrine} \\
+ \text{ascorbate} + \text{O}_2 & \rightarrow + \text{dehydroascorbate} \\
& + \text{H}_2\text{O}
\end{align*}
\]

The enzyme concerned, dopamine hydroxylase (E.C.1.14.2.1; 3,4-dihydroxyphenylethylamine, ascorbate : oxygen oxidoreductase (hydroxylating)) has been shown to be a copper-containing protein. The enzyme (E) is first reduced by ascorbate. The reduced form then reacts with oxygen and the substrate (RH).
The function of ascorbate in catecholamine synthesis is to reduce the essential metal on the enzyme so that the reduced enzyme may mediate in the hydroxylation reaction.

The interrelation of ascorbic acid and adrenaline is subject to conflict. Some workers (Banerjee, 1945; Giroud and Martinet, 1941) have reported increased adrenaline content in adrenal glands of scorbutic guinea pigs, while Karg (1957) found the adrenaline contents of normal and scorbutic guinea pigs to be the same.

Gonadal Hormones:

Vitamin C is present in high concentrations in the interstitial cells of the testis and ovary.
Stubbs and McKernan (1967) noted a sex difference in tissue concentrations of the vitamin; tissue ascorbate concentrations were shown to be significantly higher in all of the tissues from male rats with the exception of adrenals and bone. The activities of the ascorbic acid-synthesizing enzymes were also found to be higher in the male rats.

Salomon and Stubbs (1961) had demonstrated a marked hypophyseal influence on ascorbic acid biosynthesis in rats. Hypophysectomy caused no significant change in the activities of the enzymes from female rats, with the exception of a considerable decrease in gulonolactone hydrolase (Stubbs et al., 1967), in both sexes. On the other hand, the enzymic activities of gulonate NADP oxidoreductase, glucuronolactone hydrolase, and gulonolactone:oxygen oxidoreductase in hypophysectomized males were significantly diminished to levels characteristic of female rats. The effects of castration are similar, and the decrease in all the four enzymes are of a similar magnitude. From these data Stubbs and co-workers (1967) concluded that the higher enzymic activities in intact males are androgen dependent, and that the decreased activities following hypophysectomy and castration are the result of decreased secretion of testicular androgens.
Stimulation of the rabbit ovary by gonadotropin causes a rapid and marked decrease in ovarian interstitial cell ascorbate content, suggesting a correlation between ascorbate level and oestrogenic hormone production (Jennings, 1970). Hokfelt (1950) showed that gonadotropic stimulation of the corpus luteum of pregnancy caused a rapid decrease in ascorbate content. Deb and Chatterjee (1963) found that alloxan diabetes produced a disturbance in the estrous cycle of rats, and that this could be corrected by injection of ascorbic acid.

**Insulin**

Banerjee (1943) demonstrated that the insulin content of pancreas is markedly diminished in scurvy. In the scorbutic guinea pigs, \( \alpha \)-cells were found to be increased in number in proportion to the \( \beta \)-cells, and the \( \beta \)-cells were found to be mostly degranulated (Banerjee, 1944). Banerjee and Kawishwar (1959) concluded that the operation of the tricarboxylic acid cycle was impaired in scurvy, probably because of the deficiency in insulin synthesis, and that this impairment could be treated with insulin. Mazumder et al. (1973) demonstrated that insulin induces the enzyme L-gulono-\( \gamma \)-lactone oxidase.
Pituitary:

The amount of vitamin C in the pituitary is relatively high, but its function there is not known. The tissue content of vitamin C in the rat can be depleted by injection of hypothalamic extracts (Jennings, 1970). A precipitous decline in the urinary excretion of ascorbic acid was observed in hypophysectomized rats, with a new low level of excretion being established within 48 hours (Salomon and Stubbs, 1961). Hypophysectomy thus caused an immediate and irreparable defect, indicating very marked dependence of some reaction or sequence of reactions upon availability of pituitary hormone. Salomon and Stubbs (1961a) demonstrated that the enzyme, gulonolactone hydrolase, is dependent upon somatotrophic hormone. Allison (1955) showed that the stimulation of the pituitary-adrenal axis increases the blood ascorbic acid. The sex variation in ascorbic acid levels observed by Stubbs and McKernan (1967) is dependent on differences in liver biosynthetic enzyme activities, themselves under the control of pituitary-stimulated testicular androgens.

Thyroid:

Experimental hyperthyroidism is found to reduce tissue concentrations and increase requirements for
ascorbic acid (Jennings, 1970). In experimental scurvy induced in guinea pigs, haemorrhagic infiltration of the gland was observed in acute scurvy. The gland was seen to become hyperplastic and hypersecretory in chronic scurvy. All these changes were reversed by restoration of dietary ascorbate (Jennings, 1970). Neither the body pool nor the half life of ascorbic acid was affected in any detectable way by thyroidec- tomy (Salomon and Stubbs, 1961).