

### ABSTRACT

The present work has been undertaken to study the capacity of biosynthesis of L-ascorbic acid in different species of animals. Attempt has been made to explore a probable relationship between the biosynthetic capacity and species evolution in life science. The protozoan life evolved to two distinct groups - one the invertebrates, the other vertebrates. While the invertebrates ended mainly with the insects, the life of the vertebrates probably started with fishes and ended with primates. The biosynthetic capacity was investigated in one typical insect, namely, cockroach on the one hand, and in a number of other species including fish to primates on the other hand.

The cockroaches contained a significant amount of ascorbic acid. The content did not decrease when the insects were maintained on ascorbic acid free pure cane sugar. This would indicate that ascorbic acid is formed in the body of the cockroach. However, neither the fat body nor the malpighian tubules could enzymically convert D-glucuronolactone and L-gulonolactone to L-ascorbic acid. Ascorbic acid was, of course, formed when the fat body was incubated with mannose for a period of eighteen to twentyfour hours. This synthesis was again completely inhibited when the fat body from aposymbiotic cockroach was used or when chloramphenicol was added.

to the in vitro incubation system. The results indicate that the formation of ascorbic acid in the cockroach is not enzymic but carried out by some symbionts present in the insect.

A number of different fresh water fishes, namely, foli, rohit (carp), mrigala, katla, shoule and tilapia were found to contain a significant amount of ascorbic acid in the hepatopancreas, the kidney and the brain. But none of the tissues mentioned, could effect any synthesis of L-ascorbic acid when incubated with D-glucuronolactone, L-gulonolactone or L-galactonolactone.

Synthesis of ascorbic acid takes place, however, when kidney tissues from amphibia, namely, frog and toad as well as from reptiles, namely the blood sucker and tortoise, were incubated with D-glucuronolactone or L-gulonolactone. The liver tissues from these species were unable to synthesize the vitamin.

When the tissues from mammals were examined for the biosynthetic capacity, the liver and not the kidney were found to contain the enzyme system. In the guinea pig and in the more evolved primates, neither the liver nor the kidney could bring about the synthesis. Mention should also be made of two flying mammals, namely, the Indian fruit bat and the Indian pipistrelle, those were found incapable of synthesizing the vitamin.

The change of the biosynthetic pattern with evolution is more marked in the different species of birds. In the chick and the pigeon, the species that belong to the older natural order in the branched evolution of phylogenetic scale, the kidney tissues synthesized ascorbic acid. But in the more evolved passers, the liver tissue and not the kidney tissue synthesized the vitamin. However, it was unexpectedly observed that neither the liver nor the kidney tissues of quite a number of birds belonging to this natural order, namely, the swallow, sun bird, flowerpecker, flycatchers, warblers, minivet, leaf bird, bulbuls and the shrikes were able to synthesize L-ascorbic acid.

The results would indicate that the capacity of biosynthesis of L-ascorbic acid started in the kidney of amphibia, and continued to be so in the reptiles and birds of the older order. After then, with ascent of evolution, it passed from the kidney into the liver of mammals and passerines and finally disappeared from primates and some highly evolved birds. The observation presented in this thesis is at variance with the general assumption that practically all species of animals except the guinea pig and the primates are able to synthesize L-ascorbic acid.

Irrespective of the species studied, the enzyme system converting D-glucuronolactone and L-gulonolactone into L-ascorbic acid is present in the microsomal fraction of the

tissue concerned while no added factor was needed for the conversion of L-gulonolactone to L-ascorbic acid, addition of potassium cyanide was necessary for the conversion of D-glucuronolactone. However, cyanide could be replaced by other aldehyde agents, namely, semicarbazide and hydroxyl amine. The mechanism of action of the aldehyde agents <sup>was</sup> traced to trap the aldehyde form of D-glucuronolactone, the latter being inhibitory to the synthesis. Some other properties of the microsomal enzyme were also studied.

While the synthesis of ascorbic acid takes place in the microsomes, the synthesis of xylulose takes place in the soluble supernatant. It was observed that both the liver and the kidney of all the species mentioned above could convert sodium L-gulonate to L-xylulose, the conversion in the kidney was always greater than that in the liver. This capacity of synthesizing L-xylulose did not depend on whether the site of synthesis of ascorbic acid is the kidney or the liver or whether the species is at all capable or not synthesizing L-ascorbic acid: Since synthesis of L-ascorbic acid is competitive with that of L-xylulose, it was assumed that species incapable of synthesizing the vitamin would synthesize more xylulose. The assumption was true partially in the case of different bulbuls but not fully when other species were examined.