Evolution and the Biosynthesis of Ascorbic Acid

Abstract. *The ability to synthesize ascorbic acid is absent in the insects, invertebrates, and fishes. The biosynthetic capacity started in the kidney of amphibians, resided in the kidney of reptiles, became transferred to the liver of mammals, and finally disappeared from the guinea pig, the flying mammals, monkey, and man. A similar transition in the biosynthetic ability was observed in the branched evolution of the birds.*

An example of evolutionary loss of function is a loss of capacity to synthesize ascorbic acid (vitamin C). The amphibians and reptiles synthesize the vitamin in the kidney, and mammals that produce it, do so in the liver (Table 1). However, the biosynthetic ability is lost in the guinea pig and most highly evolved species, namely, the flying mammals (*Pteropus medius* and *Vesperugo abramus*), monkeys, apes, and man (*1–3*). Similar observations have been made in birds. The primitive birds synthesize ascorbic acid in the kidney. The more evolved Passeriformes produce it in the liver while a number of other highly evolved passeres are incapable of synthesizing the vitamin (*4*).

Results obtained with L-gulono-1,4-lactone as the substrate indicate that insects, invertebrates, and fishes are also incapable of synthesizing ascorbic acid (*5*). Similar observations were made with D-glucurono-1,4-lactone semicarbazone (*6, 7*), sodium D-glucuronate, and sodium L-gulonate (*2*) as substrates. Figure 1 shows how the overall pattern of ascorbic acid synthesis by different species of animals is correlated to their phylogeny.

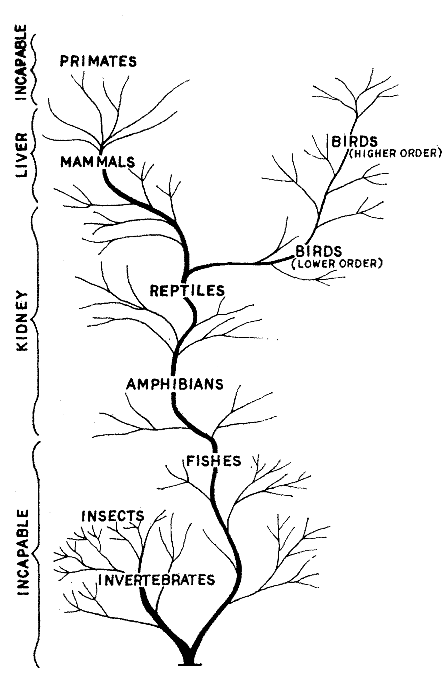

Fig. 1. Scheme of ascorbic acid synthesizing abilities of different species of animals in relation to their phylogeny.

The incapability of insects, invertebrates, and fishes to synthesize ascorbic acid apparently raises the question as to whether ascorbic acid is an essential requirement for these species. It has been reported that salmon, trout (*8*), and the desert locusts (*9*) are dependent on dietary ascorbic acid. But their need may be small, so that it is satisfied in their food. Apparently, the biosynthetic mechanism did not evolve in these earlier species of the phylogenetic tree.

Whereas the fishes are unable to synthesize the vitamin, kidney homogenate from the water-living tadpole synthesized a significant amount of ascorbic acid in vitro (200 to 250 μg per gram of kidney per hour). The evolution of ammonotelic fishes to the ureotelic amphibians thus seem to be associated with the specialized capacity to synthesize ascorbic acid.

The results in vitro (Table 1) show that, in frogs and toads, the activity of the kidney enzyme is much higher than that of the mammalian liver enzymes. The Michaelis constant for the frog and the toad kidney microsomes was approximately 1 m*M*, whereas that for liver microsomes from mammals was in the range of 4 to 5 m*M*.

Although the activity of L-gulonolactone oxidase (E.C. 1.1.3.8) of kidney microsomal fractions from reptiles is comparable to that of the liver microsomal fractions from mammals (Table 1), yet the net synthesis of ascorbic acid per kilogram of body weight per day would be comparatively much higher in mammals (*10*). This is because the weight of the liver of mammals is relatively large, about 4 to 5 percent of the body weight, whereas the kidney of reptiles comprises about 0.13 to 0.4 percent of the body weight.

There is apparently a coincidence between transition of the biosynthetic site from kidney of reptiles to the liver of mammals and the evolution of histamine action in mammals. The mast cells of the mammals not only contain high concentrations of histamine (*11*), but the histamine stored in these cells is easily released by various nonspecific stress factors (*12*). Moreover, histamine has a powerful effect on the circulatory system of mammals. In contrast, there appears to be no association between histamine and mast cells in the tissues of amphibians and reptiles, and the cardiovascular responses to histamine are absent or unimportant (*13*). Though histamine is considered to have a physiological function (*14*), an excess formation or release of histamine in the mammals may be very toxic to the body. A function of ascorbic acid has been shown to be the detoxification of histamine in rats and guinea pigs in a variety of stress conditions (*15*). The amount of ascorbic acid needed for this was about 4 to 5 times the normal need of the animals. Thus, apparently the mammals would need more ascorbic acid than the reptiles for detoxification of histamine. As indicated above (*10*), more ascorbic acid is produced in the mammalian liver than in the reptilian kidney.

Table 1. Ascorbic acid synthesis from L-gulono-1,4-lactone in microsomal fractions from tissues of different species of animals. For incubation and other details, see (*4, 6*). Each result represents an average (± S.D.) from a minimum of eight animals.

Animals	Ascorbic acid synthesized (μg/mg protein/hr) Kidney	Liver
Amphibians		
Toad (*Bufo melanostictus*)	144 ± 10	0
Frog (*Rana tigrina*)	115 ± 10	0
Reptiles		
Turtle (*Lissemys punctata*)	98 ± 8	0
Blood sucker (*Caloter versicolor*)	50 ± 5	0
House lizard (*Hemidactylus flaviviridis*)*	46 ± 6	0
Common Indian Monitor (*Varanus monitor*)	32 ± 4	0
Angani (*Mabuya carinata*)	25 ± 4	0
Snake (*Natrix piscator*)	18 ± 2	0
Tortoise (*Testudo elegans*)	14 ± 2	0
Mammals		
Goat	0	68 ± 6
Cow	0	50 ± 6
Sheep	0	43 ± 4
Rat	0	38 ± 4
Mouse	0	35 ± 4
Squirrel	0	30 ± 4
Gerbil	0	26 ± 4
Rabbit	0	23 ± 2
Cat	0	5 ± 1
Dog	0	5 ± 1
Guinea pig	0	0

* Kidneys from 12 house lizards were pooled for one determination and four such determinations were made.

In the progress of evolution, the biosynthetic capacity has been lost in the most highly evolved species. The inability of the guinea pig may be explained by the possible independent occurrence of a mutation. The flying mammals are considered to be more near, in an evolutionary sense, to the primates. The absence of biosynthetic capacity in this species supports such a contention.

The failure of the guinea pig, the flying mammals, monkey, and man to synthesize ascorbic acid is due to a common defect, namely, the absence of the terminal enzyme L-gulonolactone oxidase (*3, 16*). This in turn may be attributed to the loss of the gene or the capacity of the gene responsible for synthesizing the enzyme. Whereas the biosynthetic capacity started in the amphibians which evolved roughly about 330 to 340 million years ago, the gene mutation leading to loss of the capacity probably took place in the common ancestor of man and other primates about 25 million years ago (*17*). The mutation leading to the loss of such an essential gene was, however, neutral (*18*) and not lethal. The mutants did not become extinct because the environment furnished the vitamin and the species continued to survive.

Based on the nature of the evolutionary processes, Pauling (*19*) has put forward arguments suggesting that for optimum health the daily intake of ascorbic acid for an adult man should be 2.3 g, which could be increased to 9 to 10 g in some ailments. This is about 30 to 130 times the dose of 75 mg recommended by the Food and Nutrition Board of the National Research Council, of the United States. According to Pauling, the fact that most animals continue to synthesize ascorbic acid indicates that the need for the vitamin is greater than the amount provided by the usually available foodstuff. Pauling further considered that, although man has lost the biosynthetic ability, yet the need for ascorbic acid is similar to that of other mammals capable of synthesizing the vitamin. However, in experiments with guinea pigs, we did not get any extra beneficial effect of large doses of ascorbic acid on growth and maintenance of the animals fed a fortified wheat diet with adequate intake of protein (*20*). There are some reports (*21, 22*) that large doses of ascorbic acid are beneficial in a variety of stressful situations. We have indicated that the beneficial effect of large doses of ascorbic acid may be due to its ability to detoxify the excess histamine produced or released in stress conditions (*15*). In the guinea pig, a maximum detoxification effect was obtained with a dose of 50 mg of ascorbic acid per kilogram of body weight per day, which is approximately five times the normal need of these animals (*15*). On the other hand, when the guinea pigs were fed a low protein, high cereal diet, a daily intake of 0.3 g, or more, of ascorbic acid per kilogram of body weight was toxic as revealed by inhibition of growth and early mortality (*20*).

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10. The calculated values of net synthesis of ascorbic acid in milligrams per kilogram of body weight per day were, for reptiles: anjani, 10.7; common Indian monitor, 10.6; snake, 10.3; house lizard, 10.0; blood sucker, 9.6; turtle, 7.7; and tortoise, 6.6. The corresponding values for mammals were: mouse, 275; rabbit, 226; goat, 190; rat, 150; dog, 40; and cat, 40. The values were calculated in the following way. In the intact animal ascorbic acid is normally synthesized from D-glucuronic acid. The synthesis in vitro from sodium glucuronate in tissue homogenates from different animals was determined [for details see (*6*)]. The respective value in milligrams per gram of tissue per hour was multiplied by the weight of the organ followed by multiplication with 24 to get a hypothetical net synthesis by the animal in 24 hours. The value was then divided by the body weight (kilograms) of the animal to represent synthesis in milligrams per kilogram of body weight per day.
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23. Supported by grants-in-aid from the Council of Scientific and Industrial Research and U.S. PL-480 grant No. FG-In-416.

17 September 1973

Encoding of Geographic Dialects in the Auditory System of the Cricket Frog

Abstract. *The frequency sensitivity of the auditory nervous system of cricket frogs* (Acris) *varies geographically. This variation is closely matched to the spectral energy in their mating calls, thus enabling them to respond preferentially to the calls of their local dialect.*

The cricket frog gets its name from the cricket-like sound of the male's mating call. The two species that comprise this genus (*Acris*) are both found in the United States: *A. gryllus* occurs in the southeastern part, while *A. crepitans* is found throughout most of the country east of the Rockies. The two species are sympatric in the southeastern states from Virginia to Louisiana (*1*). Behavioral studies in the field have shown that females of each species respond preferentially to the mating calls of their own species (*2*). Thus the species-specific mating call of the male and the selective response of the female provide a reproductive isolating mechanism between these two species in their zone of sympatry. Furthermore, not only are their mating calls species-specific, but they are also geographically specific. When the mating call of a local male *crepitans* and the mating call of a male *crepitans* from a different geographical locality are presented simultaneously through separate loudspeakers,