L-Ascorbic Acid Synthesis in Birds: Phylogenetic Trend

Abstract. *The ability of several species of birds to synthesize* L-*ascorbic acid is correlated with their phylogeny. In the more primitive species, synthesis of* L-*ascorbic acid occurs in the kidney. Among the highly evolved passeriform species, kidney and liver can synthesize* L-*ascorbic acid in some, whereas in others synthesis occurs in the liver. In still others, the capacity for the synthesis of* L-*ascorbic acid is apparently lost. The pattern of evolution of the ascorbic acid pathway among birds is thus similar to that among mammals.*

In some species of birds, L-ascorbic acid is synthesized in the kidney whereas in others, it is produced in the liver (*1*, *2*). However, neither liver nor kidney tissue from *Pycnonotus cafer*, a passeriform species, can synthesize this vitamin (*2*). We have now found that *P. cafer* is not unique in lacking this biosynthetic capacity. The livers and kidneys of 15 other species of passeriform birds are also incapable of synthesizing the vitamin (Fig. 1). In our experiments, both D-glucuronolactone and L-gulonolactone were used as substrates; the microsome fraction was used as the enzyme source (*3*). Similar results were obtained by dye titration as well as by estimation of total ascorbic acid.

Other evidence (*3*) indicates that animals which can synthesize L-ascorbic acid do so according to the following sequence. D-Glucuronolactone in the presence of D-glucurono reductase (*4*), forms L-gulonolactone, which in the presence of L-gulono oxidase (*4*) forms 2-keto-L-gulonolactone, which spontaneously forms L-ascorbic acid. In an incubation system that contained the microsome fraction from passeriform species incapable of synthesizing L-ascorbic acid, neither L-gulonolactone (*5*) nor 2-keto-L-gulonolactone (*6*) could be identified as intermediates, nor did the synthesis take place in the absence of air when phenazine methosulfate was used as the electron carrier (*7*). These results indicate that these birds lack D-glucurono reductase and L-gulono oxidase. This genetic defect is common to man, monkey, the guinea pig, and the Indian fruit bat (*5*). The possibility that the microsome fractions might have contained some inhibitor was eliminated for they did not inhibit L-ascorbic acid synthesis by rat liver or goat liver microsome fractions.

Thus, there is an apparent relation between the biosynthetic capacity and phylogeny of birds. The enzyme systems concerned in the biosynthesis are located in the kidneys of birds placed in the older order. The enzymic activity then becomes localized in the liver of the more advanced Passeriformes (including a Piciformes, *Brachypternus benghalensis*) except *Corvus splendens* and *Acredotheres tristis*. The biosynthetic capacity has apparently been lost by 16 of the 28 species of passeriform birds examined (Fig. 1). *Corvus splendens* and *A. tristis* synthesize the vitamin in both kidney and liver and thus apparently stand at the border line in the transition of the biosynthetic capacity from kidney to the liver.

The change of biosynthetic pattern with evolution of birds is similar to that observed in other animals of the mammalian line. In the latter case, the enzyme resides in the kidneys of species of older order but is found in the liver of more evolved mammals and is lacking in the most evolved primates (*3*). The number of birds incapable of syn-

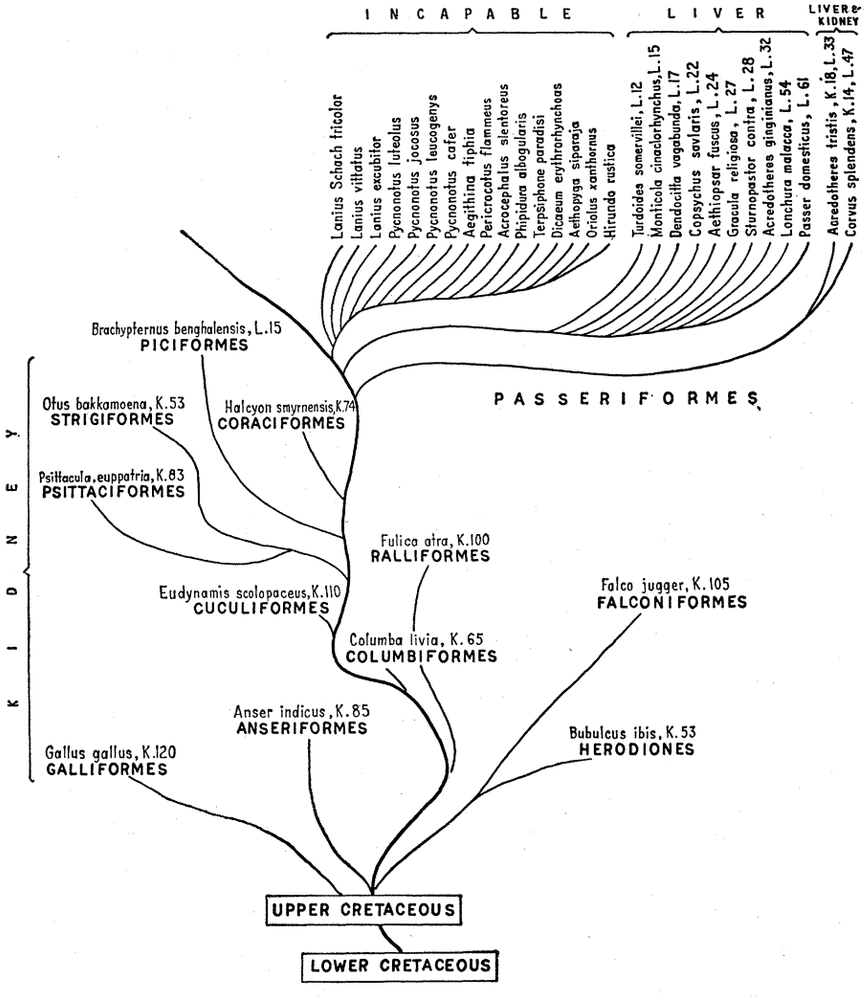

Fig. 1. Synthesis of L-ascorbic acid in the liver and kidney microsome fractions of different species of birds. The phylogenetic relationships are taken from Gregory (*8*). The positions of the Passeres are arranged according to the biosynthetic capacity. The incubation medium contained (in a total volume of 2.5 ml) 0.25 ml of microsome suspension in 0.25*M* sucrose (equivalent to approximately 5 mg of protein), 0.01*M* L-gulonolactone, 0.02*M* sodium phosphate buffer (*p*H 7.2), 0.002*M* KCN, and 0.005*M* sodium pyrophosphate. The ascorbic acid was estimated by dye titration (*7*). Abbreviations are: *L*, liver; *K*, kidney. The numbers following *L* and *K* indicate the amount of ascorbic acid synthesized in micrograms per milligram of protein. Each value represents an average of three experiments; two to six birds were used for each experiment. Tissues from equal number of males and females were pooled.

thesizing L-ascorbic acid is quite large. Therefore, this incapability may not be fortuitous. It would rather appear that the incapable Passeres are more evolved than other birds of the same branch of the phylogenetic tree.

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References and Notes

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4. D-Glucurono reductase is located in the microsome fraction and is not dependent on nicotinamide-adenine dinucleotide phosphate. The suggested enzyme commission nomenclature for this enzyme is L-gulono-γ-lactone : (acceptor) oxidoreductase. The Enzyme Commission number and nomenclature for L-gulono oxidase is 1.1.3.8, L-gulono-γ-lactone : O_2 oxidoreductase.
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26 November 1968; revised 26 February 1969 ■

Serum C'3 Lytic System in Patients with Glomerulonephritis

Abstract. *The serums of patients with hypocomplementemic glomerulonephritis contain a substance that combines with a normal serum cofactor in the presence of magnesium ion to specifically cleave the third component of complement. This lysis of C'3 is 80 to 90 percent complete in 20 minutes at 37°C and* p*H 7. Neither the nephritic factor nor its cofactor is identifiable with the complement system.*

It has always been assumed that the reduction in serum complement seen in several forms of glomerulonephritis is secondary to an ongoing immune reaction in the kidney. Recently, however, Pickering, Gewurz, and Good (*1*) demonstrated an inactivator of guinea pig complement in the serums of a number of these patients. They determined that this "anticomplementary substance" probably acted on one or more of the terminal six components of complement and theorized that the inactivating factor or factors may be partially responsible for the low levels of complement seen in such patients.

This report shows that a factor is present in these serums which, when combined with a cofactor present in normal human serum, specifically cleaves the third component of human complement (C'3 or β_{1C}-globulin) into at least two breakdown products, β_{1A} and α_{2D}. We have designated the factor found in these nephritic serums the C'3 nephritic factor (C'3NeF) to distinguish it from other nonspecific inactivators of complement (*2*) and, more specifically, from the C'3 inactivator described by Tamura and Nelson (*3*).

To demonstrate the nephritic factor and its cofactor, monospecific antiserums directed against each of the three major antigenic determinants of C'3, previously designated A, B, and D (*4*), have been utilized. As C'3 is broken down, the B antigen that is associated only with the intact native molecule disappears; ultimately, β_{1A}, containing only the A antigen, and α_{2D}, containing only the D antigen, are formed. In this study, therefore, the rate of C'3 breakdown has been quantitated by measuring the disappearance of the B antigen by use of the immunoelectrophoretic - precipitin method (*5*).

In a mixture of equal volumes of normal human serum and serum from a patient with persistent hypocomplementemic glomerulonephritis (*6*), there is a rapid decline in the concentration of the B antigen with concurrent formation of the β_{1C} breakdown products, β_{1A} and α_{2D}. The kinetics of this reaction are such that 80 to 90 percent of the B antigen disappears within 20 minutes after mixing. The rate of disappearance is the same in such a mixture even if the volume of the nephritic serum is reduced by 50 percent. Further characterization of this reaction shows that there is full activity at a temperature of 37°C over a broad *p*H range of 6 to 9. Below *p*H 6 or above *p*H 9 and at temperatures from 0° to 25°C, however, there is a marked reduction in the rate of breakdown.

Several lines of evidence make it clear that the cleavage of C'3 in the serum mixture is dependent not only on the nephritic factor but also on one or more cofactors present in fresh human serum. First, the presence of sodium fluoride or disodium ethylenediaminetetraacetic acid at the time the serums are mixed completely inhibits breakdown of C'3. However, addition of these reagents after the reaction has started, even if the interval is as short as 30 seconds, does not alter the reaction rate. Both Na_2EDTA and NaF exert their effect in this system by specifically removing Mg^{++}; Ca^{++} does not appear to be involved. Second, as shown in Table 1, nephritic serum causes only slight breakdown of the β_{1C}-globulin in a euglobulin fraction of normal serum. Breakdown at approximately the normal rate does occur, however, when normal pseudoglobulin is added to the mixture. It would appear, therefore, that at least

Table 1. Localization of C'3NeF in the pseudoglobulin fraction of nephritic serum; requirement of a cofactor for lysis of C'3.

Reactants	B antigen (unit/ml)		
	Time 0*	Time (20 minutes)†	Decrease (%)
Nephritic serum and normal serum	23.8	3.2	87
Nephritic serum and normal euglobulin	14.3	12.5	13
Nephritic serum, normal pseudoglobulin, normal euglobulin	12.9	2.2	83
Nephritic pseudoglobulin, normal pseudoglobulin, normal euglobulin	16.1	3.8	77
Nephritic euglobulin, normal pseudoglobulin, normal euglobulin	17.1	16.7	2

* Reactants mixed at 0°C without incubation. † Reactants mixed at 37°C and incubated for 20 minutes.