basis for objectively evaluating the question of differences and similarities in the shapes (and processes) of clade diversification histories. It may be that the empirical record, when analyzed according to the ways we have suggested, will support a claim of temporal directionality. We would naturally be delighted by either outcome.

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4. The results displayed in table 1 of (*2*) represent cases most biased against bottom-heaviness, that is, most biased toward symmetrical clades of $CG = 0.5$, and tightly constrained standard deviations; "all clades" simulations of table 1 are dominated by single-lineage clades whose CG statistic is 0.5 by definition. (Minimum CG for case 5 is 0.379 and not 0.505, which is the mean.)
5. We assumed a normal distribution, on the basis of the decision of Gould *et al.* (*1*) to utilize the *t* test.
6. We thank W. Ericson and T. Kramer for discussion.

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Possible Role of Carbamates in Neurotoxicity and Neurotransmitter Inactivation

John H. Weiss and Dennis W. Choi (*1*) report that the neurotoxicity of β-*N*-methylamino-L-alanine (BMAA) requires bicarbonate and suggest a "noncovalent interaction" of bicarbonate with the secondary amino group of BMAA to explain that finding. Carbon dioxide, present in the bicarbonate solutions used by Weiss and Choi, reacts rapidly with the unprotonated form of primary and secondary amines to form carbamates (*2*): $RNH_2 + CO_2 = RNHCO_2^- + H^+$ and similarly for secondary amines. Carbamates have well-established physiological roles: for example, carbamates formed at the amino terminus of hemoglogin ("carbamino hemoglobin") account for a significant fraction of the carbon dioxide transported from tissues to the atmosphere (*3*). This well-known covalent interaction may make it unnecessary to invoke a "noncovalent interaction" of BMAA and bicarbonate.

Because carbamate formation involves the nonprotonated form of amines, only a fraction of a percent of most amino acids (which have amino groups with pK_a's over 9) is in the carbamate form at physiological *p*H and P_{CO_2}. Amines with lower pK_a's (such as peptides), however, can have substantial proportions of carbamate. BMAA is a diamine, and might be expected to have one particularly low amino pK_a. For example, ethylamine has a pK_a of 10.63, while its diamino counterpart, ethylenediamine, has a pK_a for the first deprotonation of only 6.85 (*4*). A substantial proportion of BMAA may be in the carbamate form under the conditions studied by Weiss and Choi.

The results of Weiss and Choi thus have an implication for the physiology of the nervous system that is even more intriguing than simply another mechanism of neurotoxicity: they may provide a clue to an "inactivating mechanism . . . inherent in the nature of the transmitter itself," suggested by Werman (*5*) as a mechanism for terminating neurotransmitter action. Because the readily reversible formation of carbamates occurs only on unprotonated amines, the stability of carbamates depends strongly on the *p*H. Thus differences in *p*H between synaptic vesicles and the extracellular environment could provide an inactivating mechanism. For example, suppose that the carbamate but not the unmodified form of an amine were a neurotransmitter. If this amine were stored in a synaptic vesicle at a high *p*H, a significant proportion of it could be stored as a carbamate. The few microseconds required for diffusion across the narrow synaptic cleft mean that significant amounts of the carbamate should reach postsynaptic receptors after release. But in the extracellular environment, more acidic than inside the vesicle, the carbamate would decompose over a course of milliseconds [the decomposition rate is about 200 s^{-1} for the carbamate of glycylglycine at 5°C (*3* and references therein)], producing inactivation without enzymatic assistance. BMAA might represent an example of a "failure" of this inactivation mechanism, either because of a high potency of its carbamate form or because it has a high proportion of carbamate under physiological conditions.

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Response: We thank Edmund A. Mroz for his suggestion (also made by William Jencks at Brandeis University) that an interaction between the beta-amino group of BMAA and bicarbonate/CO_2 could take place covalently to form a carbamate, rather than noncovalently as we had originally proposed (*1*). We agree that such a rapid and reversible covalent interaction could also attractively account for our basic observation that BMAA activates glutamate receptors only in the presence of a bicarbonate cofactor. Further study would be required to distinguish between these covalent and noncovalent alternatives. Independent of the exact nature of the interaction between BMAA and bicarbonate, this interaction could permit certain other compounds, not themselves structurally recognizable as glutamate agonists, to serve as glutamate agonists—either as neurotoxins or, as Mroz suggests, excitatory neurotransmitters. We have now examined two other compounds structurally related to BMAA, 2,3-diaminopropionate and 2,4-diaminobutyrate, and have found that the neurotoxicity of both of these compounds can be substantially increased by adding bicarbonate to the medium.

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Possible role of carbamates in neurotoxicity and neurotransmitter inactivation

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