LETTER

Reply to Rocke and Barker: The question is not whether birds are affected by thiamine deficiency or botulism, it is about the order of events

Contrary to the suggestion by Rocke and Barker (1), we do not want to exclude botulism as a part of the investigated wildlife disorder (2), because botulism may be secondary to the thiamine deficiency syndrome. This possibility and the investigations by Neimanis et al. (3) are discussed in Text S5 in our *SI Appendix* (2).

Mörner et al. (4), who indeed examined paralyzed birds with respect to botulism, obtained only 1 positive result out of 12, whereas Neimanis et al. (3) obtained 16 positive results out of 32 (mouse inoculation test) and 15 positive results out of 53 (ELISA), respectively. These proportions are too low to support any argument that botulism would be the primary cause of the paralytic disease. Rocke and Barker point out that type C botulinum toxin is detected in <100% of experimentally intoxicated birds (5), and the actual sensitivity in the study by Rocke et al. (5) was 35 out of 40 (88%), which is considerably higher than the proportion found among Swedish gulls (3, 4). In our opinion, Rocke's own suggestion that lead poisoning and vitamin A deficiency in birds might increase the risk of botulism (6) strengthens the possibility of botulism as a secondary effect of other agents as well (e.g., thiamine deficiency).

Rocke and Barker expect to find lower tissue thiamine levels in paralyzed gulls compared with symptom-free individuals. This will often be true, but even in the dissertation by Gilman (7) the relationship between thiamine deficiency and paralytic symptoms is not that simplistic. A reason for this would be that the toxicity is not caused by the thiamine deficiency per se but rather by the accumulation of a number of intracellular toxic metabolites, which is subject to individual differences.

The omission of four thiamine-treated specimens in the evaluation of the herring gull experiment is carefully and very well motivated (2). Even if this procedure is not accepted, and all specimens are included in the evaluation, there is still a highly significant therapeutic effect of the thiamine treatment [Fisher's exact test, $P = 0.0012$; our Table S2 (2)]. The reference to a very brief and outdated description of thiamine treatment with uncertain therapeutic efficacy in a case of two botulinum-intoxicated humans (father and son) (8) by no means proves that thiamine could remedy botulinum-intoxicated birds. Moreover, the article by Mikhailov (9) describes LD_{50} experiments with botulinum-inoculated rats (not mice), whose survival increased by 13% after thiamine treatment. This very small effect was, however, not statistically evaluated or verified, and accordingly it constitutes very weak evidence, if any, for a therapeutic effect of thiamine in cases of botulism. The observation that high doses of thiamine may exert a cholinomimetic effect (10) is of some interest. No such effect was observed, however, for doses up to 50 mg/kg (10), which is the dose that we used in our experiments. An important motive for our work has been the unsatisfying sweeping conclusions that the paralytic disease is caused primarily by botulinum intoxication, and we welcome the scientific discussion about the nature of the actual etiologic agents.

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