COMMENTS ON 'CONCENTRATION BY EVAPORATION AND THE PREBIOTIC SYNTHESIS OF CYTOSINE'

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Abstract. The claim by Nelson *et al*. (2001) that the reaction of cyanoacetaldehyde and urea provides 'an efficient prebiotic synthesis' of cytosine is disputed. The authors have not dealt with the important points presented in a criticism of this reaction (Shapiro, 1999): (1) The reactants undergo side reactions with common nucleophiles that appear to proceed more rapidly than cytosine formation, and (2) No reactions have been described thus far that would produce cytosine at a rate sufficient to compensate for its decomposition by deamination, and permit accumulation over extended periods of time. Instead, Nelson *et al*. have conducted 'drying-down' experiments, in an effort to simulate evaporations on the early Earth, but the design of these experiments is flawed. The initial reactant concentrations are much higher than might be expected in a natural setting, and potentially interfering substances such as glycine, cyanide and thiols have been excluded. 'Drying beaches and drying lagoons' have been invoked as sites for such a reaction but no effort has been made to describe the characteristics of such sites or to estimate their frequency with reference to the present Earth. In the absence of contradictory data, the conclusion put forward in Shapiro (1999) remains valid: 'It was quite unlikely that cytosine played a role in the origin of life'.

Keywords: cytosine, prebiotic synthesis, concentration, cyanoacetaldehyde, urea, glycine, deamination

In a new article in this journal (Nelson *et al*., 2001), Professor Stanley Miller and his collaborators have attempted to defend their earlier proposed 'efficient prebiotic synthesis of cytosine and uracil' (Robertson and Miller, 1995) from my published criticisms. Their current article begins with the sentence: 'In a recent article, Shapiro (1999) claims that the efficient prebiotic of cytosine and uracil from cyanoacetaldehyde would not work because of the dimerization and decarbonylation of the cyanoacetaldehyde, the decomposition of urea and other factors'.

Their description diverges substantially from the following one, taken from the abstract of Shapiro (1999):

> "The reported 'prebiotic' syntheses of cytosine involve the reaction of cyanoacetylene (or its hydrolysis product, cyanoacetaldehyde), with cyanate, cyanogen or urea. These substances undergo side reactions with common nucleophiles that appear to proceed more rapidly than cytosine formation. To favor cytosine formation, reactant concentrations are required that are implausible in a natural setting. Further, cytosine is consumed by deamination (the half life for deamination at 25°C is about 340 yr.) and other reactions. No reactions have been described thus far that would produce cytosine, even

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in a specialized local setting, at a rate sufficient to compensate for its decomposition. On the basis of this evidence, it appears quite unlikely that cytosine played a role in the origin of life."

In their rebuttal, Nelson *et al.* ignore entirely the first point concerning side reactions: If other nucleophiles were present that are more reactive toward cyanoacetaldehyde or urea than cyanoacetaldehyde and urea are to one another, cytosine formation would not take place. Glycine, whose yield in spark discharge experiments can be more than sixty times greater than that of urea (Orgel and Miller, 1974) reacts with urea in 50% yield to form N-carbamoyl glycine (Sakurai and Yanagawa, 1984), under conditions that appear less severe than those employed for cytosine formation. Nelson *et al.* acknowledge that glycine is 'an amino acid formed readily under prebiotic conditions', but exclude it from their simulations. Also excluded are cyanide, which could combine with cyanoacetaldehyde, and thiols and amines, which may react with cyanoacetaldehyde dimer. The lengthy discussion of this dimer by Nelson *et al.* appears based upon a misinterpretation of my paper. They comment (using CA as an abbreviation for cyanoacetaldehyde): "Shapiro states dimerization of CA will ruin the CA + urea reaction". My only statement concerning this dimer was: "Cyanoacetaldehyde ... is in equilibrium with a dimer, which then reacts readily with thiols. Its reaction with amino groups of proteins suggests that simple amino acids will also combine with it". The possibility that that the dimer might be diverted by side reactions was neither tested nor commented upon by Nelson *et al*. Their observation that reversible dimer formation does not impede cytosine synthesis, in the absence of reactants that might trap the dimer, is not surprising.

In my article, (Shapiro, 1999), I noted that 'the assembly of a cytosine-containing replicator would require several steps beyond cytosine synthesis as well as the concurrent synthesis of the other replicator components'. For cytosine to play such a role in prebiotic chemistry, it would be necessary for cytosine to be made in quantities suitable to support further chemical transformations, in diverse locations. The need for the rate of local cytosine synthesis to equal or exceed that of cytosine decomposition on a global scale was ignored by Nelson *et al*. and earlier by Robertson and Miller (1995), despite the fact that substantial deamination of cytosine to uracil was taking place in their synthetic preparations. If their reactions had not been terminated by intervention of the experimenters, the conversion of cytosine to uracil would have gone to completion.

The central thrust of the Nelson *et al*. paper is instead directed to a single point: the defense of extreme concentration as a likely event on the early Earth. The authors take considerable pains to confirm the validity of the law of conservation of matter for the evaporation of glycine solutions, but ignore the vital distinction between glycine, a stable substance, and their less stable reactants, cyanoacetaldehyde and urea. These substances can hydrolyze at any concentration, but react appreciably with one another only at high concentrations. To bring 1L of a 10^{-5} M urea solution (an estimate of its possible concentration in a primitive ocean; see

Shapiro, 1999) to a concentration of 0.1 M in urea (the approximate threshold for cytosine formation; Ferris *et al.,* 1974), a reduction in volume of the solution to 0.1 mL would be needed. If this were to take place in nature by evaporation of a large body of water, an extended period of time, perhaps decades, would be needed. For the bulk of this period, cyanoacetaldehyde and urea could decompose by hydrolysis (or, as we have discussed, react with other substances) but their concentrations would be too low for appreciable reaction with one another. In their 'drying down' experiments, Nelson *et al*. circumvented this problem by starting with 0.01 M urea. By using this strategy, they avoided almost 99.9% of the contraction in volume needed for a realistic prebiotic simulation. Even so, their maximal reported 'drying down' yield was 0.23%, as compared to a maximum of 53% in the conventional laboratory syntheses (Robertson and Miller, 1995). What would the yield have been if a full evaporation procedure was carried out? Such a lengthy study would have been impractical, of course, because of the extended time periods required. An alternative strategy, however, would be to determine accurately the rate constants for reactant decomposition, cytosine formation, and cytosine deamination under reaction conditions, and extrapolate the results. The authors rejected this route in favor of their inadequate simulation: 'Rather than analyze the complicated kinetic argument, we decided to run the reaction under dry-down conditions'.

The authors further buttressed their position by citing a list of other published papers that supposedly used 'dry-down' procedures. Their use of the ambiguous term 'dry down', serves here to conceal substantial differences in experimental procedure and rationale. A reaction in which a solution is evaporated to a small fraction of its volume to attain elevated concentrations has little in common with one in which substances are heated together in the solid state to promote reactions that link them together by elimination of water, for example nucleoside formation (Fuller *et al.*, 1972) or the polymerization of amino acids (Fox and Dose, 1977).

Their proposals that concentrated urea solutions 'might have been found in an evaporating lagoon or in pools on drying beaches on the early Earth' invoke specific geological features. Such suggestions would best be supported by references to geological publications (or by their own field work) rather than a discussion of practices used in other laboratories. My own examination of the geological literature turned up no example of contemporary lagoons that had been concentrated to the extent required by the cyanoacetaldehyde-urea reaction (see the discussion in Shapiro, 1999). If 'pools on drying beaches' were substituted for lagoons, the rate of evaporation might be speeded, but the kinetic factors that favor decomposition over cytosine synthesis during evaporation would remain in place. I leave it to the authors to measure the number of suitable pools on contemporary beaches and to measure their evaporation rates, if they wish to pursue this line of reasoning further.

Conclusions

The reaction of urea with cyanoacetylene to form cytosine is unlikely to be of prebiotic relevance, for reasons discussed by Shapiro (1999). The majority of those reasons were not addressed in the recent article by Nelson *et al.* (2001). Extremely high urea concentrations are required for efficient reaction of urea with cyanoacetylene. The 'drying-down' experiments presented by Nelson *et al.* do not adequately model the lengthy evaporation process needed in a natural setting to create such high concentrations.

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