

PREBIOTIC RIBOSE SYNTHESIS: A CRITICAL ANALYSIS

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Abstract. The discovery of catalytic ability in RNA has given fresh impetus to speculations that RNA played a critical role in the origin of life. This question must rest on the plausibility of prebiotic oligonucleotide synthesis, rather than on the properties of the final product. Many claims have been published to support the idea that the components of RNA were readily available on the prebiotic earth. In this article, the literature cited in support of the prebiotic availability of one subunit, D-ribose, is reviewed to determine whether it justifies the claim.

Polymerization of formaldehyde (the formose reaction) has been the single reaction cited for prebiotic ribose synthesis. It has been conducted with different catalysts: numerous basic substances, neutral clays and heat, and various types of radiation. Ribose has been identified (yields are uncertain, but unlikely to be greater than 1%) in reactions run with concentrated (0.15 M or greater) formaldehyde. It has been claimed in reactions run at lower concentration, but characterization has been inadequate, and experimental details have not been provided.

The complex sugar mixture produced in the formose reaction is rapidly destroyed under the reaction conditions. Nitrogenous substances (needed for prebiotic base synthesis) would interfere with the formose reaction by reacting with formaldehyde, the intermediates, and sugar products in undesirable ways.

The evidence that is currently available does not support the availability of ribose on the prebiotic earth, except perhaps for brief periods of time, in low concentration as part of a complex mixture, and under conditions unsuitable for nucleoside synthesis.

1. Introduction

Recently, there has been great excitement in the field of RNA chemistry. It has been appreciated that certain RNA molecules, derived from biology, can take part in specific reactions and catalyze others, without enzymatic assistance. The reactions involved include hydrolyses, rearrangements, phosphate-transfers and others (Cech and Bass, 1986; Waugh and Pace, 1986; Zaugh and Cech, 1986).

Although the RNA catalysts described thus far contain hundreds of nucleotides, and the substrates are oligonucleotides or RNA, there has been speculation that smaller RNA molecules may be able to replicate themselves and catalyze a wider variety of chemical processes (Bass and Cech, 1984; Pace and Marsh, 1985; Cech, 1986; Gilbert, 1986; Nisbet, 1986; Orgel, 1986). This possibility has encouraged the advocates of an earlier theory that RNA preceded proteins in evolution (Darnell and Doolittle, 1986, and references therein). It has been suggested that life began with an 'RNA world' (Gilbert, 1986), containing only replicating RNA species and ribonucleotides (Sharp, 1985). According to Gilbert: "The first stage of evolution proceeds, then, by RNA molecules performing the catalytic activities necessary to assemble themselves from a nucleotide soup".

However attractive and versatile the properties of present-day RNA molecules may be, any theory that features their participation in the origin of life must also

document plausible chemical routes for the synthesis and accumulation of the first RNA molecules and their components. In this paper, I wish to collect and evaluate the evidence presented to date to support the availability of ribose, a critical monomer subunit of RNA. (In subsequent work, I hope to evaluate the prebiotic syntheses of other RNA components, and the paths for their assembly to RNA). I have not attempted to survey all conceivable synthetic routes to ribose, but limited myself to the reactions cited by the advocates of the RNA-first theory.

2. The Prebiotic Availability of RNA Components: An Assumption

Many scientists and writers concerned with the origin of life have assumed that the necessary parts for RNA construction were readily available on the prebiotic Earth. For example, Eigen and Schuster (1978) stated “Here we simply start from the assumption that when self-organization began, all kinds of energy-rich material were ubiquitous, including in particular: amino acids in varying degrees of abundance, nucleotides involving the four bases, A, U, C, G, polymers of both preceding classes... having more or less random sequences.” The same authors wrote at a later date (Eigen and Schuster, 1982). “The building blocks of polynucleotides – the four bases, ribose and phosphate were available too under prebiotic conditions. Material was provided from steadily refilling pools for the formation of polymers, among them polypeptides and polynucleotides”. Kuhn and Waser (1981) wrote in a similar mode: “The most important components of our model for the origin and the earliest steps of life are amino acids, ribose, and the nucleotide bases G, C, A, and U. These substances were presumably present on the primordial planet and might have accumulated in particular regions by natural concentration processes, such as evaporation of an aqueous solution and redissolution of the residue, or by adsorption and desorption.” R. E. Dickerson wrote in the *Scientific American* (1978): It is not difficult to account for the appearance of the bases and sugars of nucleic acids on the primitive earth ... current knowledge of the chemistry by which amino acids, bases, sugars and other monomers of life could have been synthesized on the primitive earth is really rather impressive”. Only occasionally does a note of caution enter, for example in Dillon (1978): “Although further experimentation toward the syntheses of sugars is needed, and probably will be forthcoming, sufficient convincing data exist to suggest that saccharide formation could actually have occurred spontaneously in the environment usually postulated for the primitive earth.”

A great many authors have voiced similarly optimistic sentiments, and they have been echoed in texts such as Lehninger (1975): “the organic building blocks of nucleotides, namely pyrimidines, purines, ribose and 2-deoxyribose, have been shown to arise under simulated primitive-earth conditions.” These views have been spread further in popular articles concerning the origin of life, for example, one by P. Trachtman in *Smithsonian* (1984): “In the past few decades, researchers have demonstrated clearly that the necessary building blocks of the cell, including amino

acids and nucleotides, can be made out of simple, ordinary compounds found in any chemistry lab and likely to have been present on the early earth”.

Why is there reason to doubt this apparent consensus which covers many of the workers in the field, and those who summarize it for a broader audience? Some objections arise from a consideration of the general background of organic chemistry, and others from a review of the specific literature cited. These topics will be discussed in separate sections.

3. Considerations from Organic Chemistry

As carbon is normally tetravalent, and can form stable bonds to itself, and to hydrogen, oxygen, and nitrogen, the number of possible organic compounds with a particular number of carbon atoms increases exponentially as the carbon number increases (Lederberg *et al.*, 1969; Smith, 1975). The relationship between a molecular formula and the number of possible isomers compatible with it is not a simple one, but it is clear from the last cited references that many thousands of substances with five or less carbon atoms are capable of existence. When greater carbon numbers are permitted, possibilities become vast. In 1983, the Chemical Abstract Service listed its six millionth chemical (Maugh II, 1983).

Under equilibrium conditions, however, most of the carbon in a mixture of C, H, O, and N will be present as carbon dioxide, carbon monoxide and methane, with the concentration of more complex organic substances smaller by many orders of magnitude (Eck *et al.*, 1966; Morowitz, 1968). The exposure of such a system to an energy flux, however, will result in the synthesis of a variety of small organic molecules. The composition of the mixture will depend on the choice of energy supply and starting materials (Morowitz, 1968). As the reaction is allowed to proceed with increasing formation of carbon to carbon bonds, valence considerations dictate that the mixture must grow more complex, with individual substances represented to a lesser extent.

D-Ribose is a substance of some complexity, with five carbons, three of which are asymmetric, and a molecular weight of 150. Unless some special circumstance existed, any prebiotic reaction leading to its synthesis would also be expected to lead to the production of a host of other substances as well, and thus give ribose in poor yield. Let us consider only its close relatives, for example. D-Ribose is one of eight straight-chain aldopentoses. Eight straight-chain ketopentoses also can exist, as well as seven branched-chain pentoses. Many other isomers can be listed if other functional groups are permitted, for example, carboxylic acids. Non-isomers (tetroses and hexoses, for example) would also be formed in reactions that lead to pentoses, and so the possibilities will be vastly multiplied.

Of course, one or more special effects might have existed to overcome the above negative considerations. For example:

(1) Geological circumstances may have favored the formation of certain classes of organic compound at the expense of others. For example, if the pool of organic

precursors was more oxidized than reduced, carbohydrates and other oxidized substances, rather than hydrocarbons, might be expected. In addition, recent models of the pre-biological atmosphere have indicated that N_2 was the principal form of nitrogen present, with ammonia and other substances containing reduced nitrogen absent (Levine, 1982; Levine and Augustsson, 1985). If this were the case, then the production of organic compounds of nitrogen would be minimized or eliminated, and greater yields of compounds with only C, H and O would result. Such conditions are reasonable, but no firm geological data exists to document them.

(2) Mechanistic paths may have favored the highly preferential synthesis of certain compounds. In the particular case of ribose, its formula $C_5H_{10}O_5$ represents a pentamer of formaldehyde, CH_2O . This suggests that a direct synthesis of ribose from formaldehyde is likely. This path could also afford isomers and homologs of ribose, but would disfavor substances of less regular composition. As we shall see, all suggested prebiotic syntheses of D-ribose have followed this assumption.

(3) Possibly, ribose could be unusually stable with respect to related substances. Thus, even though it might not be favored by any preferential synthetic route, it might accumulate as time passed by the conversion of other materials. The evidence available thus far indicates that this circumstance does not apply.

For example, D-ribose isomerizes within a few weeks at $25^\circ C$, pH 7 to form a mixture containing 75% D-arabinose, 6% D-ribulose, and only 19% D-ribose (Tewari and Goldberg, 1985). Such isomerization reactions of monosaccharides can proceed further, accompanied by various degradation reactions (C—C bond fission, dehydration, furfural formation, and other decompositions). These reactions occur most rapidly in alkali, but also occur at appreciable rates in acidic or neutral solution (Berrens, 1965; Pigman and Anet, 1972; De Wit *et al.*, 1979). D-Ribose is further vulnerable to decomposition by free radicals formed by radiation (Von Sonntag and Dizdaroglu, 1977). Thus, ribose should not accumulate, but should rather have a relatively short duration after its formation.

4. Claims Concerning Prebiotic Ribose Synthesis

Despite the above difficulties, many optimistic statements have been published (some have been cited above) concerning the ready availability of ribose on the prebiotic earth. Often, no documentation was offered with the claim, but when references were provided, only the polymerization of formaldehyde, in a process called the formose reaction, was cited. This reaction generally has been carried out under alkaline conditions, but variations, catalyzed by clays, light and other radiation have also been reported. The latter variations were felt more relevant for prebiotic purposes. We will discuss each set of conditions separately.

5. The Alkali-Catalyzed Formose Reaction

This reaction is often mentioned, usually quite briefly, to justify the prebiotic availability of ribose, for example in Lehninger's biochemistry text (1975):

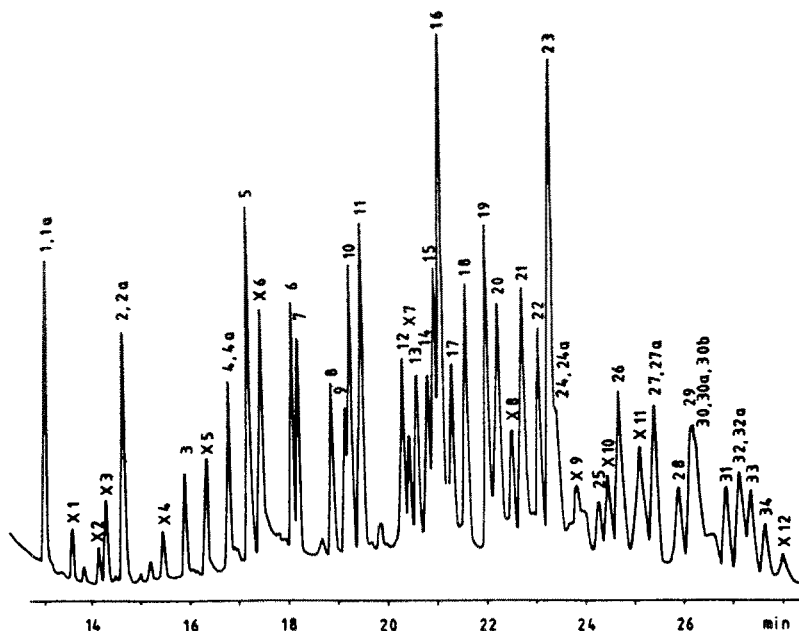


Fig. 1. Gas chromatographic separation of n-butoxime trifluoroacetyl derivatives of a formose reaction, run at 40 °C, with sodium and calcium hydroxide catalysis. Peak number 8 represents the derivatives of D- and L-ribose. Reprinted from Decker *et al.* (1982) by permission of Elsevier Publishing Co.

“Formaldehyde, also readily formed in simulated primitive-earth experiments, yields a variety of sugars when heated with limestone (calcium carbonate).” Monographs on the origin of life (Miller and Orgel, 1974; Dillon, 1978) provide a fuller account, tracing the origin of the reaction to Butlerov (1861). The most sustained interest in the reaction, however, has come not from the origin-of-life field, but from those concerned with possible practical applications. The interested reader should consult the review by Mizuno and Weiss (1974) for a full account, and Shigemasa *et al.* (1984) for access to more recent work. In this review, I shall only emphasize a number of points relevant to the prebiotic synthesis of ribose:

(1) The formose product is exceedingly complex: “The formose product can be regarded as a carbohydrate analog of petroleum, in that it contains so many carbohydrates of varying molecular weight and isomeric structure” (Weiss *et al.*, 1970). This complexity can best be appreciated by examining the separation of an appropriately derivatized formose mixture (Figure 1, Decker *et al.*, 1982). Peak No. 8 in this figure has the same retention time as ribose, and it is accompanied by peaks representing virtually every possible straight and branched chain aldose and ketose of three to six carbons (Substances of greater chain length have also been identified in formose mixtures). An outline of some of the pathways involved in generating these substances is given in Figure 2. Other products have also been observed, for example reduced sugar alcohols (formed by a cross-Cannizzaro reaction with formaldehyde), saccharinic acids (Socha *et al.*, 1981), and sugar decomposition products, as well as many unidentified products.

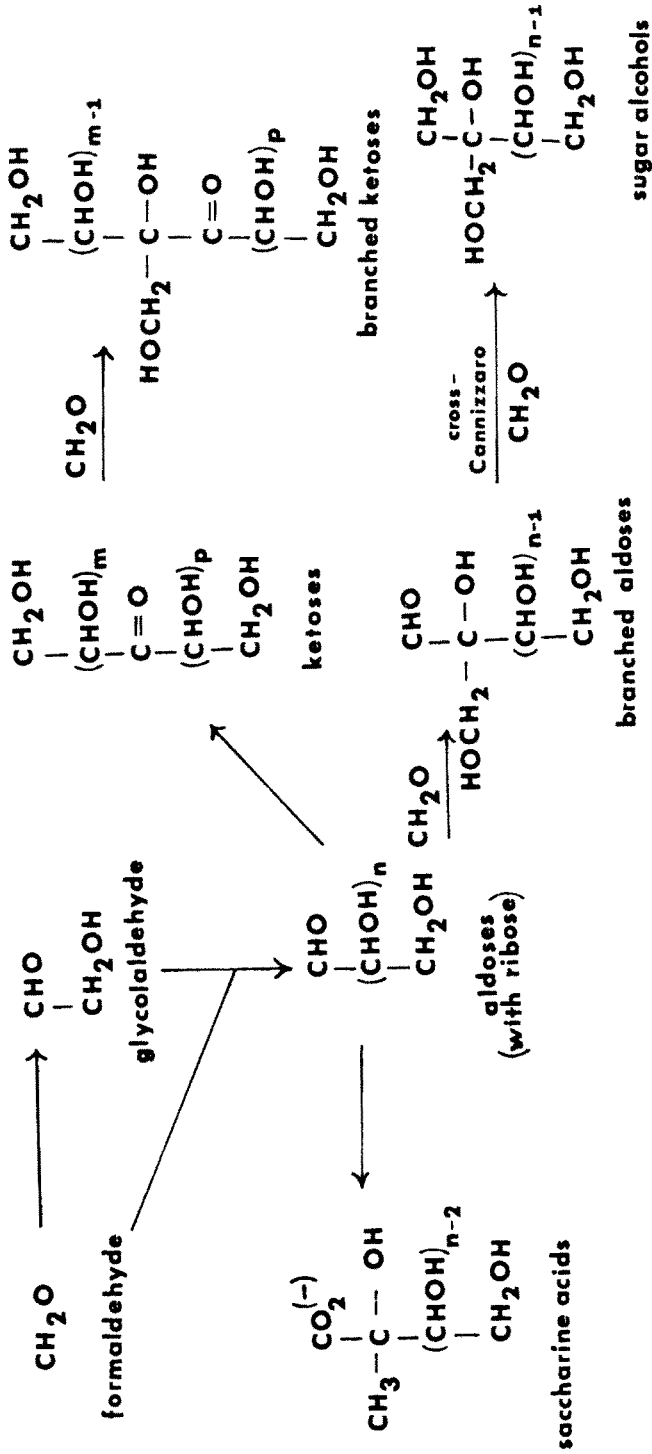


Fig. 2. Formose Reaction pathways.

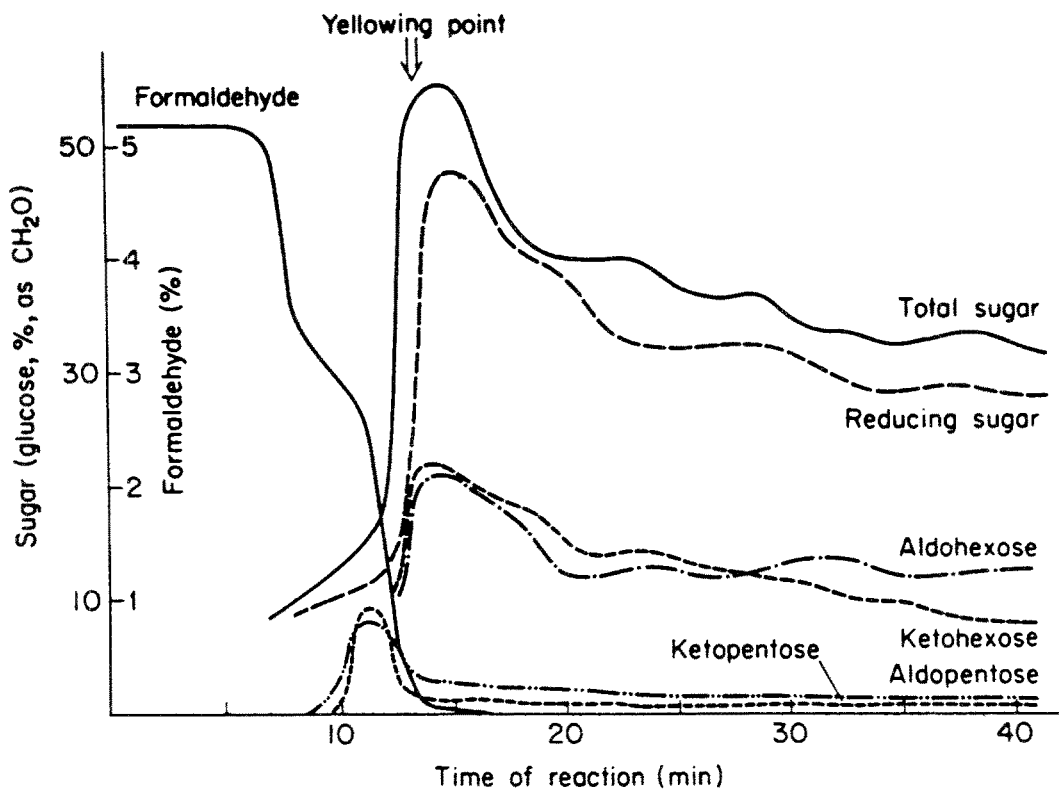


Fig. 3. Sugar formation and decomposition in a calcium hydroxide catalyzed formose reaction run at 60°C. Reprinted from Mizuno and Weiss (1974) by permission of Academic Press.

(2) No special selectivity for ribose has been demonstrated. Separation difficulties often have led workers to report product yields in terms of rough fractions such as pentoses, hexoses, etc., and there have been few reports of yields of individual substances. Recently, Harsch *et al.* (1983) have attempted a more exact quantitation. They separated prebiotic together with lyxose and ketopentoses, with a maximal yield under the optimal conditions reported by them (1.0 M CH_2O , excess heterogeneous calcium hydroxide catalyst, 50°C, 48 min) of 4.4%. In earlier work (Mizuno and Weiss, 1974), the best reported yield of the aldopentose fraction was 8.0% (2.0 M CH_2O , 0.2 M calcium hydroxide, 60°C). If the material in the above reactions were divided evenly among the various components in the isolated ribose fraction, then the maximal yield of D-ribose would be somewhat less than 1%.

Many studies have been run on the formose reaction using a variety of catalysts and conditions, to improve selectivity. Some selection has been obtained, in fact, for branched and reduced sugars (Mizuno and Weiss, 1974; Shigemasa *et al.*, 1978), and dihydroxyacetone (Shigemasa *et al.*, 1984), but none has been reported for ribose. In one report, selectivity for straight-chained rather than branched carbohydrates

was achieved (Castells *et al.*, 1980), though ribose was not listed among the products. The conditions, however, were far from prebiotic: The conjugate base of thiazolium ion served as the catalyst, in a solvent of dimethylformamide and triethylamine.

(3) Timing factors are critical to the success of the formose reaction. A lag occurs initially, as formaldehyde has little tendency to react with itself to form carbon-carbon bonds. During this time, much of the formaldehyde may be consumed by the Cannizzaro reaction (Shigemasa *et al.*, 1978). The lag period can be overcome by adding small quantities of glycolaldehyde, to initiate normal aldol-type processes. The termination point is even more important. The yield of sugars reaches a maximum at the 'yellowing point,' immediately after the initial formaldehyde has been consumed (Mizuno and Weiss, 1974), and then declines. The situation has been described by Shigemasa *et al.* (1978): "Formose sugars decompose considerably rapidly after the yellowing point. Therefore, in order to obtain formose sugars, the reaction must be stopped at this point."

The importance of timing is brought forth in the time-yield curve in Figure 3 (Mizuno and Weiss, 1974). Under the conditions employed, the maximum yield of aldopentoses (presumably containing ribose) peaks at 10–13 min and is much less at other times.

6. The Clay-Catalyzed Formose Reaction

The alkali-catalyzed formose reaction demonstrates that it is possible, in principle, to obtain some ribose from formaldehyde in a 'one-pot' process, even though it is present in modest yield as part of a complex mixture. Many writers have recognized, however, that this reaction is not a valid model for a prebiotic process: among other defects, it employs strongly alkaline conditions and high concentrations of pure formaldehyde. For example, R. E. Dickerson (1978) wrote: "There are problems with the formose reaction (the ribose produced is unstable in aqueous solution and the experimental conditions are not realistic simulations of primitive earth conditions), but some such reaction could have yielded the necessary ribose molecules." A report that certain clay minerals could catalyze the same process using lower (0.01 M) formaldehyde concentrations (Gabel and Ponnampertuma, 1967) was therefore greeted with enthusiasm and is now the principal cited reference for the prebiotic synthesis of ribose. (An accompanying paper (Reid and Orgel, 1967) confirmed the general reaction but added little new specific data.) For example, Miller (1984) wrote: "Particularly attractive is the finding that at 100°C, clays such as Kaolin serve to catalyze formation of monosaccharides, including ribose, in good yield from dilute (0.01 M) solutions of formaldehyde." Because of the particular importance of this paper to the subject of this review, it will be discussed in some detail.

It must be noted to start that the work appeared only as a letter in *Nature*, less than two pages in length, so that the details of the experimental procedures are lacking. The conduct of the reaction was simple. Aqueous formaldehyde (0.33 M, with ¹⁴C

label) was refluxed with a suspension of alumina for five hours. The reaction was worked up by two-dimensional thin layer chromatography, and products located by use of autoradiography and by aniline-hydrogen phthalate spray, on separate duplicate plates. Sugar groups were identified by comparing their R_f value and spray color with monosaccharide standards, and the 'pentose' fraction worked up by electrophoresis. "In these conditions, the total conversion of formaldehyde to ribose was 3.8%"

This last claim must be viewed with some doubt. The final identification of ribose apparently rested only upon its mobility in one paper electrophoresis run. The reference cited for the procedure (Mills, 1961/1962) listed R_f values for only five substances; no further calibrations were offered in the *Nature* paper. An early review of ribose had recommended that any identification of that substance should rely on at least three R_f values, since natural sugars may have similar R_f's (Jeanloz and Fletcher, Jr., 1951). While the Mills electrophoretic procedure is somewhat specific, responding to a *cis*-glycol group, the complexity of formose mixtures is much greater than that of natural monosaccharide mixtures, as it contains many unnatural and unidentified substances. Much more caution should therefore be exercised in claiming an identification, at least in cases where the information is used to support more than a routine tabulation.

In retrospect, it seems likely that some ribose was formed in the alumina-catalyzed formose reaction, if only by analogy to the alkali-catalyzed reaction. It also seems likely that the yield reported was inflated by the presence of other substances, as subsequent workers on the alkali-catalyzed reaction have not yet reported a yield of purified ribose, despite the use of elaborate derivatization and gas chromatographic procedures.

Experiments with 0.01 M formaldehyde were also reported in the *Nature* paper, with alumina and kaolinite catalysts. The yield of all formaldehyde condensation products (10.7% in the best experiment) was less than that reported for the reaction with 0.33 M formaldehyde (78%). No identifications or yields of ribose or other individual substances were reported. No carbohydrate product other than glycolaldehyde was detected in a reaction with 0.001 M formaldehyde.

The accompanying paper (Reid and Orgel, 1967) reported the production of 'sugars' from 0.5 M and 0.01 M, but not 0.001 M formaldehyde, using similar conditions. No analysis for individual substances was given. When reaction times were extended beyond the optimal times, the products decomposed. This factor, and the need for concentrated formaldehyde solutions led the authors to conclude: "We do not believe that the formose reaction as we and others have carried it out is a plausible model for the prebiotic accumulation of sugars. ... The formation of sugars in plausible conditions and their incorporation into nucleosides have not been achieved. Until the problem is solved or by-passed, it remains a weakness in theories of abiotic nucleic-acid synthesis."

Little has happened in the decades since that report to alter the above judgement. No full papers have appeared to provide the experimental details of the above studies.

The work has not been taken up and extended by others; we still have no conclusive identification of the ribose claimed in the reaction with 0.33 M formaldehyde, nor any report of the formation of ribose at lower concentration of formaldehyde, using this procedure.

7. Formose Reactions Catalyzed by Radiation

Occasional reports have appeared which describe the polymerization of formaldehyde under the influence of radiation. One such result, which was announced at two meetings in 1963 (Ponnamperuma and Mariner, 1963; Ponnamperuma, 1965), has been cited intermittently but continually over the years, often to provide support for the probiotic availability of ribose (Dillon, 1978). Other texts, for example Miller and Orgel (1974), have omitted any discussion the work. The first report (Ponnamperuma and Mariner, 1963) appeared in print only as a brief eight-line abstract. The second (Ponnamperuma, 1965) was the edited transcript of a talk given at a meeting, and provided more details. We shall again discuss this work at some length because of the numerous citations of it.

The following words introduce the section that deals with ribose: "Recent experiments completed on the eve of my departure for this meeting provide strong evidence for the formation of two sugars, ribose and deoxyribose, during the electron irradiation of methane, ammonia, and water. While further experimentation would be necessary to substantiate this result on irrefutable grounds, the evidence available is compelling enough to warrant my presentation of the results at this meeting."

The products of the above procedure were identified by comparison of their positions on a two-dimensional paper chromatogram with those of authentic standards. It was deduced that formaldehyde had been an intermediate in the above conversions, so further experiments were started with formaldehyde.

Radioactive formaldehyde (0.0003 M) was exposed to either gamma radiation or ultraviolet light. The reaction mixture was fractionated by paper chromatography, and ribose and deoxyribose identified by two-dimensional paper chromatography, as above. No yield was reported.

If correct, these results would be quite noteworthy, particularly with respect the low formaldehyde concentrations used. Ribose is a pentamer of formaldehyde (deoxyribose is not one; its formation also requires an unusual reduction step). The rate of formation of ribose should therefore depend on the fifth power of formaldehyde concentration. All reports of the conversion of ribose to formaldehyde, save the above, have specified much higher formaldehyde concentration. This report suggests that some unusual and specific process is at work.

The circumstances, however, provoke skepticism. Despite the admonition of the author quoted above, no regular publication has appeared in the intervening years to substantiate the claim; it has been left in the above form. As stated in an earlier section, the simple coincidence of a spot with ribose standard, even in a two-dimensional procedure, is insufficient to establish its identity (particularly in a

formose-type reaction). Other workers in fact, have exposed neutral formaldehyde solutions to ultraviolet light (though under conditions that varied from the above, in one detail or another) and reported no ribose (Shigemasa *et al.*, 1977; Halmann and Bloch, 1979; Folsome *et al.*, 1983). The above claim of prebiotic ribose synthesis cannot be accepted, therefore, without further detailed experimental verification, including a much more substantial characterization of the claimed products.

The polymerization of formaldehyde by radiation, under somewhat different circumstances, has been documented however. Ultraviolet light, in the presence of alkali, afforded a photochemical formose reaction (Shigemasa *et al.*, 1977). This procedure employed concentrated formaldehyde, and failed in the absence of a basic catalyst. It therefore followed normal formose conditions, except that light had been substituted for heat. The product distribution differed, however, with branched products predominant (ribose was not reported).

Other workers have employed very different conditions (Seel *et al.*, 1981). In their study, two types of zeolite minerals were dried under vacuum at 250°C, exposed to formaldehyde gas, then subjected to ultraviolet irradiation. The adsorbed formaldehyde was polymerized to a complex mixture, which was analyzed by high pressure liquid chromatography or, preferably, by gas chromatography after derivatization. The latter technique afforded an overlapping series of peaks superimposed on a substantial background. One partly resolved peak, in one run, was noted to have the same retention time as the derivative of D-ribose. Much more work would obviously be needed for adequate identification. At any event, this work would rationalize the occurrence of formaldehyde polymers in extraterrestrial sources such as meteorites (if any should be reported), but its relevance to the origin of life is obscure at this time.

8. Reactions of Formaldehyde and Aldoses With Nitrogenous Substances

One way, not yet discussed, in which the above experiments were unrealistic as models for processes on the early earth was their use of pure formaldehyde, in the absence of other likely prebiotic substances. The presumption has been made, in many prebiotic experiments, that cyanide, amines, and other nitrogen-containing organic substances were present on the early earth (Miller, 1984). Many nitrogen-containing substances react readily with formaldehyde, without any lag period, to afford stable products. For example, cyanide and formaldehyde combine quite rapidly at 25°C to form glyconitrile. The reaction is reversible, but the dissociation constant of glyconitrile is extremely small (2.1×10^{-6} at 25°C), so that under most circumstances the formaldehyde would largely exist in bound form. The facile hydrolysis of glyconitrile to glycolic acid would then abolish the possibility of formaldehyde release (Schlesinger and Miller, 1973).

Formaldehyde reacts readily with many other nitrogenous substances which have been implicated in prebiotic schemes, including ammonia, primary and secondary amines, amides, imides, aminonitriles, urethanes and urea (Walker, 1940), amino

acids and peptides (Ivanov *et al.*, 1983), and bases and nucleosides (Chaw *et al.*, 1980; Hemminki, 1981). Attempts to run formose condensations in the presence of these substances have not been reported. If however, one were attempted and some oligomerization took place, loss of material due to side reactions could be expected with formaldehyde, at every other step on the path to ribose, and with ribose itself.

The reactions of aldoses with hydroxylamine, ammonia and ammonium ion (Yanagawa *et al.*, 1980; Weber, 1985), cyanide ion (Serianni *et al.*, 1979), amines (Chavis *et al.*, 1983), and amino acids (Candiano *et al.*, 1985) have been well documented. Amino acids and carbohydrates, when heated together, initiate a cascade of transformations with the ultimate formation of polymeric pigments. This process is termed the Maillard Reaction (Paulsen and Pflughaupt, 1980).

The list of prebiotic substances which would interfere with the formose reaction could be extended, and the reactions documented to a much greater extent, but there is no need to do so at this point (the above references will lead the interested reader into the relevant literature). If there are chemists who wish to maintain that ample supplies of ribose can be generated in a prebiotic environment containing nitrogenous substances, then the burden of proof (hopefully experimental in nature) must rest upon them.

9. Possibilities for Ribonucleoside Synthesis

Recent models have suggested that the prebiological atmosphere had very low levels of reduced nitrogen compounds (Levine, 1982; Levine and Augustsson, 1985). In that circumstance, small amounts of reduced nitrogen compounds probably would have been removed by formaldehyde produced by photochemical reactions (Kasting and Pollack, 1984). Thus, in some environments on the prebiotic earth, organic synthesis could have taken place without nitrogen fixation (Ferris and Chen, 1975). There is uncertainty about the levels of formaldehyde that might accumulate in a situation of this type (Fegley, Jr. *et al.*, 1986; Canuto *et al.*, 1983). If however, these levels rose above a certain threshold (which might vary with the exact conditions), and if appropriate catalysts were present, then the formaldehyde could polymerize to afford a complex mixture. It is likely that one component of this mixture would be D-ribose (with the L-form also present), but its yield would probably be a fraction of a percent, at best, and it would quickly be destroyed.

We can imagine a situation where a continuous influx of formaldehyde, sufficient to balance its destruction, was maintained on the prebiotic earth. If appropriate catalysts were present, then continual production of ribose as part of a formose-type mixture could occur. It would be destroyed as produced and would not accumulate, but a low steady-state concentration might be maintained. It is hard to estimate this concentration. Miller and Orgel (1974) noted that "the source of such concentrated formaldehyde solutions [0.01 M] on the primitive earth could prove a problem". Yet thirty-fold higher amounts were necessary to produce D-ribose in yields below 1%.

Although experimental data would be most welcome, it is hard to imagine steady-state D-ribose concentrations of as much as 10^{-5} M on the early earth. This ribose would be surrounded by a thousand-fold excess of related carbohydrates and entrapped in a much larger amount of decomposition products. Such substances would be likely to interfere with subsequent steps that would be needed to generate a genetic material of regular structure. For example, activated nucleotides containing L-ribose inhibit the template-directed polymerization of their D-ribose containing counterparts (Joyce *et al.*, 1984). If a hypothetical genetic material were made only of D-ribose residues, connected together in some stereospecific manner, it would still be hard to envision how this substance could be formed under the above circumstances. (It is not easy to imagine some physical process which would fortuitously separate D-ribose from its contaminants of similar structure, let alone its enantiomer). The actual situation is much worse, however.

In order for a spontaneous prebiotic synthesis of RNA to take place, the ribose in the above mixture must first be combined specifically with the appropriate bases under somewhat different conditions, and further specific transformation must then take place. The prebiotic base reaction mixtures, however, are rich in all those substances whose rigorous exclusion was necessary to run the formose reaction. Adenine, for example, can be prepared from HCN and ammonia in a maximal yield of 0.1% under "prebiotic" conditions (Voet and Schwartz, 1983). Intermediates and by-products of this reaction include an enormous variety of amines, amino-acids and substituted ureas (Oro and Kimball, 1962; Ferris *et al.*, 1978). Thus, an environment suitable for the prebiotic synthesis of bases would consume the formaldehyde needed for a formose reaction, as well as any intermediate products of such a reaction.

This problem has been noted by some workers in the field. Schlesinger and Miller (1973) commented: "The great stability of glyconitrile shows that the two types of compounds (sugars and bases) could not have been synthesized at the same time on the primitive earth unless there was a mechanism to concentrate the formaldehyde and hydrogen cyanide in different areas. It is more plausible to think that the adenine was synthesized during one period and the sugars during another period." The authors note the instability of sugars and suggest that the adenine was prepared first. They did not discuss the need for adenine to be made in another place, even if the timing requirement were met. Only one suggestion has been made, to my knowledge, which addresses this difficulty (Schwartz, 1981; Schwartz *et al.*, 1982). It was proposed that glaciers transported the products of HCN oligomerization (bases) to heated locales, free of nitrogenous compounds, suitable for the formose reaction.

The above speculation certainly shows a vivid use of imagination. It is backed by no evidence, and cannot be tested scientifically, except perhaps by the exploration of young earthlike planets in the remote future. It has been invented only to rescue a theory that otherwise faces apparently insurmountable difficulties. A sounder scientific alternative remains, however: to set aside the theory until better evidence appears.

10. Conclusions

The evidence that is currently available does not support the availability of ribose on the prebiotic earth, except perhaps for brief periods of time, in low concentration, as part of a complex mixture, under circumstances that are unsuitable for nucleotide synthesis. This situation could change if some alternative pathway for ribose synthesis were discovered: one that produced it in better yield and was not as vulnerable to interference from nitrogen-containing substances.

The discovery of such a scheme would improve the prospects for a prebiotic 'RNA world', but would still not make it likely, as other synthetic difficulties would still remain. Another possible alternative for prebiotic RNA synthesis would be one that bypassed ribose entirely and produced nucleosides from simple chemicals. Considerations from organic chemistry of the type discussed in an earlier section make such a reaction seem considerably less likely, even, than the prebiotic synthesis of ribose. Any such claim should therefore require meticulous experimental documentation and confirmation, before it could be accepted.

In the interim, one other possibility deserves serious consideration: that RNA was not present at the start of life, but was first produced by biosynthetic processes, and subsequently took on a hereditary function by 'genetic takeover' (Cairns-Smith, 1982). A critical research priority in this case would be the identification of possible alternative genetic systems that were precursors to RNA (Orgel, 1986).

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