

FORMATION OF ADENINE BY ELECTRON IRRADIATION OF METHANE, AMMONIA, AND WATER*

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Several reports have described the effect of different forms of energy on gaseous mixtures of methane, ammonia, hydrogen, and water—the kind of mixture that probably comprised the atmosphere of the prebiotic earth.¹⁻³ While the formation of amino acids in such experiments has been clearly demonstrated, there is little evidence for the formation of the heterocyclic bases which are major constituents of the nucleic acids. Apart from Oró's synthesis of adenine by the action of heat on a concentrated solution of ammonium cyanide⁴ and Fox's synthesis of uracil by a thermal reaction between malic acid and urea,⁵ there appears to be no report in the literature of the formation of a purine or pyrimidine under simulated prebiotic earth conditions. Palm and Calvin suggested that adenine was a probable product of the electron irradiation of a mixture of methane, ammonia, hydrogen, and water.³ They had preliminary but not conclusive evidence for the formation of adenine.

The primary object of the present investigation was to examine the possibility of the synthesis of heterocyclic bases from mixtures of primitive gases. We have established that (1) adenine is indeed a product of electron irradiation of a mixture of methane, ammonia, and water, (2) there is an inverse relationship between the amount of adenine synthesis and the amount of hydrogen gas present, and (3) of the five nucleic acid bases, adenine is the one most readily synthesized under prebiotic conditions.

Materials and Methods.—Mixtures of methane-C¹⁴, ammonium hydroxide (4 N), and, in some experiments, hydrogen were irradiated with electrons in the glass apparatus (volume approx. 750 ml) shown in Figure 1. The source of ionizing radiation used in these experiments was, simply as a matter of convenience, a linear electron accelerator. Four separate experiments were performed.

Twenty ml of 4 N NH₄OH and two or three boiling chips (SiC) were introduced into the irradiation tube. The flask B was cooled to -78° and the tube evacuated. One-quarter mM of C¹⁴H₄ (containing 0.5 mc; sources: Tracerlab, Inc. and New England Nuclear Corp.) was introduced, followed by nonlabeled methane (12 mM) until the pressure in the tube was 300 mm. During the methane addition, care was exercised to exclude all air from inlet tubes. In two experiments, no H₂ was used. In a third experiment, 50 mm of H₂ was added; in a fourth experiment, 100 mm of H₂ was introduced into the irradiation tube.

During the irradiations the tube was kept in a horizontal position. The electrons entered the tube through the concave end window, which was larger in diameter than the cross section of the electron beam. The electrons had an energy of 4.5 Mev and were delivered in 60 pulses per sec, each pulse lasting 6 μsec. The integrated dose rate was 18 μamps, and the time of irradiation was 45 min. The current delivered during this time was 0.0486 coulombs. Cobalt glass dosimetry at the center of a similar irradiation tube indicated that 1.5×10^4 rads were absorbed per microcoulomb. The total energy absorption was therefore about 7×10^8 rads, or 7×10^{10} ergs/gram.

The liquid in flask B (Fig. 1) was boiled during the irradiations; heat was supplied by two infrared lamps. The irradiations took place in both the gas and liquid (on the cold-finger surface)

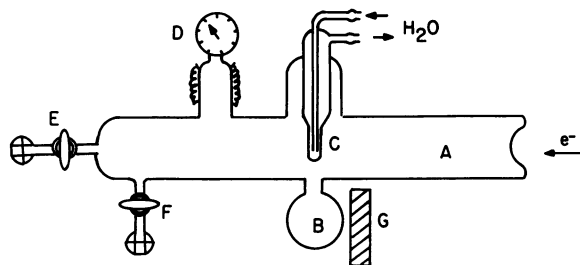


FIG. 1.—A, Irradiation tube. B, flask containing NH_4OH . C, cold finger condenser. D, vacuum-pressure gauge. E and F, vacuum-seated stopcocks. G, lead shield.

phases. The boiling caused a continuous washing back into B of the condensate on C. During the irradiation the pressure in the tube rose to 1.0–1.5 atmospheres.

At the end of the reaction the liquid in B was removed. The entire tube was washed with about 20 ml of water and the washings were added to the reaction products. Volatile products were not investigated.

Analyses for the nonvolatile products were carried out on aliquot portions by means of paper chromatography on oxalic acid-washed Whatman No. 4 paper. An aliquot portion of the product was placed on the paper together with carrier adenine. The paper was developed with *n*-propanol-16 *N* NH_4OH -water (6:3:1 by volume) in one direction and *n*-butanol-glacial formic acid-water (77:10:13 by volume) in the other. The area corresponding to the carrier adenine was cut out and eluted. The eluted material was rerun in two other solvent systems: *n*-butanol-water (86:14 by volume) and isopropanol-2 *N* HCl (65:35 by volume). The distribution of radioactivity on the chromatograms was recorded by autoradiography with X-ray film. The adenine was located on the chromatograms through the use of shadowgrams.^{6,7} The percentage of adenine formed from CH_4 was determined by elution of the adenine spots from the chromatograms, and counting the radioactivity in a liquid scintillation counter.

Other series of chromatograms were run in which the other purine and pyrimidines commonly found in the nucleic acids (guanine, cytosine, uracil, and thymine) were used as carriers.

Results and Discussion.—In all four experiments, and in all four paper chromatographic solvent systems, there was perfect coincidence in both position and shape between the inactive carrier adenine (shadowgrams) and one of the radioactive-product spots (X-ray film darkening). An example of such coincidence is shown in Figure 2.

The following conditions were all held constant through the four experiments: CH_4 pressure (300 mm), C^{14}H_4 activity (0.5 mc), NH_3 and H_2O pressure was a total of about 1.5 atm (from 20 ml of 4 *N* NH_4OH at about 100°), energy absorption about 7×10^{10} ergs. The one variable was the amount of added H_2 . The amount of adenine produced as an apparent function of this variable is shown below.

Experiment	1	2	3	4
H_2 pressure, mm	0	0	50	100
% of C^{14}H_4 converted to adenine- C^{14}	0.012	0.016	0.002	0.001

The results of this investigation clearly establish adenine as a product of the irradiation of methane, ammonia, and water. Furthermore, it appears that the production of adenine is enhanced by the absence of H_2 . This is not surprising since methane carbon must be oxidized in order to appear finally in purines and amino acids. The hydrogen would be expected to interfere with the oxidative processes. In our system, the principal species affecting the oxidations are probably

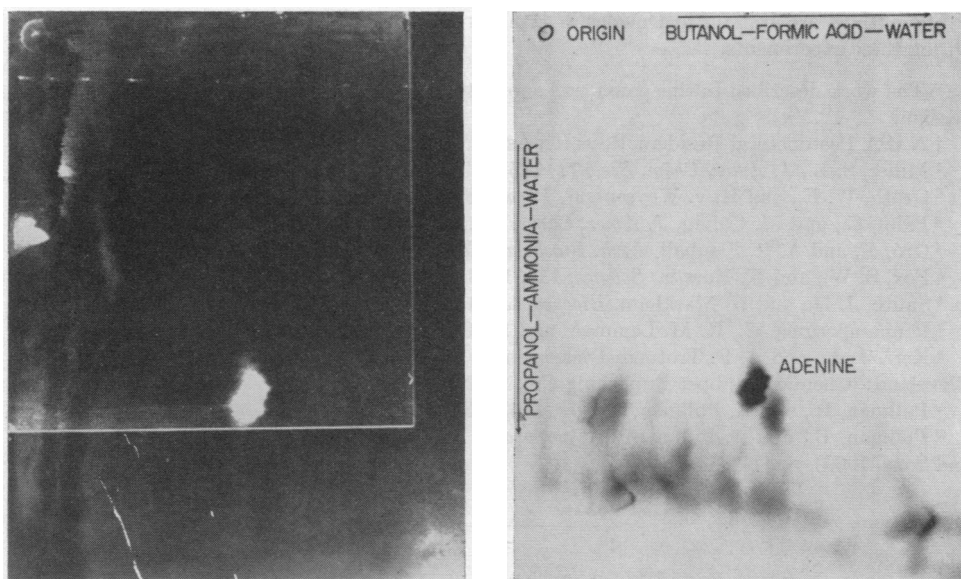
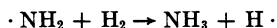
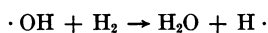


FIG. 2.—Formation of adenine by electron irradiation of simulated prebiotic earth atmospheres.

OH and NH₂ radicals, and these radicals would revert to the starting materials (water and ammonia) on reaction with hydrogen:



The first reaction is energetically favored since the H—H bond energy is 104.2 kcal and the HO—H bond energy is 119 kcal.⁸ The second reaction is slightly unfavored (H₂N—H bond energy is 103 kcal) and may not occur. Another way in which H₂ may interfere with the production of purines would be through the back reaction $\cdot\text{CH}_3 + \text{H}_2 \rightarrow \text{CH}_4 + \cdot\text{H}$. Here, the bond energies are very similar: H—CH₃ (104 kcal), H—H (104.2 kcal).⁸ In any event, the high concentration of organic matter on the prebiotic earth probably arose only at a time when most of the hydrogen had escaped from the atmosphere. Other results of our present work have indicated that there is also an inverse relationship between the presence of hydrogen and the synthesis of amino acids.

No guanine, cytosine, uracil, or thymine was detected on any of our chromatograms. Any one of these bases would have been detected if it had been present in one hundredth the amount of the adenine. The apparent preference for adenine synthesis may be related to adenine's multiple roles in biological systems. Not only is it a constituent of both DNA and RNA, but it is also a unit of many important cofactors—for example, ATP, ADP, DPN, TPN, FAD, and coenzyme A. In addition, molecular orbital calculations have shown that, of all the biologically-important purines and pyrimidines, adenine has the greatest resonance energy.^{9,10} This would not only make adenine's synthesis more likely but would, in addition, confer radiation stability upon it. Thus, after formation, the adenine is more likely to survive the radiation fields of our experiments.

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DECOMPOSITION AND EQUIVALENCE OF LOCAL VECTOR FIELDS*

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Let R^n be the real n -space with the coordinates $x = (x^i)$. Denote by \mathfrak{A} the totality of the germs of C^∞ vector fields about the origin 0. For any $X = \sum a^i(x) \partial / \partial x^i$ of \mathfrak{A} , the $n \times n$ matrix $(a_j^i(0))$ will be referred to as the jacobian of X .

THEOREM 1. *If $X \in \mathfrak{A}$ vanishes at 0 and if none of the characteristic roots of the jacobian of X has vanishing real part, then*

$$X = S + N$$

such that (a) $S, N \in \mathfrak{A}$ with $[S, N] = 0$, (b) with respect to a suitable C^∞ coordinate system y about the origin 0, S is linear and semisimple, i.e.,

$$S = \sum c_j^i y^j \partial / \partial y^i$$

with the matrix (c_j^i) similar to a complex diagonal matrix, (c) the jacobian of N is nilpotent.

The above theorem is a nonlinear analogy of the usual decomposition of a linear transformation into semisimple and nilpotent parts.

Denote by A the totality of the formal vector fields of the form $\sum f^i(x) \partial / \partial x^i$ where each $f^i(x)$ is a formal power series in x with real coefficients. We may define formal transformations, which are substitutions of the form:

$$x^i \rightarrow \sum h_j^i x^j + \sum h_{jk}^i x^j x^k + \dots, \quad i = 1, \dots, n,$$

with $\det(h_j^i) \neq 0$. Then $\hat{X}, \hat{Y} \in A$ are said to be equivalent if there exists a formal transformation, which carries \hat{X} to \hat{Y} .^{1, 2} Similarly, $X, Y \in \mathfrak{A}$ are said to be equivalent if there exists a C^∞ local homeomorphism about 0 which carries X to Y .