THE SYNTHESIS OF SOME PURINES AND PYRIMIDINES BY A FISCHER-TROPSCH-LIKE PROCESS UNDER POSSIBLE PRIMITIVE EARTH CONDITIONS

A Thesis

Presented to the . Faculty of the Department of Chemistry College of Arts and Sciences University of Houston

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In Partial Fulfillment of the Requirements for the Degree . Master of Science

by

Cheng Chu Yang January 1969

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ABSTRACT

A Fischer-Tropsch-like process has been employed to synthesize some biologically significant purine and pyrimidine bases, under the conditions which was believed possibly existing in the early solar nebula.

The reaction conditions were intended to simulate conditions in the solar nebula; brief thermal spikes superimposed on a general downward trend. Carbon monoxide, hydrogen, and ammonia (1:2:0.4 mole ratio) were the reactants. Iron-nickel alloy powder, alumina and silica gel were used as catalysts. In a typical run, adenine, guanine, cytosine, guanylurea, melamine, urea, biuret and cyanuric acid were synthesized in.a yield 3.828%. The presence of these compounds were positively identified by paper chromatography, ultraviolet and infrared spectrophotometry with comparison to the authentic standards.

The agreement between the synthetic products in this work and the nitrogen compounds identified in the meteorites shows that the nitrogen compounds in meteorites probably formed by a Fischer-Tropschlike process under the conditions which existed in the early solar nebula.

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I.

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INTRODUCTION

I. INTRODUCTION

The question of how life began is one that has inspired much speculation throughout history, but only in the past few decades has it become the object of widespread serious scientific investigation.

Twenty-three centuries ago it was suggested by Aristotle that living things could originate from inanimate matter (1). From that time this concept has been referred to as spontaneous generation and has been widely debated (2,3). In spite of much opposition to such an idea, it was subscribed to for centuries by many poets, philosophers, and naturalists (3).

In 1828 Wöhler (4) synthesized urea from organic materials without the assistance of a living organism. This was clearly the synthesis of a molecule of biological significance from an organic reagent and was a key to abandonment of the theory that a "vital force" was necessary in the formation of organic substances (5). Yet after two decades leading chemists of the time such as Berzelius (6) and Gerhardt (7) still expressed a belief in supernatural powers which were presumed necessary for the synthesis of truly organic substances (8).

In the meantime the theory of evolution flourished and advances durings this century in astronomy, nuclear physics, and chemistry have given evolution a broader scope, involving the universe (9,10), the galaxies, and stars (11), the chemical elements (12), and the chemical compounds (13) from which the simplest living organisms may have evolved. The interest here is focused on the chemical evolution and the transition

to simple molecules of biological significance from simpler components which exist in various cosmic environments. Hydrogen, carbon, nitrogen, and oxygen, the most abundant reactive elements in the universe, are also most abundant in living organisms and in organic molecules. It does not seem illogical, therefore, to assume that mixtures of these elements, sufficiently excited, might combine under appropriate conditions to yield a wide variety of products. It seems just as logical that some of these simple products might be sufficiently reactive to combine further under appropriate conditions to produce more complicated molecules, some of which might be important to biological systems. The first experimental evidence supporting such reasoning became available in 1913 (14,15), but four decades passed before an experiment was conducted specifically to test this data. This was Miller's classic synthesis of amino acids by exciting a mixture of methane, ammonia, water, and hydrogen with an electric discharge. This simple experiment revived the concept of spontaneous generation which had been subdued by Pasteur. Other experiments with electric discharge followed (16-23), and in addition ultraviolet radiation, ionizing radiation, and thermal energy were utilized as energy sources. Whereas most of these experiments were based on models of the primitive Earth, the intent here was to investigate the role of thermal energy in such reactions in any cosmic environment involving sufficiently high temperatures. In view of the wide variety of compounds which might be formed from such processes, the scope of this investigation must be restricted and is concerned primarily with the synthesis of purine and pyrimidine bases.

ASTRONOMICAL BACKGROUND AND PREVIOUS SYNTHESIS OF BIOCHEMICALS FROM SIMPLE MOLECULES

II.

II. ASTRONOMICAL BACKGROUND AND PREVIOUS SYNTHESIS OF BIOCHEMICALS FROM SIMPLE MOLECULES

The four most abundant elements in the universe, with the exception of the most noble gases, are hydrogen, oxygen, carbon, and nitrogen, which are also precisely the four major constituent elements of organic compounds and of living matter. Indeed, as has recently been said, "the composition of living matter turns out to be a better sample of the universe than the dead earth" (16).

These four elements exist mainly as atoms and diatomical combinations such as CN, CH, C_2 , CO, NH and OH, in the atmospheres of relatively cool stars, including the sun, and in interstellar or circumstellar space. They also exist as di- and polyatomic combinations in planets, comets, and meteorites. Thus, simple and complex compounds of carbon are found widely distributed in the universe. In principle, these compounds will exist wherever the prevailing temperatures are compatible with the stability of the bonds between carbon and other elements. If the carbon containing diatomic combinations CN, CH, C_2 , and CO are considered, it is observed that their thermal stability ranges from the low temperatures of interstellar space to the relatively high temperatures prevailing above the surface of stars. In fact, such diatomical combinations have been detected in the atmospheres of supergiant carbon stars at temperatures of the order of 6000°K at which some of the most thermally stable oxides, namely titanium and zirconium oxides, are dissociated into their metallic ions (17).

Observations bearing on the distribution of simple and complex compounds of carbon in cosmic bodies and on the natural formation of these compounds, form part of a space science which is called "organic cosmochemistry." Because of the limited observational data so far obtained and the importance of the fundamental problems involved, attempts have been made to follow an experimental approach in this study.

As a result of the initial experiments of Garrison <u>et al</u>. (18), Miller (19,20), and the most recent ones carried out in this (21) and other laboratories (22), it has become apparent that processes of organic synthesis which may have occurred in the primitive Earth's atmosphere, or may be occurring in certain cosmic bodies such as comets, can be partially reproduced in the laboratory. These experiments have opened a field of investigation which was called "experimental organic cosmochemistry."

A. <u>Models for Organic Synthesis</u>

In order to duplicate an incompletely known natural process, any experimental approach requires the formulation of assumptions about the experimental model to be used. It is recognized that it would be difficult to determine with certainty all the conditions applying to a cosmic model for organic synthesis. However, if it is understood that organic reaction pathways are determined by rather general laws, then it becomes possible to obtain significant knowledge about natural organic synthetic processes even with only partially complete models.

We have focused our attention on a cometary model (21) primarily because comets are supposed to contain large amounts of reactive

carbon compounds and because it is considered that their composition reflects approximately the composition of the primordial solar nebula and protoplanets (23). Indeed, a recent model for the protoplanets of the solar system (24) as suggested by Fowler is almost identical to a cometary model proposed some time ago by Whipple (25) and recently revised by the same author (26). On the basis of this physical and chemical similarity it is reasonable to assume that the chemical processes which occur in comets by the action of solar radiation, when these bodies are at distances of less than 3 A. U. from the sun, may have also occurred, but on a much larger scale in the Earth protoplanet.

Furthermore, it is possible that the conditions for organic synthesis were quite favorable during the transformation of the gravitationally undifferentiated protoplanet into the primitive planet. This would result from the mixing of the reactive precursors of organic compounds with inorganic particles such as silicate and metallic grains which could have acted as surface catalysts. Due to the low density of the synthesized organic compounds, these compounds would migrate toward the exterior of the planet during the process of gravitational differentiation. The nonvolatile combinations, ionic or high molecular weight compounds, would accumulate on the surface of Earth, whereas the gases and the compounds volatile at the prevailing temperatures would be evaporated into the outer region of the solar system where comets originate presently. The difficult problem of the escape of gases and volatile compounds from primitive planetary atmospheres had been discussed mainly by Suess (27) and Urey (28).

With regard to the composition of the model, it is known that the spectra of comets show fluorescence emission bands corresponding to the molecules or radicals CN, CH, CH₂, C₂, C₃, NH, NH₂, and OH to the ions CH⁺, OH⁺, CO⁺, N₂⁺, and CO₂⁺ and to the atoms of Fe, Ni, Cr, and other elements (28). These emission bands are observed in the heads or in the tails of comets when these bodies are at less than 3 A. U. from the sun. The band corresponding to the CN radical is generally the first emission band to appear on the tails of comets during the travel of these bodies toward the sun, and it is also the band with the largest degree of extension into the comet's head followed in intensity by the C_2 and C_3 bands.

The above compounds exist in the nuclei of comets either as frozen free radicals or as "ices" (25,26) (or crystalline clathrate type hydrates) of molecules which are vaporized and dissociated into radicals by the solar radiation. In general, it is considered that the parent molecules of CN, NH₂, and OH are hydrogen, cyanide or cyanogen, ammonia and water, respectively. The parent molecules of the carbon radicals are supposed to be methane, acetylene, and other hydrocarbons. Therefore, a simplified experimental model could be made of hydrogen, cyanide, ammonia, and water. A slightly more complex model could contain in addition cyanogen, acetylene, carbon monoxide, carbon suboxide, and other compounds. There are certain relations between this model and the two atmospheric models, namely, the "primitive planetary atmosphere" model and the "volcanic atmosphere" model. These models should not be

considered as providing alternative, but rather complementary approaches to the study of the formation of organic compounds on the abiotic Earth. In fact, they present progressive stages in the development of the Earth. An important condition which is common to all of these models is that they are essentially reducing or at least non-oxidizing in character, of which we have cosmochemical and geochemical evidence. Additional evidence for the reducing conditions of the atmosphere of magmatic origin is provided by the fact that the terrestrial rate of oxygen production by photolysis of water is less than the rate of volcanic carbon monoxide production (29).

B. Energy Sources

Several sources of energy were available for the synthesis of organic compounds during the transformation of the Earth from protoplanet into planet. The main source was, of course, the sun providing ultraviolet light and ionizing radiation at a rate of 10^7 times as high as that observed at the present time (24). A second source was the Earth itself with its natural radioactivity and the heat derived from gravitational compression and radioactivity.

However, as indicated above, since some of the primordial constituents of the Earth protoplanet were radicals or reactive chemical compounds, then organic synthesis could have occurred spontaneously at relatively low temperatures during the melting of the protoplanetary ices in the absence of highly activating forms of energy. It is surmized that these spontaneous syntheses were responsible for the formation of

substantial amounts of organic and biochemical compounds. Furthermore, due to the relatively low prevailing temperatures and the reducing conditions of the protoplanetary environment, the compounds thus formed would have been preserved for a very long time.

During the further stages of geological development, additional sources of energy were available on the surface and atmosphere of the Earth. It is likely that in addition to ultraviolet light and ionizing radiation, electric discharges and the heat from plutonic processes contributed also to the formation of organic compounds.

TABLE I

ENERGY DISTRIBUTION ON EARTH (30)

Source	<u>Energy (cal./cm²/yr)</u>
Total radiation from sun U. V. light	260,000
λ < 2500 Ű	570
λ < 2000 A°	85
λ < 1500 Ű	3.5
Electric discharges	. 4.0
Cosmic rays	0.0015
Radioactivity	0.8
Volcanoes	1.13

C. Synthesis of Purines and Intermediates

The formation of purines on the primitive Earth or in cosmic bodies poses a priori difficult conceptual problem because it requires

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the formation of two fused heterocylic structures on imidazole and pyrimidine.

In principle, there are, however, two relatively simple mechanisms or pathways which can be visualized for the formation of purine ring. One involves condensation of a 3-carbon compound with a 1-carbon reactant to form a 4, 5 disubstituted imidazole and the other involves condensation of a C_3 compound with a C_1 reactant to form a 4, 5 disubstituted pyrimidine. The reaction terminates by the cyclization of either the disubstituted imidazole or the disubstituted pyrimidine with another mole of the C_1 reactant.

It is known that the formation of purines in living organisms occurs by a pathway involving 4, 5-disubstituted imidazole derivatives, and it has also been observed that the acid degradation of adenine yields 4-aminoimidazole-5-carboxamidine as an intermediate. On the one hand, in the very mild conditions of enzymatic synthesis, and the very drastic conditions of acid hydrolysis; yet in both cases a 4, 5-disubstituted imidazole shows as an intermediate. Shortly after these observations were made it became apparent that if a non-enzymatic synthesis of purines under possible primitive Earth conditions was discovered, it may likely proceed through the imidazole pathway. The first demonstration of the spontaneous synthesis of adenine from hydrogen cyanide under conditions presumed to have existed on the primitive Earth was made in our laboratory (35) and in line with the above reasoning 4, 5disubstituted imidazoles were found in the reaction product as intermediates.

Adenine was synthesized in substantial amounts by heating a solution of hydrogen cyanide (1 to 15 M) in aqueous ammonia for one or several days at moderate temperatures (27° to 100°C). The insoluble black polymer of hydrogen cyanide was removed by centrifugation and adenine was isolated from the red-brown supernatant solution by chromatographic methods. The main ultraviolet absorbing compound of the reaction product was identified as adenine by a number of different procedures including ultraviolet spectrophotometry and the melting point of its picrate derivative.

The synthesis was found linear with time at room temperature and in a typical experiment at the end of four days more than 1000 mg of adenine per liter of reaction mixture was obtained (36).

Since adenine is an essential building block of nucleic acids and of the most important coenzymes, and since hydrogen, cyanide, ammonia, and water are presumed to be common natural constituents of the solar system, these findings were considered to be of special significance in relation to the problem of the origin of life.

In addition to adenine several precursors, namely 4-amino-imidazole-5-carboxamide (AICA), 4-amino-imidazole-5-carboxamidine (AICAI), formamide, and formamidine were also found in the reaction product (37,38). The mechanism of adenine synthesis is supposed to be initiated by the base catalyzed polymerization of hydrogen cyanide into nitriles. The role played by ammonia in the synthesis is twofold. It acts as a basic catalist and it causes the ammonolysis of hydrogen cyanide into formamidine and of nitriles into amidines. One of the resulting nitriles,

possibly aminomalonodinitrile, condenses either directly or after transformation to its mono- or diamidine with formamidine to form AICAI; in the last step, AICAI condenses with another mole of formamidine to yield adenine. This last step has been confirmed in a separate experiment in our laboratory (39).

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The other purines were postulated to be formed from 4-aminoimidazole-5-carboxamide (38). Recent experiments in our laboratory have confirmed this assumption (40). It has been observed that AICA and guanidine condense in aqueous ammonia systems to yield guanine. Moreover, when AICA is allowed to react with urea under similar conditions, guanine and xanthine are formed (40). The formation of the 1-carbon reactants, guanidine and urea in the absence of free oxygen poses no special problem because compounds of this oxidation level, such as urea, were detected by Miller (31), Berger (41), and Palm and Calvin (32), in their respective experiments with electric discharges, high energy protons, and high energy electrons, which were carried out under reducing conditions. Other workers have also observed the formation of guanidine (33) and urea (33) from cyanides, cyanogen, or cyanates.

The above experiments on the synthesis of adenine from mixtures of hydrogen cyanide, ammonia, and water have been confirmed by Lowe <u>et</u> <u>al</u>. (33), who have found in an additional purine, hypoxanthine among the reaction products. A significant extension of these experiments has been carried out recently by Calvin (13), and Palm and Calvin (32), who have observed the formation of adenine by irradiating with 5 MeV electrons, a mixture containing methane, ammonia, and water among other

reduced compounds. In summary, it seems to be well estabilished that the four major biological purines can be synthesized from very simple precursors in aqueous systems under possible Earth conditions.

From a historical point of view it should be said that at the turn of the last century, cyanogen and hydrocyanic acid were thought to be involved in the synthesis of proteins and purines in living organisms. These have since been found to be erroneous concepts. Nevertheless, it is of interest that such early ideas may apply to the abiogenic formation of these compounds. Studies on the polymerization of hydrocyanic acid were initially carried out more than 150 years ago (42) and, therefore, it is highly probable that purines, purine intermediates, and other compounds of biological significance were synthesized in the laboratory many times since then, yet have remained unidentified until the present time. Interesting observations bearing on the synthesis of purines from hydrogen cyanide were made by Gautier (43), Fischer (44), Salomone (45), and Johnson and Nicolet (46), and they are discussed in some detail in a recent paper from our laboratory (38). Aside from these early unsuccessful attempts on the synthesis of purines from hydrogen cyanide, it should be added that uric acid was synthesized from glycine and urea by Horbaczewski (47) and purine from formamide and other simple compounds by Bredereck et al. (48). However, none of the biochemical purines found in nucleic acids were isolated or identified in these experiments.

D. Synthesis of Pyrimidines

With regard to the formation of pyrimidines it was proposed (21) that derivatives from the C_3 molecular species found in comets could be the source of these heterocyclic compounds. One of these C_3 derivatives is malonamide semialdimine or its isomer β -aminoacrylamide which by condensation with urea could be expected to yield uracil.

Because β -aminoacrylamide was not available to us we tested some of the C_3 compounds which are formed in the experiments with electric discharges and which are considered to be intermediates in the formation of β -alanine. These intermediates are acrylonitrile, β -aminopropionitrile, and β -aminopropionamide. When each of these compounds was allowed to react with urea in aqueous ammonia systems at 130°C, the formation of small amounts of uracil was observed in each case. Uracil was characterized by paper and ion exchange column chromatography and by ultraviolet spectrophotometry. The yields obtained from β-aminopropionamide were approximately two and five times higher than those obtained from β -aminopropionitrile and acrylonitrile, respectively. This is what would be expected if acrylonitrile has to undergo first amination into β -aminopropionitrile and this, in turn, has to undergo hydrolysis into *β*-amino propionamide. Because this amide is, in fact, the dihydroderivative of *β*-aminoacrylamide, it is obvious that the mechanism of the reaction must involve a dehydration step either before or after the cyclization. The mechanism of uracil formation involving β-aminoacrylamide or is isomer, malonamide semialdimine is in line with the well known chemical synthesis of uracil from malic acid and urea

in the presence of a strong mineral acid (49). A strong mineral acid transforms malic acid into malonic semialdehyde which then condenses with urea to form uracil. Also, in line with the above mechanism, it is known from the work of Bredereck <u>et al.</u> (50), that the pyrimidine ring can be formed in good yield from either aminoacrolein or malonodialdehyde. In theory the three pyrimidines found in nucleic acids could conceivably be formed in aqueous solution under possible primitive Earth conditions by the mechanism described above. In addition to β -aminoacrylamide yielding uracil, β -aminoacrylamide could be expected to condense with urea into cytosine and α -methyl- β -aminoacrylamide into thymine.

A possible pathway for the conversion of the symmetrical C_3 species of comets into β -aminoacrylamide or malonamide semialdimine is through the formation of carbon suboxide (C_3O_2) which has been suggested to exist in several cosmic bodies (51). By addition of hydrogen and ammonia to carbon suboxide, malonamide semialdehyde or malonamide semialdimine might be obtained. In fact, malonic acid derivatives have been obtained recently in the laboratory from carbon suboxide. In addition to purines and pyrimidines, preliminary data have been obtained on the synthesis of other heterocyclic compounds. Recently, by using Fischer-Tropsch process in a closed vycor vessel with a cold trap, Hayatsu <u>et al</u>. (34) was able to synthesize adenine, quanine, and cytosine (tentatively identified) in the same experiment. He used carbon monoxide, hydrogen, and ammonia as reactants, iron meteorite powder as catalyst; the temperatures under which the

the experiments were carried out range from 300° to 1000°C.

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THE FISCHER-TROPSCH PROCESS

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III. THE FISCHER-TROPSCH PROCESS

During the first half of the twentieth century, the chemistry of the catalytic hydrogenation of carbon monoxide has been explored at a steadily accelerating rate. The work of Sabatier and Senderens (52) on the synthesis of methane, using nickel catalysts, and the discovery that hydrogen and carbon monoxide react at 100 to 200 atmospheres of pressure in the presence of alkali-activated cobalt or osmium oxide supported on asbestos to form a liquid product containing alcohols, aldehydes, ketones, fatty acids, and some saturated and unsaturated aliphatic hydrocarbons, was followed by the development of the industrial synthesis of methanol in 1923-1925 and the extensive laboratory and pilot plant work on the Fischer-Tropsch process from 1923 to the present. An important by-product of the Fischer-Tropsch process development, the "oxo" process (hydroformylation of olefins and alcohols), has been the subject of much scientific and industrial research.

Franz Fischer and Hans Tropsch (53) discovered in 1923 that at 100 to 150 atmospheres and 400° - 500°C alkalized iron turnings functioned as catalysts for the synthesis of a product consisting of alcohols, aldehydes, ketones, fatty acids, and some saturated and unsaturated hydrocarbons. They observed also that at lower pressures of about seven atmospheres, the portion of hydrocarbons in the products was much greater and that of oxygenated compounds correspondingly lower than at 100 to 150 atmospheres, although the productivity of the catalyst was very much lower at seven atmospheres. This observation led to an

intensive search for more active catalysts for synthesis at lower pressures. Most of the catalysts tested by Fischer and Tropsch in 1925-1930 were of poor activity and durability (54). This result was in part due to the fact that at atmospheric pressure even the most active catalysts for this synthesis must be periodically washed with a good solvent for the paraffins of very high molecular weight that accumulated on them. In 1930-1933, highly active nickel and cobalt catalysts were developed by Fischer and his coworkers (54) and subsequently the cobalt-thoria-magnesia-kieselguhr catalyst was used in industrial operation. In recent process development, iron catalysts have been employed.

In hydrogenating carbon monoxide over iron catalysts, it is essential that a certain amount of potassium salt must be present on the catalysts (55). Not only does this promoter increase the CO conversion, but it also shifts the selectivity toward the more useful long chain hydrocarbons. In the absence of potassium salt, the main hydrocarbon product is methane when the catalyst is run under normal commercial synthesis conditions.

The Fischer-Tropsch synthesis is not an equilibrium process. The nature of products is at least partly determined by the reaction mechanism. So the proposed mechanism must be consistent with the results of thermodynamic studies, which show that the isomer distribution is far from equilibrium, whereas secondary reactions, such as that between carbon monoxide and water to yield carbon dioxide and hydrogen, are closer to equilibrium on iron catalysts. The reaction mechanism must

be in accord also with the extensive chemical effect on activity or product distribution. Although no generally accepted mechanism exists, kinetic studies combined with X-ray diffraction and magnetochemical measurements on catalysts have indicated how more active and more durable catalysts can be prepared (56).

A. Thermodynamics of the Fischer-Tropsch Synthesis

In the discussion of thermodynamics of Fischer-Tropsch synthesis, it should be emphasized that these data indicate only the possible extent to which the reactions can proceed as defined by their equilibrium constants. How nearly the reaction approaches equilibrium depends upon the rate of reaction and elapsed time, and in flowing systems upon the rate and space velocity. If two or more reactions are thermodynamically possible from the same reactants, the products formed depend upon rates of the possible reactions and rates of transformations of the products to more stable forms. The purpose of a catalyst is to accelerate the rate at which one or more of the thermodynamically possible reaction approach equilibrium. The ideal catalyst in a system in which several reactions are possible is one which accelerates only the reactions producing the desired products.

The reactions producing paraffinic and olefinic hydrocarbons in the Fischer-Tropsch synthesis may be represented by equations 1A, 1B, 2A, 2B where 1 and 2 represent reactions yielding paraffins and olefins, respectively, and A and B denote reactions yielding water and carbon dioxide, respectively.

(2n + 1) H ₂	+ $nC0 = C_n H_{2n+2} + nH_2 0$	1A
2n H ₂	+ nCO = $C_n H_{2n}$ + nH ₂ O	2A
(n + 1) H ₂	+ 2nCO = $C_n H_{2n+2}$ + nCO ₂	1B
n H ₂	+ 2nco = $C_n H_{2n}$ + nco ₂	2B

Similar equations can be written for the production of other hydrocarbons such as cyclic compounds and diclefins. Since most of these reactions are exothermic, removal of this heat from the reactor is one of the most difficult engineering problems of the synthesis (because the synthesis is carried out at a relatively constant temperature).

From the thermodynamical data for these reactions, the following conclusions can be drawn (57):

1. Reactions that form carbon dioxide (type B) have larger equilibrium constants (more negative values of ΔF°) than corresponding reactions yielding water (type A) at the same temperatures. Thus, reaction of type B are thermodynamically possible at higher temperatues than corresponding reactions of type A.

2. The free-energy changes per carbon atom of the hydrocarbon of reactions forming methane are more negative than those for corresponding reactions yielding higher hydrocarbons. Below 500°C the reactions producing carbon (graphite) are less negative than those yielding methane, but more negative than reactions yielding higher hydrocarbons.

3. The practical upper temperature limit of the formation of

higher hydrocarbons of any type is about 500°C.

4. For molecules containing the same number of carbon atoms the free energy of the synthesis reactions become more negative in the following order: diolofins, mono-olefins, and paraffins. The formation of sizable amounts of acetylene is thermodynamically impossible at usual synthesis temperatures and pressures ($150^{\circ} - 350^{\circ}C$, 1 - 20 atms.).

5. The formation of branched-chain, cyclic, and aromatic hydrocarbons is thermodynamically possible.

6. The hydrocarbons formed in the Fischer-Tropsch synthesis, chiefly normal hydrocarbons and α -olefins, indicate that the process does not proceed to the state of lowest free energy. The course of the reaction is determined by the catalytic mechanism.

As to the thermodynamics of the decomposition of hydrocarbons to carbon and hydrogen, all hydrocarbons except methane and ethane are thermodynamically unstable with respect to decomposition to carbon and hydrogen at temperatures above 100°C, the free energy of formation of methane and ethane from the elements becoming positive at about 550°C and 200°C, respectively (free energy of the decomposition reactions is negative).

The olefins in the Fischer-Tropsch products are formed by a primary process and not by a subsequent dehydrogenation of paraffins. Similarly, straight chain paraffins cannot be produced in sizable amounts by isomerization of branched paraffins, nor α -olefins from their isomers. The

presence of chiefly straight chain paraffins and large amounts of α -olefins indicate that these reaction products are formed by primary processes and not by isomerization (58). Hydrogenation of olefins and the hydrocracking of saturated hydrocarbons are thermodynamically possible at all synthesis temperatures.

B. Development of Fischer-Tropsch Catalysts

In the Fischer-Tropsch synthesis, carbon monoxide is hydrogenated in the presence of a catalyst of which the chief constituents are nickel, cobalt, iron, or ruthenium. The optimum temperature ranges are 170° - 205°C for nickel and cobalt, 200° - 325°C for iron, and 160° -225°C for ruthenium. Nickel catalysts are best operated at atmospheric pressure, and cannot be successfully used at pressures much above this because of excessive corrosion due to nickel carbonyl formation. Cobalt and iron catalysts may be used up to about 20 atms. before appreciable corrosion by carbonyl formation occurs. Ruthenium is only slightly active at atmospheric pressure; 100 atm. pressure is necessary for a space-time-yield of the order of that obtained for other catalysts at pressures in the range of 1 - 20 atm. All catalysts yield detectable amounts of metal carbonyls at optimum reaction temperatures and pressures. Pichler (59) thinks that this fact is important for discussions of the probable mechanism of the reaction.

In a highly exothermic reaction like Fischer-Tropsch synthesis, the heat produced by a dense, unsupported catalyst may exceed the capacity of the reactor to remove the heat of reaction, and the catalysts

may be quickly destroyed. The first satisfactory catalyst used in the Ruhrchemie pilot plant units contained approximately 100 Co:18 ThO₂: 200 kieselguhr:2 Cu, parts by weight. The advantage of incorporating a small amount of copper into the catalyst was that it made possible the use of a low reduction temperature. The standard, or normal, commercial catalyst in 1938 had the approximate composition 100 Co:15 ThO₂: 200 kieselguhr. Although the cobalt catalyst was used in the German plants because its development occurred earlier than that of the iron catalysts, research on iron catalysts in the laboratory and pilot plants was continuously being done and is still in progress. Important advantages of iron over cobalt are that first, it imparts flexibility to the Fischer-Tropsch process, and second, it is easily available from many sources, whereas the supply of cobalt is limited.

Significant improvement in catalyst activity and the life of iron catalysts was produced by the addition of alkali. The effect of alkali in producing higher molecular weight products had been observed when the synthol process was discovered. The same effect was observed in the Fischer-Tropsch synthesis on alkalized iron catalysts. With increasing content of the catalyst, a large yield of paraffin wax is possible. However, catalysts containing large amounts of alkali were short lived. The optimum alkali content, with respect to the life of the catalyst, was observed by U.S. Bureau of Mines workers to be 0.6 part of K₂O per 100 part of Fe. Since a higher surface basicity correlates with a lower methane selectivity in the Fischer-Tropsch synthesis, impregnating the catalyst with alkali after fusion rather than addition of the alkali

at the fusion stage of preparation, results in the catalysts with higher surface basicity and consequently lower methane selectivities. Silica decreases surface basicity and results in an increased methane production (60). It has been demonstrated (61) that the effectiveness of the alkali promoter decreases in the order Rb, Na, Li. High pressure pilot plant synthesis tests shown that promotion with CaO or Li_20 is quite ineffective in comparison to promotion with K_20 . Clearly then a strongly basic alkaline promoter is required. All the catalysts used in the synthesis of higher hydrocarbons from hydrogen and carbon monoxide are very readily poisoned by sulfur. As all sulfur or other sulfur containing compounds must be removed from the catalysts.

The second important advance in the development of Fischer-Tropsch iron catalysts was the discovery of the medium pressure synthesis. The development of pretreatment techniques was the third significant advance in the evolution of iron catalysts. It was preferable to pretreat fused, sintered, and "cemented" (iron oxide particles cemented into granules by the addition of a binding agent) catalysts by reduction with hydrogen at 450°C.

C. Temperature and Pressure Coefficients of Rate of Synthesis

The temperature dependence of the rate of the synthesis is approximately the same for all catalysts, the activation energy being in the range of 20 - 25 kilocalories per mole of carbon monoxide reacted. For the synthesis on Ni and Co, a rate equation that adequately represents the data (62) has not yet been found. For the synthesis on iron catalysts

the equation

$$r = rate = \frac{K P_{H_2}}{a P_{H_20}}$$

$$1 + \frac{P_{H_20}}{P_{C0}}$$

adequately represents the data for wide ranges of conversion and gas composition, where K and a are constants, P_{H_2} , P_{H_20} , P_{C0} are partial pressures of hydrogen, water vapor, and carbon monoxide, respectively. Durability of cobalt and iron catalysts is much greater at 7 - 20 atmospheres pressure than at atmospheric pressure. The marked increase in durability at immediate pressures of 7 -20 atm. probably is due to the formation of liquid oil films of relatively low molecular weight hydrocarbons on the catalyst surface. Such liquid oil would dissolve wax and high boiling oxygenated compounds from the pores of the catalyst granules and thereby provide a continuous extraction and condequent regeneration of the catalyst activity (63).

D. <u>Selectivity</u> - Determining Factors of the Synthesis

As the pressure was increased above atmospheric, the total yield of hydrocarbon at first increased and then (at about 15 atm.) decreased. The rapid decrease in yield of hydrocarbons for pressures above about 16 atm. was probably due to corrosive of active catalyst surface by carbonyl formation and by strong adsorption of the oxygenated organic compounds which were produced in larger amounts at the higher pressures. At an operating pressure of 1 atm. and at about 230°C, the
products from iron catalysts contains much more wax than that from cobalt catalysts (64).

The carbon number and isomer distribution in the products of the synthesis can be calculated with fair accuracy from certain assumptions as to the mechanism of the growth of carbon chains. From simple kinetic pictures of chain growth, there were stepwise additions of one carbon atom (65) at one end or adjacent to end carbon atom of the growing chain. Addition was not postulated to occur on any carbon atom that would result in producing structures containing ethyl branches or quaternary carbons because such compounds were not found in early analysis of Fischer-Tropsch products. The equation for calculation of isomer distribution is $\phi_n = k F_n a^{n-2}$, where ϕ_n is the number of moles containing n carbon atoms, k is a constant, and F_n is a function of f, a constant representing the ratio of chain branch b, over chain lengthening a. If b = af, the lengthening and branching of chains is illustrated as follows:



The agreement between the calculated and the experimental values of isomer distribution is the hydrocarbon fraction of the products from the fluidized iron-catalyst when f = 0.115 is satisfactory in view of

the simplicity of the assumption made.

E. A Possible Mechanism of the Synthesis

Storch, Golumbic, and Anderson (66) suggested that groups such as A and B:



are the intermediates on the catalyst surface (M). Addition at terminal carbon atoms may occur as follows:



The chain growth may be ended by desorption of aldehyde after hydrogenation to yield alcohols, or after dehydration to yield olefins which may then be hydrogenated to paraffins. The experiments of Eidus (67) by using radiochemical studies found that in the Fischer-Tropsch synthesis on iron catalysts the CO and H_2 form primary complexes similar to the absorbed complexes of primary alcohols.

From the recent review (67) it was concluded that in the preparation of hydrocarbons from CO and H_2 , the latter form a primary complex (the C_1 complex) on the catalyst surface, and that the growth of the carbon chain begins from this complex. Chain growth involves the successive attachment of structural units containing one carbon atom to the primary complex. Experiments with the addition of a small quantity of compounds labelled with $^{14}\mathrm{C}$ to the CO - $\mathrm{H_2}$ mixture show that these compounds can be divided into two groups. The reaction with compounds of the first group gives liquid products whose molar radioactivity increases regularly with increase in the carbon number of the fraction. These compounds include formaldehyde, methanol, methyl-formate labelled in the C=O group. The reaction with gas (CO + H_2) containing compounds of the second group gives liquid products whose molar radioactivity remains constant with change in the carbon number. The compounds include: ethanol propanol, acetaldehyde, propanol, ethylene, propene labelled in the CH_2 group. These apparently take part in the formation of a primary complex which is analogous to or identical with the ${\rm C}_{\rm l}$ complex either as the complete molecule or as a part of the molecule containing the labelled ¹⁴C atom.

Substances of the first group undergo preliminary decomposition with the formation of CO, which reacted further taking part in chain growth. When added in larger quantities, ethylene may be included in the chain during its growth. This shows that a structural unit with two or more carbon atoms may also take part in chain growth. In the same way, the primary complex may contain not merely one, but also two or more carbon atoms. The initiation of the chains by methylene radicals formed by dissociation of keten shows convincingly that the presence of an oxygen containing radical is not essential for chain initiation. This process also takes place with the participation of a hydrocarbon radical. The primary complex apparently may or may not contain oxygen, depending on the conditions; there is then an increase in the probability of the condensation or polymerization scheme for carbon bond formation.

The fact that chain growth under certain conditions takes place exculsively according to the polymerization scheme and not by condensation with the liberation of water is shown by new data, according to which the hydropolymerization of ethylene under the influence of CO takes place under certain conditions with almost no consumption of the latter, and hence without the liberation of water.

It has been shown that degradation processes play an important part in the formation of carbon chains.

F. The Future Application of the Fischer-Tropsch Process

Data on the composition of natural substances are important in the study of possible interrelationships. A similarity exists between the low molecular weight alkane isomers in crude oil and Fischer-Tropsch catalytic synthesis products (68). There is a possible significance for the origin of petroleum (instead of organic origin in nature, then degraded by thermal and bacteria processes). In recent years Fischer-Tropsch processes have been developed (69) in some countries to convert

coal to a light crude distillate in an ebullated bed of catalyst. The distillate produced can be converted to gasoline by conventional refinery operations. Economics is used to justify conversion of bituminous and subbituminous coal to a light crude distillate by the Fischer-Tropsch process with subsequent upgrading to gasoline by conventional refinery processes. This process is economically competitive with the conversion of petroleum crude oil to gasoline in a comparably sized grass roots refinery.

G. Possible Fischer-Tropsch-Like Process in the Solar Nebula

The early primitive atmosphere and ocean was hypothesized as a result of planetary outgassing (70). Volatiles from outgassing interacted with the alkaline crust to form an ocean having a pH 8-9 and to produce an atmosphere consisting of CO, N_2 , CO_2 , and H_2 . N_2 and H_2 are in equilibrium with NH₃ at a temperature as low as 200°C, and a pressure up to 1000 atm. Catalysts such as SiO₂, Al₂O₃, Mo, Fe, and Co are also available on the primitive earth. So a Fischer-Tropschlike process among CO, H_2 , and NH₃ seems to be quite possible with or without catalysts. Besides this catalytic thermal synthesis, radiation interacting with such a mixture yields HCN as a principal product (70). It is likely that the abiogenetic formation of some substances of biologic interest synthesized by a Fischer-Tropsch-like process.



IV. EXPERIMENTAL

Insofar as possible, the experiments were designed to approximate conditions in the solar nebula (71). Carbon was included as its highest temperature form CO, nitrogen as NH₃, and hydrogen as H₂, while solid phases of possible catalytic activity were represented by iron nickel alloy powder. To maintain an adequate concentration of carbon, both CO:H_2 ratio and the total pressure had to be kept well above their values in a solar nebula (CO:H₂ $\approx 10^{-3}$ P $\approx 10^{-2}$ -10^{-4} atmospheres). Hence the result of these experiments may not be strictly valid for the solar nebula. The C:N ratio was kept close to the cosmic value, however.

In this work the emphasis was put on accumulating enough samples to identify the purine and pyrimidine derivatives synthesized by using infrared spectrophotometry in order to confirm Hayatsu's report (72); but in these experiments iron nickel alloy powder used instead of iron meteorite powder.

The experimental conditions and the compounds identified in these experiments are summarized in Table II. Fine temperature variation was made possible by using a potentiometer and temperature readings were taken from a calibrating thermometer (the electric furnace used had its lowest temperature reading at 450°C). In a typical run the reaction vessel was rapidly heated to a peak temperature of 630°C, followed by gradual cooling to room temperature (630°C for 1.5 hrs., 310°C for 6 hrs., 200°C for 12 hrs., 120°C for 24 hrs., and 70°C for 12 hrs.). These

TABLE II. REACTION CONDITIONS AND PRODUCTS IDENTIFIED

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Run	Temperature (in Centigrade)	Catalysts	Total Pressure at Room Temperature and Gas Composition	Compounds Identified
]	600° 1 hr. 140° 3 hrs. 410° 8 hrs. 100° 1 hr. 310° 24 hrs. 200° 6 hrs.	Fe-Ni alloy (58-42%) 1.474 g SiO ₂ 0.650 g Al ₂ O ₃ 0.580 g	25 psig CO:NH ₃ :H ₂ = 1:0.4:2 (mole ratio)	adenine urea melamine guanine biuret cyanuric acid cytosine guanylurea
2	600° 1 hr. 280° 6 hrs. 340° 7 hrs. 160° 20 hrs. 300° 20 hrs. 75° 4 hrs.	Fe-Ni alloy (58-42%) 2.140 g	26 psig CO:NH ₃ :H ₂ = 1:0.4:2 (mole ratio)	adenine urea melamine guanine biuret cyanuric acid cytosine guanylurea
3	650° 1.2 hrs. 125° 19 hrs. 308° 9 hrs. 75° 4 hrs. 220° 7 hrs.	SiO ₂ 1.047 g Al ₂ O ₃ 0.916 g	26 psig CO:NH ₃ :H ₂ = 1:0.4:2 (mole ratio)	urea melamine biuret cyanuricacid guanylurea
4	950° 0.5 hrs. 120° 5.5 hrs. 310° 9 hrs. 75° 14 hrs. 210° 28 hrs.	no catalyst	l atm CO:NH ₃ :H ₂ = 1:0.4:2 (mole ratio)	adenine urea melamine biuret cyanuric acid guanylurea
5	450° 1.5 hrs. 105° 7 hrs. 300° 24 hrs. 70° 10 hrs. 220° 15 hrs.	Fe-Ni alloy (58-42%) 1.370 g SiO ₂ 0.56 g Al ₂ O ₃ 0.47 g	26 psig CO:NH ₃ :H ₂ = 1:0.4:2 (mole ratio)	urea melamine biuret cyanuric acid guanylurea
6	Repeat run #1 ten times in order to obtain enough sample for infrared (in KBr) spectrophotometry.			

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temperature variations were intended to simulate conditions in the solar nebula: brief thermal spikes superimposed on a general downward trend (73).

The total pressure of reactant gases varied from 1 atm. to 3 atm. (at room temperature); the temperature varied from room temperature to 950°C. No higher pressure or temperature were tried because of the strength of the reaction vessel. According to Hayatsu's work (72), only sufficiently high temperatures can initiate the synthesis of some biologically significant compounds. Also, only at a temperature of 600°C or higher can HCN be synthesized from a carbon monoxide and ammonia mixture in a considerable yield (74). No isothermal type temperature was tried because, even in that case, the reaction vessel would have to be cooled to room temperature before any analytical work could begin. During the cooling period the temperature was not isothermal.

A. Reaction Conditions and Separating Procedures

The reaction vessel is about 15 inches in length, 1 inch inside diameter quartz tube, having a volume of 244 cc, sealed at one end and joined at the other to a stainless steel control valve (corrosive service type) by a grade seal. The sealed end was inserted to a depth of 4.5 inches in the electric furnace which was heated to the desired temperature. The rest of the vessel was left outside the furnace. The system was not strictly isothermal, however, as one end was kept close to room temperature. In this arrangement, volatile compounds could distill away from the high temperature zone, thus remaining protected to some extent from further reaction or destruction.

The total reaction time varied from 48 hrs. to 72 hrs. At the end of the reaction, the reaction vessel was cooled to room temperature and the inside pressure was in partial vacuum (no leakage occurred during the reaction period. Therefore the reactions were undergone to a considerable extent). After the reaction the color of the catalysts changed from gray to deep black, near the cold end, some liquid formed on the bottom of the reaction vessel, and some white powder deposited on the upper inner surface of the reaction vessel.

All the catalysts (58% - 42% Fe-Ni alloy, Al_2O_3 , SiO_2) used in these experiments were reduced in H_2 flow at 400°C for 2 hrs. then baked out under vacuum in a torch (maximum temperature 1600°C) for two hours.

Since NH_3 was proportionally lowest in the reactant mixture $(C0:NH_3:H_2 = 1:0.4:2)$, it was filled into the evacuated reaction vessel first to the desired partial pressure, followed by the CO and H_2 mixture (1:2 mole ratio) to the desired total pressure. During the reaction or filling reactant gases, leaking of NH_3 was detected when it turned the red litmus paper blue.

Separation and identification procedures used in this work were designed for the nitrogen compounds only. Volatile products (at room temperature, 1 atmosphere) were not analyzed. The separation procedures are presented by the flow chart (In Table III). In a typical run the volatile products were discarded because the nitrogenous compounds in which we are primarily interested, are in solid state at room temperature. The non-volatile products were extracted by a one-hour

treatment with 50 ml of 50% ethanol and 50% distilled water at 60°C (made acidic 0.05 N - 0.1 N by adding required amounts of dilute HCl). After the solvent mixture was evaporated under vacuum at 35°C, a white residue remained. (Fraction A). Four compounds were identified in this fraction by two dimensional paper chromatography: urea, biuret, melamine, and adenine (Table IV and V). Four unidentified spots were also present.

Although the presence of cyanuric acid was suspected in this fraction, we consistently failed to identify it by paper chromatography because it shows no characteristic color reaction. It was finally isolated by a solubility method. Half of fraction A was acetylated with acetic anhydride according to the method developed by Cason (75). Fraction A was refluxed with 75 ml of acetic anhydride for one hour under these conditions; cyanuric acid was not acetylated (melamine formed diacetylmelamine 98% conversion). The resulting slurry was filtered and the residue washed with water three times, dried, and extracted with 5 ml hot methanol (at 60°C). The methanol-insoluble fraction was dissolved in 3 ml of hot water and filtered rapidly. A white powder soon separated from the filtrate. After two recrystallizations from water, a residue of 0.6 mg remained. Cyanuric acid did not have any characteristic absorption peak in 0.1 N HCl, but it had a maximum absorption at 221 m μ in 0.05 M borax solution. Its infrared spectrum . agreed with that of an authentic sample, showing the following absorption features in KBr: 3020 (broad), 2780, 2440, 2100, 1950, 1780-1720 (triplet), 1460, 1400, 1050, and 770, 750 cm⁻¹. Owing to the high

TAB	LE	I	I	Ι
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SEPARATING FLOW CHART



TABLE IV. NON-VOLATILE SYNTHETIC NITROGEN COMPOUNDS: SUMMARY

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Fraction	
A	Urea, biuret, melamine, adenine, cyanuric acid.
С	Urea, biuret, granylurea, adenine (trace).
D	Urea, melamine, guanylurea, adenine, cytosine, guanine.
E	Urea, melamine, adenine, guanine.
E	orea, meramme, adennie, guanne.

TABLE V. IDENTIFICATION OF SYNTHETIC COMPOUNDS AND YIELD (Product from Experiment #1 Repeated 10 Times)

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Compound	Reagent ^(a) and Spectrophotometry	R _f x 100 in two dimensional paper chromatogram ^(b)		Amount	% Yield ^(c)
		HAC:n-butanol H ₂ 0 (lst)	HCl:isopranol H ₂ O (2nd)	(mg)	
Urea Biuret Melamine Guanylurea Adenine Guanine Cytosine Cyanuric Acid	DAB DAB DAB,U.V.,I.R. FCNP U.V.,I.R. U.V.,I.R. U.V.,I.R. I.R.	54(53) 59(55) 28(29) 37(37) 38(35) 28(29) 31(29)	75(73) 82(80) 29(29) 21(20) 40(38) 29(29) 52(48)	0.49 0.21 0.88 0.30 0.47 0.19 0.16 1.20	0.475 0.178 1.220 0.353 0.507 0.158 0.127 0.810
	. TOTAL		- <u></u>	3.90	3.828

(a) DAB = P-dimethylaminobenzaldehyde FCNP = Alkaline ferricyanide-nitroprusside

- (b) Values in parentheses refer to standards. 1st dimension: glacial acetic acid n-butanol-water (20:60:20). 2nd dimension: isoproyl alcohol conc. HCl water (65:16.6:18.4) on Whatman No. 3 MM paper. R_f values for melamine and guanine are the same. These two compounds have been separated in 1.25% phenolic solution (in water) with R_f values of 0.79 and 0.43 respectively. Cyanuric acid is identified by its infrared spectrum alone.
- (c) Gas mixture: $CO/H_2/NH_3 = 1/2/0.4$; 244 ml at 3 atm. Yields are calculated based on conversion of NH_3 .

melting point of cyanuric acid (>360°C), it decomposed before its melting point, and we could not check its purity by a melting point determination. However, from the comparison of the infrared spectra of the standard and sample, the absence of extraneous features in the infrared spectrum suggested a rather high purity.

Three further fractions were obtained from the insoluble residue remaining after removal of fraction A with slightly acidic 50% ethanol. The residue, consisting largely of catalysts and free carbon, was extracted with 10 ml concentrated HCl at 0°C for 15 minutes. The solution was diluted with 20 ml H_{2} 0, filtered, and evaporated to dryness under vacuum below 30°C. The pale greenish yellow residue was dissolved in 40 ml of a 5:1 mixture of chloroform and methanol at 40°C, and the opaque solution was passed through a 20 x 0.8 cm alumina column pretreated with 1 N HCl. Fraction C, eluted with 80 ml $CHCl_3$ - CH_3OH (5:1); after evaporation the solvents contained adenine, urea, biuret, and guanylurea, along with 3 unidentified spots. Fraction D, eluted with 80 ml of 4:1 mixture of $CHCl_3$ - CH_3OH , gave an especially large variety of compounds: adenine, guanine, cytosine, melamine, urea, guanylurea, and 6 unidentified spots on the paper chromatogram. The last fraction E, was eluted with 100 ml of a 2.5:1 mixture of CHCl₃: It contained adenine, melamine, guanine, urea, and 3 unidenti-CH₂OH. fied spots.

In separating and identifying the products, the column step is necessary to remove $FeCl_2$, $FeCl_3$, and $NiCl_2$, derived from the reactions of catalysts with concentrated HCl during extraction. These metallic

chlorides can cause misidentification on paper chromatograms. All three compounds showed up as dark spots under short wave length ultraviolet light ($\lambda = 253.7 \text{ m}\mu$) and the first also gave blue spots with alkaline ferricyanide-nitroprusside. Their R_f values are similar: 0.31 - 0.39 in first dimension, n-butyl alcohol-acetic acid-water (60:20:20), and 0.72 in second dimension, isopropyl alcohol - concentrated HCl-water (65:16.6:18.4). However, they were strongly adsorbed by acid-treated alumina and were not eluted even with a methanol-rich solvent (CHCl₃ - CH₃OH 1:1). The column procedure used here was based on preliminary tests with authentic samples.

The preparation of an alumina column (Al_2O_3) is basic in nature) (76), consisted of first, putting Al_2O_3 powder in three to four times the amount of carbon dioxide-free water, stirring the solution for a few minutes and then letting the milky solution stand until the Al_2O_3 precipitated. The solution was carefully decanted. This washing was repeated two times. The alumina was then treated with three to four times the amounts of 1 N HCl, stirring thoroughly. After decanting the 1 N HCl solution, the Al_2O_3 was washed with carbon dioxide-free water several times until the washing was slightly acidic to the blue litmus paper.

In packing the column with "acidic Al_2O_3 ", Al_2O_3 was used in slurry form and a weak suction was applied on the other end of the column. When the Al_2O_3 column had reached the desired height (20 cm), the suction was increased until the whole column was nearly dried.

B. Identification of Compounds

Paper chromatography was the only technique used to separate the products. Both uni-dimensional and two dimensional paper chromatography were tried. One dimensional paper chromatography was unable to separate these compounds in good resolution. Even in two dimensional paper chromatography guanine and melamine were not separated in the solvent mixtures used. (First dimension: glacial acetic acid, n-butanol, water [20:60:20]; second dimension: concentrated HCl, isopropanol, H_20 [16.6:65:18.4] on Whatman #3 MM paper, $8\frac{1}{2}$ " x $8\frac{1}{2}$ " in size. Both dimensions took about 10 hrs. and 23 hrs., respectively to be developed.) One specific direction of paper was always used for one dimension because different directions of paper would affect the R_f values remarkably. Before developing paper chromatograms, solvents were put in a closed chamber for at least 24 hrs. in advance to let the vapor phase saturate.

After the paper was developed in two dimensions and dried, ultraviolet light sensitive compounds were detected when irradiated by short wave length ($\lambda = 253.7 \text{ m}\mu$) ultraviolet light. The ultravioletabsorbing spots were circled in pencil. The R_f values of synthesis products were compared with the authentic standards in Figure 1,2 (one dimensional) and Figure 3,4 (two dimensional).

An ultraviolet-absorbing spot had R_f values (0.28, 0.29) quite close to the standard guanine, but its ultraviolet spectrum (in 0.1 N HCl) showed absorption maximum at 275 mµ. It also showed another absorption peak at 238 mµ. This indicated that the spot contained more than one component. Then, from the comparison of one dimensional

ONE DIMENSIONAL PAPER CHROMATOGRAM OF EXP'T.-48 (Glacial acetic acid: n-butanol;water, 20:60:20)

FR-E (Fraction-E)	FR-D (Fraction-D)	FR-C (Fraction-C)	FR-A (Fraction-A)
1,2,3,4	1,2,4	1,2,3,4	1. melamine
unidentified	unidentified	unidentified	2. unidentified
5. adenine	3. guanine and , melamine	5. adenine	 unidentified (violet color)
	5. cytosine		4. adenine

STD (Standards)

- A Adenine
- C Cytosine
- G Guanine



ONE DIMENSIONAL PAPER CHROMATOGRAM OF EXP'T.-48

(Isopropanol: Conc. HCl: H₂0, 65:16.6:18.4)

FR-A FR-E FR-D FR-C (Fraction-E) (Fraction-D) (Fraction-C) (Fraction-A) 1,3 1,2 (both in 1. melamine and 1. unidentpale green) ified guanine unidentified 2. adenine 3,4 (colorless) 2. adenine 2. adenine but all unident-3. Cytosine ified 4,5,6. unidentified

> STD (Standards) A - adenine C - Cytosine G - guanine



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TWO DIMENSIONAL PAPER CHROMATOGRAMS

(lst dimension: n-butanol: Acetic Acid: H₂0 60:20:20, vertical direction)

(2nd dimension: Isopropanol: conc. HCl: H₂O 65:16.6:18.4, horizontal direction)

Upper Figure: TWO DIMENSIONAL PAPER CHROMATOGRAM OF STANDARDS.

- 1. guanylurea
- 2. guanine + melamine
- 3. adenine
- 4. Cytosine
- 5. urea
- 6. biuret

Lower Figure: TWO DIMENSIONAL PAPER CHROMATOGRAM OF EXP'T.-48 FRACTION-D

- 1. guanylurea
- 2. guanine + melamine
- 3. Adenine
- 4. Cytosine
- 5. urea
- 6,7,8,9,10,11.

unidentified





TWO DIMENSIONAL PAPER CHROMATOGRAMS

(lst dimension: n-butanol: Acetic acid: Water, 60:20:20, Vertical direction)

(2nd dimension: Isopropanol: Conc. HCl: Water, 65:16.6:18.4, Horizontal direction)

Upper Figure: TWO DIMENSIONAL PAPER CHROMATOGRAM OF EXP'1.-48 FRACTION-A

- 1. Adenine
- 2. urea
- 3. biuret
- 4,5,6,7.

unidentified

Lower Figure: TWO DIMENSIONAL PAPER CHROMATOGRAM OF EXP'T.-48 FRACTION-E

- 1. adenine
- 2. guanine and melamine

3. urea

4,5,6.

unidentified



paper chromatograms in two solvent mixtures (used in two dimensional paper chromatography), both guanine and melamine had the same R_f values in both dimensions. So, we tried to find a solvent mixture to separate these two components into single spots after extraction from the two dimensional paper chromatogram. A 1.25% phenolic (in water) solution could separate guanine and melamine in one dimension chromatogram with R_f values of 0.43 and 0.79, respectively.

Every ultraviolet-absorbing spot was cut off and extracted in 0.1 N HCl for at least one day (each spot paper had been torn into very small pieces). The paper chromatograms were then used for the spraying of DAB solution (p-Dimethylaminobenzaldehyde), or FCNP solution (Alkaline ferricyanide-nitroprusside), to detect the presence of nonultraviolet-absorbing products.

DAB solution: (Erhlich reagent) Two grams of p-dimethylaminobenzaldehyde were dissolved in 100 ml of 1.2 N hydrochloric acid. This reagent could be kept for three to five days in a cold place without deterioration. Urea, biuret, and melamine gave yellow color spots immediately after spraying (77). The quantities of biuret and urea synthesized were determined by extracting the yellow paper chromatogram spots with pyridine and measuring them spectrophotometrically at 449 mµ.

FCNP: (Alkaline ferricyanide-nitroprusside) (77) Equal volumes of 10% sodium hydroxide, 10% sodium nitroprusside, and 10% potassium ferricyanide were mixed and diluted with three volumes of distilled water and allowed to stand for 20 minutes. The dark brown solution turned pale yellow and the reagent was ready for use. This reagent was unstable

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at room temperature, but could be kept for two or three weeks in the cold without deterioration. Guanylurea gave a very strong purple spot immediately after spraying with the reagent.

All solvents and reagents for spraying paper chromatograms were analytical reagent grade.

 $R_{\rm f}$ values of paper chromatography are not very positive identification for an organic compound. For example, guanine and melamine (both authentic standards) can have the same R_{f} values in two dimensional paper chromatography (glacial acetic acid - n-butyl alcohol - water [20:60:20] for first dimension; isopropanol - concentrated HCl water [65:16.6:18.4] for second dimension on Whatman No. 3 mm). Therefore, further spectroscopic evidence (for example, mass spectrum, infrared, or ultraviolet spectrum) seemed to be not only desirable but necessary in order to confirm the presence of these biologically significant compounds. We failed to find references in literature about the mass spectrometry of adenine, guanine, and cytosine, (probably because of their high melting points, 365°C, 360°C, 325°C, respectively. All these bases decomposed at their m.p., although adenine could sublime at 220°C, 1 mm Hg) or their derivatives (acyl, picryl, trifluoroacetyl, etc.). These derivatives decomposed at their respective melting temperatures, too.

Only infrared and ultraviolet spectroscopic work was done. Each ultraviolet-absorping spot and a spot from the blank paper chromatogram (paper developed in the same solvent mixtures, both spots having the same size, were cut off and extracted in two test tubes with 0.1 N HCl for at least one day. The paper spot was torn into pieces in 0.1 N HC1. In all ultraviolet-absorbing spots only four were identified: adenine, guanine, cytosine, and melamine. Their ultraviolet spectra in comparison with authentic standard spectra are shown in Figures 5, 6, 7, 8. No ammeline was detected in any of these experiments.

If the ultraviolet spectrum showed the characteristic absorption and without too much impurity absorption, the sample was accumulated in order to obtain enough to make a KBr pellet. We considered the infrared spectrum as the most positive and final confirmation of the products.

Based on the Sadtler standard infrared spectra, free adenine, adenine monochloride, and adenine di-hydrochloride, all were quite different. Therefore, it was desirable to remove HCl from these bases before making pellets. Concentrated ammonium hydroxide was added to the sample solution (in 0.1 N HCl), until the solution was slightly basic. Due to the relatively low solubilities of these nitrogen compounds in alkaline solutions, adenine, guanine, melamine, cytosine could be precipitated out by centrifuging (NH_4Cl dissolved in the solvent). The samples were then washed twice with distilled water. After obtaining enough sample, these samples and the spectroscopic grade KBr were dried in the oven for 24 hrs. at 100°C. A pellet was made out of well-ground KBr and sample under a pressure of 20,000 psi for two minutes. The infrared spectrum was measured for each sample, and then compared with a standard free base spectrum (Figures 9,10,11,12,13). Cyanuric acid was detected and identified by its infrared spectra (Figure 13) only because it did not show color reaction with any reagent used in these experiments

ULTRAVIOLET SPECTRA OF ADENINE AND UNKNOWN IN

0.1 N HC1



ULTRAVIOLET SPECTRA OF CYTOSINE AND UNKNOWN IN

0.1 N HC1



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ULTRAVIOLET SPECTRA OF GUANINE AND UNKNOWN IN

0.1 N HC1



ULTRAVIOLET SPECTRA OF MELAMINE AND UNKNOWN IN

0.1 N HC1


INFRARED SPECTRA OF STANDARD ADENINE AND UNKNOWN (Both in KBr)

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Upper Spectrum: Standard Adenine

Lower Spectrum: Unknown





WAVELENGTH IN MICRONS

INFRARED SPECTRA OF STANDARD CYTOSINE AND UNKNOWN (Both in KBr)

Upper Spectrum: Standard Cytosine

Lower Spectrum: Unknown



WAVELENGTH IN MICRONS

PERCENT TRANSMITTANCE

INFRARED SPECTRA OF STANDARD GUANINE AND UNKNOWN (Both in KBr)

Upper Spectrum: Standard guanine

Lower Spectrum: Unknown



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INFRARED SPECTRA OF STANDARD MELAMINE AND UNKNOWN (Both in KBr)

Upper Spectrum: Standard melamine

Lower Spectrum: Unknown

PERCENT TRANSMITTANCE



WAVELENGTH IN MICRONS

INFRARED SPECTRA OF STANDARD CYANURIC ACID AND UNKNOWN (Both in KBr)

Upper Spectrum: Standard cyanuric acid

Lower Spectrum: Unknown



TRANSMITTANCE ERCENT

WAVENUMBER CM⁻¹

on the paper chromatograms. Only the qualitative work was done by infrared spectrophotometry. The quantitative measurements of adenine, guanine, cytosine, and melamine were done by ultraviolet violet light spectrophotometry. Comparisons of the absorption coefficients at their characteristic peaks were always used.

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DISCUSSION

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V. DISCUSSION

A. <u>Significance of Results</u>

It is remarkable that biologically important compounds (adenine, guanine, cytosine) are formed among the synthesis products. Adenine, a nucleotide base of DNA and RNA, is made in about the same yield as urea, a compound of lesser importance, simpler structure, for longer history of abiotic synthesis (4), and is not biologically important.

What lends particular significance to the present synthesis is the fact that it was achieved under the same conditions which seem to be required for the meteoritic hydrocarbon reaction: carbon monoxide and hydrogen in the presence of iron meteorite, iron or nickel powder catalysts (71,78). Now it has been shown that the same synthesis in the presence of ammonia also produces the nitrogen compounds found in meteorites (except ammeline): adenine, guanine, melamine, ammeline, guanylurea.

A few remarks concerning energetics of abiotic processes may be in order. The products sought in abiotic syntheses (purines, pyrimidines, amino acids, etc.) are thermodynamically unstable with respect to the presumed constituents of a "primitive atmosphere": CH_4 , NH_3 , H_2O . For example, the equilibrium constant for the formation of adenine from CH_4 and NH_3 is vanishingly small at 298°K (room temperature) and 1 atmosphere. [Free energy data were taken from Dayhoff <u>et al.</u> (79)].

$$CH_4 + NH_3 \rightarrow 1/5 C_5H_5N_5 (g) + 3H_2 K_{298} = 5x10^{-29}$$

In the Miller-Urey synthesis, this difficulty was circumvented by infusion of energy from an outside source (ultraviolet light, electric discharges, etc.), which produces highly unstable free radicals and molecules. These can react spontaneously to give amino acids, etc.

An alternative approach is to use reactions with a more favorable free energy change, involving reactive molecules [CO, HCN, (CN)₂] as the starting material. These molecules are thermodynamically stable at high temperatures, but become increasingly unstable at lower temperatures. They can thus transform spontaneously to a host of biologically important compounds if a suitable reaction pathway is available. For example, the equilibrium constant for the formation of adenine from carbon monoxide and ammonia is quite appreciable:

$$CO + NH_3 \rightarrow 1/5 C_5 H_5 N_5 (g) + H_2 O (g) K_{298} = 6 \times 10^{-4}$$

This explains why synthesis of adenine from CO and NH_3 proceeds spontaneously (7), while synthesis from CH_4 and NH_3 requires an external energy source (32,80).

Exoergic reaction with such reactive molecules has often been called "thermal" with the implication that "thermal energy" somehow supplies the driving forces. However, this term is misleading. The driving force is the inherent instability of these molecules at low temperatures, which permits them to transform to a wide variety of organic compounds with a net decrease in free energy of the system. The principal way in

which "thermal energy" enters into the picture is in speeding up reaction rates. Thus it would seem best to call such reactions "spontaneous" rather than "thermal." "Quasiequilibrium," "metastable," or "limited equilibrium" are alternative names (79,71,81).

One may rightly ask whether it is realistic to postulate reactive molecules as the starting materials. Actually, these molecules can be produced in large amounts under a variety of natural conditions. In the solar nebula, CO rather than CH_4 is the dominant form of carbon down to 1000°K if the gas is of solar composition, and to lower temperatures if it is depleted in hydrogen. On the primitive earth, CO can be produced by the equilibrium:

 $C + CO_2 \stackrel{2}{\leftarrow} 2 CO$

and

$$CO_2 + H_2 \neq CO + H_2O$$

which proceed to the right at high temperatues. Large amounts of CO can thus be brought into the primitive atmosphere by degassing of the crust and mantle (70). Furthermore, CO would be generated in the impact of carbon-bearing planetesimals or comets, as suggested by Studier et al. (71).

If CO and NH_3 are available, HCN can be produced spontaneously. This reaction is thermodynamically feasible and proceeds with halftimes of a few seconds between 500° and 700°C (82). Other forms of

nitrogen also had been tried. To obtain an indication of the kinds of chemicals that might be synthesized, T. C. Hoering and P. H. Abelson had conducted experiments under ultraviolet irradiation (70). Starting mixtures with pressures measured in centimeters of Hg were (1) N_2 , 8; CO, 8; H_2 , 2; (2) N_2 , 4; CO, 4; H_2 , 4; (3) N_2 , 2; CO, 4; C_2 , 6; (4) N_2 , 2; CO, 4; H_2 , 24. The principal products formed in the last three mixtures were HCN and H_2O . Small amounts of CH_4 and CO_2 were also made. In the first mixture, CO_2 was the major product, with HCN and H_2O second. The products were analyzed by T. C. Hoering in a mass spectrometer. Other products such as formaldehyde, nitriles, acids, and hydrocarbons (except CH_4) were not detected, which meant that if made, they were present in amounts no more than about 10^{-3} those of HCN. When hydrogen was not present, the principal products from N_2 + CO or CO alone were CO_2 and C_3O_2 . In summary, irradiation of a variety of CO, $\rm H_2, \ N_2$ produces HCN as the major product and little else except $\rm C_3O_2,$ $\mathrm{CO}_2,$ and $\mathrm{H}_2\mathrm{O}.$ Since HCN is a key intermediate for many nitrogen compounds of biological interest, it would seem that spontaneous chemical reaction may have produced an important share of the prebiotic organic matter on the earth.

B. Possible Reaction Mechanisms

The mechanisms of reactions were proposed, but no intermediate was isolated to confirm the mechanisms because of the low yields of reactions. The formation of adenine presumably took place by Oro's mechanism (39), involving the polymerization of HCN in aqueous ammonia solution. Oro and Kimball (36,38) had synthesized adenine in this manner (Figure 14) using 1 to 15 M NH₄CN solutions in NH₄OH. A similar reaction was also observed to take place in one of Hayatsu's experiments (34), under rather less restrictive conditions. An aliquot of the volatile fraction, consisting of HCN, $(CN)_2$, NH₃, CO₂ and light hydrocarbons were taken off at 500°C and condensed at -190°C. It was redistilled at 5°C, recondensed, and passed through 10 ml of H₂O at 10°C. The solution was then heated to 40°C overnight. Adenine was detected by paper chromatography after hydrolysis in 3 N HCl at 40° - 50°C.

Formation of adenine from NH_4CN goes smoothly in the laboratory. However, Sanchez <u>et al.</u> (83) have pointed out a difficulty that might arise in nature. They note that polymerization requires HCN concentration of at least 0.1 M; at lower concentrations, hydrolysis to formic acid predominates. Given the reactivity of HCN, steady-state concentrations as high as 0.1 M are not likely to arise in the large water masses. Sanchez <u>et al</u>. (83) therefore propose that the required high concentrations were achieved by partial freezing of more dilute HCN solutions.

The present experiments suggest that this difficulty might be circumvented in an alternative manner by keeping most of the water in a vapor phase so that the small amount of liquid phase would be disproportionately enriched in HCN. At the lowest temperature reached in these experiments (75°C), the vapor pressure of water (289 torr) was close to its partial pressure (<500 torr), so that water could condense only a

PATHWAY FOR DIRECT ADENINE SYNTHESIS

- AMN aminomalononitrile
- AMNSA aminomalononitrile semiamidine
- AICN 4-amino-5-imidazolecarbonitrile
- AICAI 4-amino-5-imidazolecarboxamidine
- FAICAI 4-formamidino-5-imidazolecarboxamidine



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PATHWAY FOR DIRECT ADENINE SYNTHESIS

limited extent, mainly in the cold part of the vessel adjoining the control valve. Such partial condensation of liquid water is a realistic possibility for the primitive earth. For the solar nebula, rather specialized conditions would be required, e.g. a partial H₂O pressure of several torr. Such conditions would be met only in the denser or more highly fractionated parts of the nebula.

The formation of guanine might be through condensation of guanidine and 4-amino-5-imidazole-carboxamide (AICA, one of the isolated intermediates in the Oro adenine synthesis mechanism) in aqueous ammonia solution (reaction shown in Figure 15). Cytosine could be synthesized in the condensation between β -amino-acrylamidine and urea (or guanidine) by losing 2 moles of ammonia. It is not clear whether the guanylurea found in the meteorite was initially present as such or was formed from a labile precursor during the hydrolysis procedure. Cyanoguanidine might be such a precursor. It is easily converted to melamine above 205°C, and gives guanylurea on hydrolysis in dilute acid. However, melamine may have been synthesized directly. Two pathways are known: a direct one from CO and NH₃ or HCN and NH₃, and an indirect one from CO₂ and NH₃ via urea and cyanuric acid (84,85,86). Possible reaction mechanisms shown in Figure 15.)

POSSIBLE REACTION MECHANISMS

POSSIBLE REACTION MECHANISMS

(a) Urea and Biuret

$$5 \text{ CO}_{2} + 10 \text{ NH}_{3} \xrightarrow{\text{heat}} 5 \text{ NH}_{4} \text{NH}_{2} \text{CO}_{2} \xrightarrow{\text{heat}} 5 \text{ (NH}_{2})_{2} \text{CO} \xrightarrow{\text{heat}} 4 \text{NH}_{3}$$

$$+ \text{NH}_{2} \text{CONHCONH}_{2} + \underbrace{\text{NHCONHCONHCO}}_{\text{biuret}} (1)$$

$$= \begin{array}{c} \text{biuret} \\ \text{acid} \end{array}$$

 $(NH_2)_2C=NH + NH_2COCN=CHNHC(NH_2)=C -2NH_3 N=C(NH_2)NHCOC=CN=CHNH$ guanidine AICA guanine(4-amino-5-imidazole-carboxamide) (2)

(d) Melamine

FIGURE 15 CONTINUED

(8)

VI.

CONCLUSION

VI. CONCLUSION

In this work, a Fischer-Tropsch-like process, under the conditions which possibly existed in the solar nebula, has been used to synthesize some purines and pyrimidine derivatives. All compounds confirmed in this work are positively identified by paper chromatography, ultraviolet and infrared spectrophotometry, the agreement between the standards and unknowns in U. V. and I. R. spectra is excellent. From these spectroscopic evidences and R_f values of two dimensional paper chromatograms, the presence of adenine, guanine, cytosine, melamine, cyanuric acid, urea, biuret, guanylurea are positively demonstrated.

In an attempt to confirm Hayatsu's report, the emphasis was put on getting more evidence (preferably spectroscopic evidence) to identify the synthetic compounds. We were able to accumulate enough sample to identify these compounds by infrared spectrophotometry (in KBr).

The experimental conditions were designed to approximate the conditions in solar nebula. A rapid temperature increase to a high temperature and then followed by a gradual temperature drop were intended to simulate conditions in the solar nebula: brief thermal spikes superimposed on a general downward trend. Iron-nickel alloy powder (sometimes combined with alumina and silica gel) was used as catalysts, this catalyst had been demonstrated by Gelpi and Hayatsu that it had the same catalytic effect on the synthesis of hydrocarbons as iron meteorite powder. Again from this work, it can be concluded the iron-nickel alloy and meteorite powder having the same catalytic effect on the synthesis of nitrogen compounds too. All compounds (except ammeline and cytosine) synthesized in this Fischer-Tropschlike process were also present in the meteorites. They are: adenine, guanine, melamine, ammeline, and guanylurea (no cytosine was detected in meteorites, but no ammeline was identified in this work).

From the agreement of this result and the nitrogen compounds present in meteorites, it is reasonable to believe that the formation of these biologically important nitrogen compounds in meteorites probably was through a Fischer-Tropsch-like process. A similar synthetic process may have occurred on the primitive Earth.

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