Self Organization Of Ammonium Thiocyanate Under Ultraviolet Irradiation

by

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ABSTRACT

The results reported from many laboratories over the past decade make it clear that abiotic synthesis of many compounds could have been formed under conditions believed to have existed on the primitive earth.

It was proposed and experimentally demonstrated here that ammonium thiocyanate may have played a role in the types of condensations occuring on the primitive earth. In dilute, aqueous solution, ammonium thiocyanate is able to promote the synthesis of a variety of amino-acids and to form microspheres having cell-like structure and organization property. The effect of different parameters such as UV irradiation, time and Ph. variation were investigated. The integrity of the microspheres was tested under different media. A morphological investigation was also carried out, both with light and electron microscopes. Some striking resemblances between our microspheres and Precambrian microfossils led us to consider the possibility of an abiotic origin of those microfossils. It was also shown tha+, under any kind of atmosphere (reducing or oxidizing) and with no specific energy, the appearance of protocells was considered an inevitable event on the primitive earth. Because catalytic activity is one of the primordial properties of a cell, we tried to search for "ATPase"-like activity from our microspheres.

As experimental evidence indicated that the evolution of macromolecules (Proto-cells) from C,O,H,N seems to be an inevitable event; we have examined the central feature of this process, the emergence of higher levels of order. The ammonium thiocyanate system was shown to deviate from randomness in chemical composition and morphology.

Following the idea of emergence of order, we come closer to the living state since behavior may be considered as an imbalance in a system of chemical reactions.

INTRODUCTION:

No question has aroused the curiosity of man as long a time as the primeval source of the persistent, localized, chemical reactions which are now recognized as living organisms. It is only very recently, however, that this problem can be approached by scientific methods and the advanced experimental techniques. As Bernal pointed out in 1949, the subject is so wide that it would be good - for simply formulating the problem - to be at the same time physicist, organic chemist, to have thorough knowledge of geology, geophysics, geochemistry and to be perfectly familiar with all biological disciplines.

However there is a growing appreciation that the evolution of highly ordered systems from a chaos or cosmos poses an important fundamental problem in its own right, independent of the physical or chemical representation of the systems.(1) The general postulates of the biological theory of evolution, which in effect define what we mean by living matter (Crick 1961)(2) have been reduced to the molecular level.

Many experimental results show that hypothetical primitive earth environments can produce most classes of molecules which are essential for present living organisms. Starting from the simplest molecules, such as water, carbon dioxide and ammonia, it is possible to produce abiogenetically, many of the essential biochemicals from common, non-specific energy sources in a matter of hours or minutes. Most of these experiments are variations and extentions of experiments first conceived by Calvin (Garrison et al 1951)(3) and Urey (Miller 1955)(4). The phrase "chemical evolution" is often used to describe this level of reaction in contrast to biological evolution. (Calvin 1956, Blum 1961)(5-6)

It is now a reasonable hypothesis that volcanic regions of the primitive earth produced significant quantities of high molecular weight heteropolyaminoacids from simple gases.(7) Some of these abiogenic polymers easily organize themselves into structured spheres which occasionally cleave or aggregate into more complex forms.(8)

It is our purpose to propose and demonstrate that ammonium thiocyanate may have played a role in the occurrence of organic compounds needed for the origin of living systems. It is believed that this simple starting material could have been found in volcanoes.(9)

HISTORICAL REVIEW AND THEORETICAL CONSIDERATIONS

Origin of the Earth

How can we be sure the earth has an origin? One indication that the earth cannot be indefinitely old comes from the analysis of the process of energy production in the sun and stars: radiation from the sun originates in nuclear reactions by which hydrogen is converted into heavier elements, and the current ratio of remaining hydrogen to other elements indicates that the sun cannot have existed as a star for more than 5 or 6 billion years. The presence of radioactive elements in rocks of the earth with long half-lives, is another indication of finite age, for no known process is capable of producing these elements on or in the earth today.(10)

All current hypotheses about the earth's beginning postulate a time when its substance was part of a homogeneous sample of average cosmic matter. Hypothesis of a generation ago put the homogeneous sample in the sun or a similar star; mechanical difficulties with generating a planetary system from a fully formed star have led recent speculations back to an earlier hypothesis, in which the earth's material was once part of a huge cloud of gas and small particles spread thinly over a volume larger than the present orbit of Pluto. The cloud at first was cold, and the nucleus of solid matter that grew into the primitive earth was formed as an aggregate of cold particles and larger fragments, often called planetesimals. One justification for postulating cold accretion of planetesimals, is based on a striking difference between average compositions of cosmic and terrestrial matter, the far greater amount of volatile elements in the former, a difference that is especially pronounced for the inert gases. A low temperature is indicated even more by the fact that the inert gases have been lost in much greater amounts than more active volatile materials such as water, carbon dioxide, and ammonia. At temperatures of several hundred degrees the active volatile compounds would be just as easily lost as inert gases, since their molecules would be free to move and their molecular weights are similar. At low temperature, however, the active compounds would have been partly retained as mineral hydrates and carbonates.(10) The current hypothesis on the formation of the earth derived from the original theories of Kant, Laplace, Von Weizsäcker, Ter Haar, Chandrasekhar.(11-12-13-14-15-16)

Kant suggested that the raw material has been acquired from interstellar space. Planets were formed by agglomeration of metallic and stony dust, starting from buds whose mass increased more and more. According to the majority of the present authors, the cloud of dusts and gases which gave rise to the solar system had condensed by self-rotation, whereas the protoplanets were formed by agglomeration of cold solid particles: Urey,(17-21) Brown,(22) Latimer,(23) Hoyle,(24-25) Yu Levin(26).

Laplace envisaged a rotating nebula that cools and contracts and in doing so throws off rings of material that go to form the planets. The difficulty is that such a process will not associate the main part of the angular momentum with only a small fraction of the total mass. Granting, however, that condensation into planets is somehow possible, Kuiper(27-28) attempted to work out a general evolutionary history of the system.

Alfven(29) postulates a suitable general magnetic field of the Sun; he envisaged a complicated scheme of ionization and neutralization of atoms falling through the field towards the Sun. This was intended to provide material of graded chemical composition for planetary formation. He was the first to envisage an evolutionary role of a magnetic field.

Note: A sphere of the material compressed to the density of the Sun and retaining its angular momentum would have a peripheral speed greater than the speed of light! Thus we may not think of the solar system as having been formed simply by the isolation and contraction of the material within a particular region of an interstellar cloud.(30)

One of the modern theories is based on the fact that if the Sun passed through a cloud of gas and dust, it must certainly capture some material and a part may remain for a time in orbit around the star. This is the basis of the theory that postulate a solar nebula. It was studied by O. Yu Schmidt(31) and pursued by B. Yu Levin(32). Also Von Weiszäcker sees the original dust cloud as a system of vortices rather than a single rotating mass.(33) Planets would then form in smaller eddies acting as a kind of "roller bearing" between the large ones. But the theory does not predict the total mass and angular momentum about the Sun. This has been confirmed by Lyttleton in 1960(34). We may take the view that the "angular momentum" difficulty means that, if the Sun was formed by contraction of a portion of interstellar matter then this did not proceed in isolation from the surrounding matter; a mechanism of transfer of angular momentum must have operated. The possibility of magnetic braking remains to be considered. If the conductivity is great enough, the magnetic lines of force that originally traversed a contracted portion and the surrounding medium, remain attached to both. So they behave like elastic strings tied to the rotating portion and its surroundings; the lines tend to get wound around the former and at the same time they exert a torque upon the latter. In this way angular momentum is transferred from the rotating portion to its surroundings. This has been studied by Hoyle (1960).(24) But with the actual value of the angular momentum, this process would not give rise to a solar nebula at all.

Urey(18-20) then brought a massive knowledge of physical chemistry to bear upon the problem of the formation of planets. He says that the terrestrial planets were formed cold by the agglomeration of planetesimals in the presence of certain gases. He thinks that the first ones were mixtures of silicates, water, and ammonia. These materials could have been irradiated by energetic photons from the Sun to give rise to heavier elements. This would explain the difference abundance between terrestrial and meteoric matter. It is seen that much current thinking suggests that the planets were formed by aggregation of planetesimals which were produced in orbits going around the Sun in one sense and nearly in one place. But a mechanism for the direct aggregation of orbiting planetesimals has apparently not yet been discovered. Speculation and exploratory investigations of the most value are those exemplified on the mechanical side by Lyttleton (1960)(34), and on the magneto hydrodynamical by that of Mestel (1959).(35)

History of the Atmosphere

The scarcity of the inert gases on earth compared with their cosmic abundance is most plausibly explained by escape of gases from the growing earth at a time when its materials were still spread through such a large volume of space that gravitational attraction was much smaller than it is today.

The fact that other gases with similar molecular weights, particularly H_2O, HCl, NH_3, CH_4, CO_2, H_2S, SO_2 were not lost in nearly the same proportion as the inert gases, is good evidence that original temperature of the earth was low, so that these substances could be retained in the planetesimals either as frozen particles or in the form of compounds. The cold primitive earth must have had very little atmosphere. As the heating increased, gases would be accumulated at the surface. Gravity would now be sufficient to retain all except the two lightest gases H_2 & He. Thus our present atmosphere is largely of secondary origin having formed by degassing of the solid body of the planet.(36)

Good evidence regarding the degassing process comes from simple calculations about the amounts of carbon in various geologic materials. The carbon of sedimentary rocks was nearly all derived from CO_2 that once existed in the atmosphere: the carbon of organic materials was fixed in organic compound by photosynthesis and the carbon of precipitated carbonates represents atmospheric CO_2 added to sea water. Rubey's (1951)(37) inventory of total carbon in the atmosphere, hydrosphere, organisms and sedimentary rocks expressed in units of 10^{20} gr. of CO_2 is respectively 0.023, 1.30, 0.145 & 920.

One interpretation (extreme) is that the atmosphere at some early period was very dense, consisting mainly of CO_2 at a partial pressure of about 12 atm. which was gradually reduced to the amount of its present low value 0.0003 atm. This is an unlikely hypothesis; it would mean that we are at the end of life on our planet.

An obvious additional source of carbon dioxide is volcanic activity. It makes no difference that some of the carbon expelled by volcanoes has the form of CH_4 and CO rather than CO_2, because they would be oxidized by atmospheric oxygen. A mechanism for continuous degassing and for maintenance of adequate CO_2 in the atmosphere seems assured. Sagan(39) submitted the question of escape and accumulation of planetory atmospheres to rigorous examination. He confirmed the hypothesis that the original atmosphere of the earth was of a reducing nature, however, it was lost to space before the planet was fully formed. This is further brought out by the fact that the noble gases are considerably less abundant in the atmosphere than in the universe at large. A secondary atmosphere was formed by the outgassing of trapped and combined volatiles from the interiors of the earth.(39) The difference between 12 and 0.0003 could be interpreted by the fact that there is more life which can keep CO_2 and that there is less carbon dioxide in the air. This is the opposite extreme which supposes that a balance between life processes and volcanic activities has maintained an approximately constant partial pressure of CO_2 throughout geologic history.

One other gas for which degassing seems clearly demonstrable is argon (Kulp, 1951).(38) In the air this gas is chiefly the isotope Ar^{40} (a product of the radioactive decay of K^{40}). In stellar atmosphere it is Ar^{36}. So it has to come from the K^{40} in deeper levels of the earth.

In the absence of contrary evidence and because the hypothesis is consistent with current ideas about the earth's origin, it is common to assume that most volatile materials now at the surface, including water, have come from the interior. This means that the atmosphere and the oceans have grown during geologic time and that they are still receiving additions from present day volcanic activities. Water (H_2O) vapor, hydrogen (H_2), carbon oxides (CO), sulfur gases (S), hydrogen halides, nitrogen or ammonia (N_2,NH_4) possibly hydrocarbons some such mixture is the primary material that has come out of the earth and that presumably constituted the primitive atmosphere.

If free hydrogen was a prominent gas at the beginning, the earliest atmosphere would have consisted of strongly reduced compounds like methane, ammonia, and hydrogen sulfide. It most of the initial hydrogen escaped during the accretion of planetesimals, the first atmosphere would have had more free nitrogen and oxides of carbon and sulfur. In either case H_2O would be decomposed at high levels in the atmosphere by UV producing a tiny concentration of O_2.

Poole(40) has suggested that an initial supply of free oxygen sufficient to support life and permit photosynthesis by green plants, may have been produced by photochemical dissociation of water vapor in the upper ionized layer of the atmosphere and by subsequent escape of hydrogen. In this connection, it is of interest to note that purple sulfur bacteria and certain other present day forms can synthesize organic matter from CO_2 in an anaerobic environment that contains some H_2S. Then the atmosphere under these reducing conditions would contain some hydrogen (H_2), methane (CH_4), nitrogen (N_2), and ammonia (NH_3);

with smaller amounts of carbon dioxide (CO_2), carbon monoxide (CO), hydrogen sulfide (H_2S), and of course, mainly of water vapor (H_2O), also with some hydrogen chloride and hydrogen fluoride. This composition has varied in the opinion of different authors. For example: Oparin(41) and Urey(18) believed that the atmosphere was composed of (CH_4, NH_3, H_2O, H_2); Bernal(42) thought that it was made of ($CH_4 \rightarrow CO_2$, $NH_3 \rightarrow N_2$, H_2O, H_2) simultaneously with a hydrosphere composed of (CO_2, NH_3, H_2S, H_2O); Rubey(43) also has given for the atmosphere (CO_2, N_2, H_2S, H_2O) and for the hydrosphere (CO_2, NH_3, H_2S, H_2O); Revelle(44) on his side gave for the atmosphere (CO, CO_2, N_2, H_2S, H_2O); finally Dauvillier(9) has proposed for the atmosphere ($CH_4 \rightarrow CO \rightarrow CO_2$). But as we can see these hypotheses are only sightly different.

The ulterior evolution of the atmosphere can be outlined in three general stages:(45)

1) The atmosphere was highly reducing. Duration: the first period began about 4.5×10^9 years ago and lasted 5×10^8 years after the earth's formation. The absence of oxygen made ultra-violet radiation more significant. Most chemical evolution occurred at this time.
2) Because of the great conversion of the methane into carbon dioxide and the ammonia into nitrogen, the atmosphere is neither strongly reducing nor oxidizing.
3) Present oxidizing atmosphere.

The transition between 2 and 3, about 1.8×10^9 years ago, was suggested by the oxidation states of Uranium minerals. According to Szabo et Coll(46) this would have been made around 700 or 800 million years ago.

Volcanic Gases

During volcanic eruptions, the magma having ascended into a region of relatively low pressure is unable to retain its volatile constituents in solution. The sample gases available are all from the smaller gas vents or fumaroles. They are not so good for at least two reasons: a) they may represent only the residual gases, b) if the fumaroles are at a distance from the central conduit, the gases may contain contamination due to volatile products distilled from vegetation and sedimentary rocks buried by the lava.(47) Nevertheless, water vapor is nearly always the dominant gas. The carbon gases (CO_2, CO, COS, CH_4) are usually next in order. The sulfur gases (SO_2, H_2S, S_2, SO_3) follow, HCl is also reported. Nitrogen is present chiefly as the free element, but NH_4Cl is common sublimate. The amount of each one depends on the temperature.(48) With its content of HCl, CO_2, and H_2S, the fumarole gas could hardly be anything but acid, but a higher pressure in a liquid in contact with silicates and perhaps with carbonates, alkaline conditions seem at least equally likely. Ammonia is often reported as a minor constituent of volcanic gases. It is assumed to be a "juvenile" (magmatic origin) gas, originally dissolved in the magma. If we consider the reaction $N_2 + 3H_2 \rightleftharpoons 2NH_3$, $\Delta F = -22.0 + 0.0474T$. Hence if all gases are at 1 atm partial pressure, the equilibrium is displaced to the left at temperature over about 200°C. Therefore, the amount of NH_3 is appreciable only at high pressures, fairly low temperatures, and with abundant H_2. Gautier (49) reported the presence of sulfocyanates in volcanic exhalations due to the following process: $CO_2 + H_2S + 2NH_3 \rightleftharpoons SCN.NH_4 + 2H_2O$. Because the hypothesis of a cold primitive earth, the presence of ammonia was likely and therefore, we are justified to consider the NH_4SCN, as an early constituent of the original earth; in our synthesis of organic compounds.

Note: $\Delta F^o = \Delta H^o - 0.001 \, T \, \Delta S^o$

In a reaction, the maximum work which can be done is given by $-\Delta F$. In other words, ΔF serves as a criterion for the occurrence of a spontaneous process. If $\Delta F = 0$, the mixture is in equilibrium and no reaction can occur, but if $\Delta F < 0$, a reaction can take place. If $\Delta F > 0$, energy must be supplied in order that the reaction may occur at all.

Energy Sources

At the present time the direct or indirect source of free energy for all living organisms is the sunlight utilized by photosynthetic organisms. But before the evolution of photosynthesis other sources of free energy must have been used.

The reducing primitive environment was subjected to several energy fluxes: thermal energy, ultraviolet radiations, radioactivity, cosmic rays, electric discharges, and volcanoes. Miller and Urey gave a nice summary of those in a table:(50)

		Energy (Calory cm^{-2} $year^{-1}$)
Total radiation from Sun		260,000
Ultraviolet light	$\lambda < 2500$ Å	570
	< 2000 Å	85
	< 1500 Å	3.5
Electric discharges*		4
Cosmic Rays		0.0015
Radioactivity (to 1 km deph)**		0.8
Volcanoes***		0.13

* : Includes 0.9 cal. cm^{-2} yr^{-1} from lighting and about 3 cal. cm^{-2} yr^{-1} due to corona discharges from pointed objects.
** : The value was 2.8 cal. cm^{-2} yr^{-1}, 4 x 10^9 years ago.
*** : Calculated on the basis of an emission of lava of 1 km^3 ($Cp = 0.25$ cal/gr., $P = 3.0$ gr/cm^3) per year at 1000°C

It is evident that sunlight is the principal source of energy, but only a small fraction of this is in the wave-lengths below 2000Å which can be absorbed by CH_4, H_2O, NH_3, CO_2, and so on.

The effective wave-lengths would be for:

$CH_4 < 1450$Å $H_2O < 1850$Å $NH_3 < 2250$Å
$CO < 1545$Å $C_2O < 1690$Å $N_2 < 1100$Å
$H_2 < 900$Å

Most of the photochemical reactions at these low wave-lengths would have taken place in the upper atmosphere. The compounds so formed would have absorbed at longer wave-lengths and therefore might have been decomposed by this U.V. before reaching the oceans. The question is whether the rate of decomposition in the atmosphere was greater or lesser than the rate of transport to the oceans.

Next in importance as a source of energy are electric discharges, such as lightening and corona, which occur closer to the earth's surface and hence would have effected more efficient transfer to the oceans. (Whipple(51)) In addition electric discharges produce parts of the electromagnetic spectrum, create strong electric fields and generate heat in which ionization of gases can take place. Moreover, the energized particles may, in turn, emit energy in characteristic wave-lengths which can serve as a secondary activating source for other atoms and molecules. (This complexity may be the basis for the greater efficiency of electric discharges over other forms of energy).(52)

Cosmic-ray energy is negligible at present, and there is no reason to assume it was greater in the past. The radioactive disintegration of uranium, thorium, and potassium was more important 4.5 x 10^9 years ago than it is now, but still the energy was largely expended on the interior of solid materials such as rocks and only a very small fraction of the total energy was available in the oceans and atmosphere.

Thermal energy or heat raised many objections, mainly because the solar system was formed from a cold cloud of cosmic dust. The mechanical energy was from the gravitation released during the condensation of the dust to form the earth. Anyhow, Fox has maintained that organic compounds were synthesized on the earth by heat!(53)

Ultrasonic vibrations have also been suggested as an energy source in the activation of reactants.(54) A recent addition to this array of energies is hypersonics. Hochstim(55) has calculated the effect of the entrance of meteorites into the atmosphere and has come up to some surprising and impressive results. For example, Figure 1, a meteorite of 500 meters radius and with a velocity of 11 km/sec. will have 400,000 tons of gas in its stagnation region, compressed to 1,500 Atm. pressure, and will create a temperature of 16,300°K. Its kinetic energy will be equivalent to 40,000 megatons of TNT.

Biogenesis Theories

Before discussing the problem of the origin of life, it is important to be certain that a problem exists. Many learned men; like Spinoza, Lord Kelvin, Buffon, Liebig, Pasteur, and Arrhenius, have thought that this was a false problem, that life was as eternal as the world itself, and therefore, it had never had a beginning. Man discouraged by the complexity of the problem, could only fall back on the supernatural. What was more obvious than to attribute to each

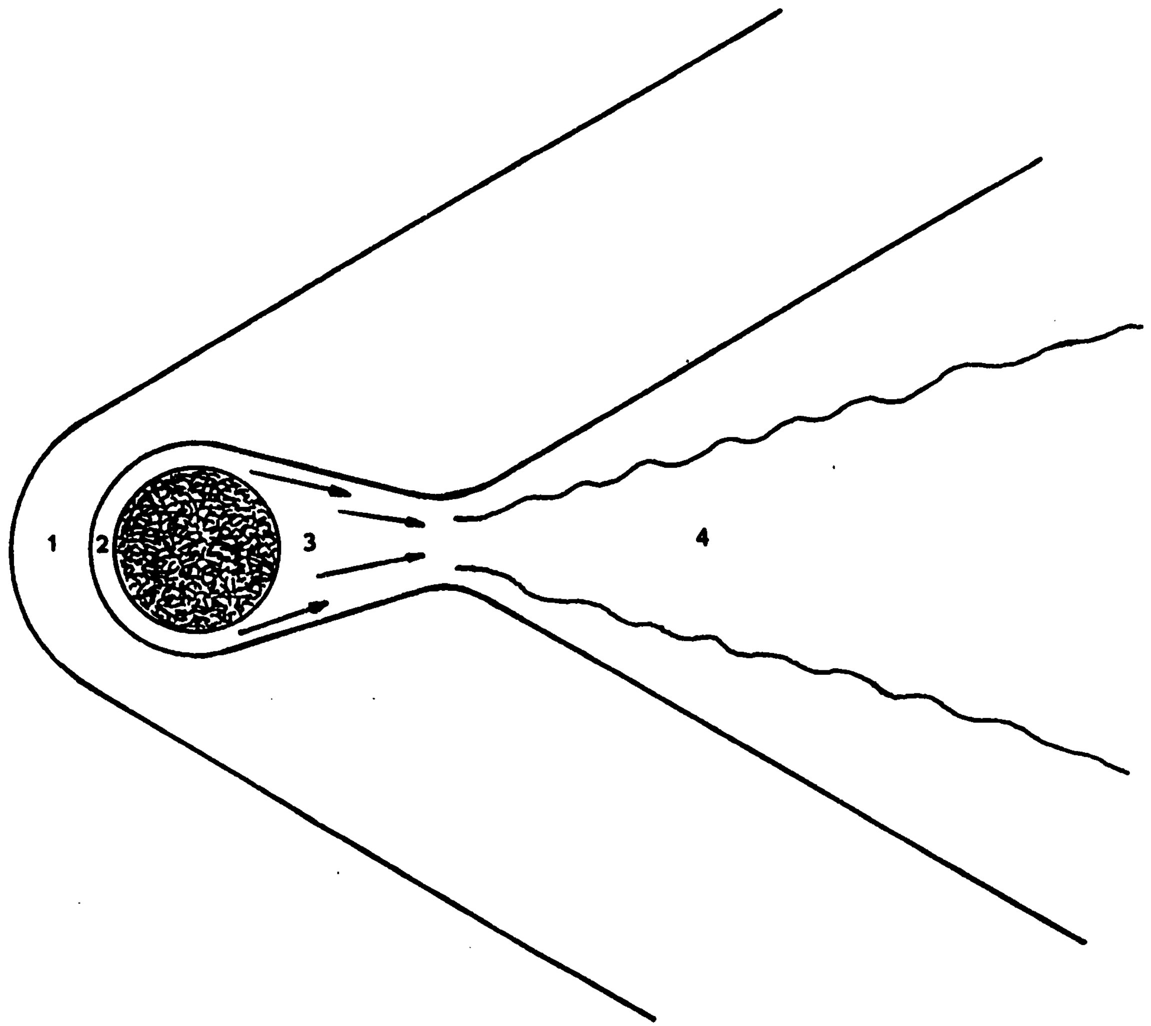


Figure 1: Hypersonic flow around meteorite 1) Stagnation region 2) Boundary layer 3) Ablating (evaporating) species 4) Inner wake.

living creature a tiny "genie", intangible and intelligent, controlling its growth, evolution, and reproduction.(56)

In 1790, Kant had a supernatural and finalist conception of life. Nowadays, not only philosophers such as A. Comte, Bergson, and Le Roy, but paleontologists, such as Teilhard de Chardin, and biologists, such as Vandel and Rouvière, look upon it with favor. In 1929, Vernadsky declared in his "Biosphère" that research on the cosmic era of the earth and the genesis of life are useless, illusory, and indeed, harmful and dangerous. This has been said before every epoch, about questions that scientific investigations has still not elucidated. It is a sterile attitude the prudence of which may appear excessive, and such reserve only hides a lack of enthusiasm, curiosity, and imagination.

During many centuries, the spontaneous generation theory (Arristote)(57) was the only rational explanation for the origin of living creatures. The first series of experiments, to show that this was completly wrong, was done by Redi in 1668(58). With the discovery of the micro-organisms, Van-Leeuwenhoek,(59) by microscopic observations, the fight was more intense. The controversy between Needham and Spallanzani is still famous..(60-61) In 1859, Pouchet(62-63) gave a considerable amount of data in favour of the spontaneous generation. These results were contradicted by Pasteur,(64-67) which showed that the spontaneous generation of micro-organisms was only a contamination of cultures by exterior germs (Bacteriology). The works of Pasteur, showing that life comes only from another life, and those of Darwin,(68-69) demonstrating that the living forms came one from another by slight and very slow modifications.

Three principal theories are still present:

1) Panspermia
2) Creative Chance
3) Materialistic or evolutionistic theory;

Cosmic Panspermia

Montlivault(70) had suggested that life came to us from germs carried by celestrial bodies, this was developed by Richter,(71) and was supported by Lord Kelvin(72) and Helmotz.(73) The experimental basis was established by Berzelius,(74) Wöhler,(75) Daubree,(76) Cloez,(77) and Berthelot,(78) by showing that organic compounds were present in meteorites. According to Arrhenius,(79-80-81) cosmic germs from other planetary systems were expelled by the pressure of the stellar radiation and reached the earth on interstellar cosmic dust. Becquerel(82), Esnault-Pelterie(83) refuted this possibility. They showed that the germs would be destroyed before reaching the earth, by the U.V. radiation.

Creative Chance

Herodotus (484-406, B.C.) declared: "Length of time may bring anything to pass". The "mechanic" hypothesis proposed that the first living thing was a living macromolecule, that was formed by the chance coming-together of its constituent elements in the proper proportions and arrangements. It was thought that this was probably a molecule of protein. In this hypothesis, the emergence of life would be due to a fluctuation of a very rare type. This was supported by many authors like: Lipman,(84) Alexander,(85) Muller(86) and Wald(87) for whom, the most improbable event becomes a statistical certainty.

Evolutionistic Theories

From the start this theory rejected the contention that only living things could make organic compounds. It proposed that organic compounds were formed abiotically (that is, without the intervention of living things) before organisms came into being. The chemical evolution which would have been done by successive steps resulted in a higher level of organization of matter. At this level, biological principles came into being which did not exist at a lower level. A number of authors started to speculate about the gradual evolution of life from nonliving matter. Among them, were: Engels(88) in his "Dialectics of Nature", Schaffer,(89-90) Spencer,(92-94) Tyndall,(95-96) Haeckel,(97) Bastian,(98) and Moore.(99) This theory was also examined at a more philosophical level by many authors.(100-108) The theories which are the most fruitful on the experimental level are the ones of Oparin and Haldane.

The fundamental modern theory of chemical evolution was first promulgated by A.I. Oparin in 1924(109) and was expanded in 1938.(41) Similar speculations were presented by J.B.S. Haldane in 1928.(110) Oparin's ideas followed from the theory of fiery origin of the Earth proposed by Jeans and thus considered high temperature reactions.

Abiotic Synthesis of Organic Compounds

The abiotic synthesis of organic compounds has become a concept almost universally accepted. We tried to summarise in Table 1, all the principal synthesis under simulated primitive earth conditions of many important compounds, (and some organic compounds not associated with living things). This table does not pretend to be complete, however, the list gives impressive evidence of the strides that have been made in a little more than a dozen years. It has been unequivocally demonstrated that all kinds of organic compounds could have been formed abiotically under a variety of conditions, even if there is no general agreement on what constitutes "primitive earth conditions" and on which energy sources should be used. The organigram following this table (Figure 2) gives a general scheme for the molecular evolution and abiotic synthesis starting from simple chemicals.

Table I - Abiotic synthesis of many important compounds under simulated primitive Earth conditions.

Energy Source	General compound synthesized	Authors	Year	Ref.	Starting materials	Synthesized compound
Sun light or artificial light (Electric bulb)	Amino Acids	A.L. Herrera	1942	(111)	NH_4SCN & formaldehyde	
		K. Bahadur	1954	(112)	Aqueous solution of paraformaldehyde, potassium nitrate, and ferric chloride	Serine, aspartic acid, aspargin, glycine, alanine, threonine, valine, lysine, histidine, leucine, ...
		K. Bahadur et al (500 watt elect. bulb)	1958	(113)	Aqueous suspension of colloidal molybdenum oxide and paraformaldehyde	Glycine, alanine, valine, histidine...
		E.C.C. Baly et al	1922	(114)	Formhydroxamic acid and formaldehyde	
	Small Peptides	Perti et al	1961	(115)	Aq.Sln. of glycine, glutamic acid, tyrosine	Diglycine, peptides.
Spontaneous	Simple Compound	T. Waldsten et al	1959	(116)	HCN, OH^-	HCN dimers.
	Small Peptides	G. Steinman et al	1965	(117)	Glycine, dicyandiamide, HCl	Diglycine.
	Nucleotides	G. Steinman et al	1965	(118)	ADP, kaloin	Adenosinetriphosphate (ATP).
					Alanine, HCl, dicyandiamide	Guanylurea
		C. Ponnamperuma et al	1964	(119)	Adenine, deoxyribose CN^-	Deoxyadenosine.
	Sugars	G. Steinman et al	1964	(120)	Glucose, phosphoric acid, dicyandiamide	Glucose-6-phosphate.
	Porphyrins	A. Szutka et al	1965	(121)	Pyrrole, benzaldehyde	Tetraphenylporphin.

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compound
U.V.	Amino Acids	Berthelot & Gaudechon	1910	(122)	CO_2, NH_3	Oxygen, formaldehyde
		Berthelot & Gaudechon	1917	(123)	CO_2, H_2O	formamide, glycine formaldehyde, glucides
		Caltera et al	1959	(124)	Various ternary	Glycine, serine and
		G. Ferrari et al	1961	(125)	Organic compounds in aqueous solution with KNO_3	alanine, α-amino-butyric acid.
		Deschreider	1958	(126)	Aqueous solution of NH_4 salts and organic carboxylic acids (NH_4SCN)	Alanine, glycine, aspartic acid.
		T.E. Pavlovskaya et al	1957-60	(127) (128) (129)	HCHO, NH_4 in aq. solution with and without catalysts	Serine, glycine, valine glutamic acid, alanine...
		Ellenbogen	1958	(130)	Suspension of FeS in aq. NH_4Cl in CH_4 atmosphere	Phenylalanine, methionine valine.
		Groth	1957	(131-132)	CH_4,NH_3,H_2O	(Amine) glycine and alanine, formic acid
		Groth & Weyssenhoff	1960	(133-135)		α-amino-butyric acid
		B. Franck	1960	(136)	Replacing methane in CH_4,NH_3,H_2O atm. with methanol.	Same as Groth, with most yield
		K. Heyns et Coll	1957	(137)	CH_4,NH_3,H_2O,H_2, N, H_2S or CO_2	Normal ones (glycine)... α-alanine, β-alanine α-amino-butyric acid sarcosine, NH_4SCN, thiourea, thioacetamine (no sulfur amino acid)
		Terenin	1957	(138)	CH_4,NH_3,CO in contact with H_2O	Alanine, β-alanine and other A.A. non identified
		Dodonova et al	1959	(139)	H_2,CH_4,NH_3,CO^3	Alanine, amino-butyric acid, valine, norleucine

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compound
U.V.	Amino Acids	O. Baudish	1913	(140)	Aqueous solution of potassium nitrate, ferric chloride, carbon monoxide	Few normal amino-acids
		Dhar and Mukherjee	1934	(141)	Glycol, Nitrate, titanium oxide	Glycine.
					Glucose, nitrate, titanium oxide	Arginine.
		Master	1957	(142)	Formaldehyde and hydrazine in aq.sln.	Glycine, valine and lysine.
		Steinman and Smith and Silver	1968	(143)	NH_4SCN	Methionine
		Smith and Steinman and Galand	1969	(144)	NH_4SCN	Glycine, alanine
	Small Peptides	G. Steinman *et al*	1964	(120)	Dicyandiamide.	Various peptides
		C. Ponnamperuma *et al*	1965	(145)	Glycine, leucine, H_2O, cyanamide.	Glycylglycine, dileucine
		Ellenbogen	1958	(130)	Suspension of FeS in aq. NH_4Cl in CH_4 atm.	Peptides.
		Bahadur and Ranganayaki	1958	(146)	Aq. sol. of amino acids	Glycylglycine, glycylalamine, and others
	Polypeptides	E. Fisher	1903	(147)	Glycine	Polypeptides, glycylglycine.
	Purines Pyrimidines Derivatives Nucleotides	C. Ponnamperuma *et al*	1963	(148)	Adenine, ribose, phosphate.	Adenosine.
		C. Ponnamperuma *et al*	1965	(149)	Adenine, ribose, EtMtP.	Adenosine. Adenosine, AMP,ADP,ATP, A4P.
				(119)	AR,AMP,ADP+EtMtP	AMP,ADP,ATP,A4P

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compound
U.V.	Purines Pyrimidines Derivatives Nucleotides	G. Steinman *et al*	1964	(120)	Adenosine, OP, dicyandiamide	Adenosine, monophosphate AMP.
		C. Ponnamperuma *et al*	1963	(150)	HCN	Adenine, guanine
		C. Ponnamperuma	1966	(151)	Purines, riboses, deoxyribose, phosphate.	Nucleosides, nucleotides
		F. Hoyle	1957	(25)	Purines, pyrimidines	Nucleosides, nucleotides
		C. Ponnamperuma	1964	(119)	Aq. sol. of adenine and deoxyribose and phosphate	Deoxyadenosine
	Sugars	Stoklasa	1913	(152)	Sol. of $KHCO_3$ with H_2	Sugars
		G. Steinman *et al*	1964	(120)	Glucose, dicyandiamide	Dissacharides.
					Glucose, phosphoric acid, dicyandiamide	Glucose-6-phosphate
		E.C.C. Baly J.C. Irvine and G.V. Francis	1924	(153) (154)	Aqueous solution of formaldehyde.	Hexoses and hydroxy acids
		C. Ponnamperuma	1965	(149)	Aqueous solution of formaldehyde.	Pentoses and Hexoses (ribose and dexoyribose)
		C. Ponnamperuma *et al* (plus γ-irradiation)	1963	(155)	Dilute solution of formaldehyde	Ribose, deoxyribose.
		C. Ponnamperuma (plus β-rays lin.acc.)	1966	(151)	Formaldehyde or HCN formaldehyde, H_2O	Ribose, deoxyribose.
	Simple Compound	Stoklasa	1913	(152)	Solution of $KHCO_3$ with H_2	Formaldehyde
		Taylor and Marshall	1925	(156)	Acetylene, CO_2, with H_2	Formaldehyde
		Groth	1937	(157)	CO and H_2 or CO and CH_4	Formaldehyde and glyoxal

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
U.V.	Simple Compound	Berthelot and Gaudechon	1910	(122)	CO_2,NH_3	Formamide, formaldehyde
					CO_2,H_2O	Formaldehyde and carbohydrates.
					CH_4	Quaternary compounds
		E.C.C. Baly, O. Baudish Heilbron	1922	(114)	Nitrites and aldehydes	Formhydroxamic acid
		C. Sagan <u>et al</u>	1960	(158)	CH_4,NH_3,H_2O, H_2	Formaldehyde
		G. Steinman <u>et al</u>	1965	(118)	Orthophosphate, kaolin, dicyandiamide	Pyrophosphate
		A. Schimpel <u>et al</u>	1965	(159)	Cyanide solutions or CH_4,NH_3,H_2O	Dicyandiamide
		N. Getoff	1966	(160)	H_2,H_2O	NH_3
	Organic Acids and Hydrocarbons	D.H. Kenyon <u>et al</u>	1965	(161)	Phenylalanine, tyrosine	Polymeric melanin-like like compounds
	Porphyrins	A. Szutka	1963	(162)	Pyrrole and benzaldehyde	Porphine-like compounds
Electrical Discharge Sparking	Amino acids (derivatives and precursors)	S.L. Miller	1953	(163)	CH_4,NH_3,H_2,H_2O	Glycine, alanine, α-amino-butyric acid cyanidric acid.
			1955 1957	(4) (164)	CH_4,NH_3,H_2,H_2O and carbon was added as CH_4	Same as before, plus aspartic acid, glutamic acid, β-alanine (sarcosine)
		W. Löb	1913	(165)	CO,NH_3,H_2O	Glycine
		K. Peters and H. Kuster	1929	(166)	NH_3,CH_4	Cyanhydric acid
		E. Brinner <u>et al</u>	1919	(167)	N, CH_4 or	Amino acids
			1938	(167)	CO, N, H_2	
			1940	(167)		
		L. Hough and A.F. Rogers	1956	(168)	H_2,CH_4,NH_3	Glycine, alanine, urea amino-butyric acid.

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Electrical Discharge Sparking	Amino acids (derivatives and precursors)	P.H. Abelson	1956- 1957	(169) (170)	CO, N_2, H_2, H_2O or CO, NH_3, H_3, H_2O	α-alanine, β-alanine, glycine, (sarcosine)
		T.E. Pavlovskaya and A.G. Pasynskii	1959	(127)	NH_3, CH_4, CO, H_2O	Miller's amino acids plus lysine.
		B. Franck	1960	(136)	CH_4, NH_3, H_2O, H_2 or methanol, NH_3, H_2O or iso-octane, NH_3, H_2O or iso-octane, H_2O	Glycine, alanine, aspartic acid, aspargine, isoleucine, glyciamide.
		K. Heyns *et al*	1957	(137)	CH_4, NH_3, H_2O, H_2S	Abelson's amino acids plus NH_4SCN, Thiourea, thioacetamide.
		F. Woeller *et al*	1966	(171)	CH_4, NH_3 (corona discharge)	Aminonitrile.
	Polymerization of amino acids Small Peptides Polypeptides	K. Otozai *et al*	1954	(172)		Amine, amino-nitrile amino-amine, peptide,
		J. Oro'	1963	(173)	Ethane (C_6H_6), NH_3, H_2O.	glycine, alanine, aspartic acid, asparagine, isoleucine, leucine, valine glycinamide.
		K.A. Grossenbacher *et al*	1965	(174)	Methane-ammonia-H_2O atmosphere	Threonine, serine, isoleucine, leucine, lysine, glycine, A.A.
		C. Ponnamperuma *et al*	1966	(175)	CH_4, NH_3, H_2O	Polypeptides.
	Polymers	S.L. Miller	1953- 1955	(163) (4)	CH_4, NH_3, H_2O, H_2	Unidentified polymers
		K.A. Grossenbacher *et al*	1965	(174)	NH_3, H_2O, CH_4, H_2	Polymeric cyanide.
		C. Ponnamperuma *et al*	1966	(175)	CH_4, NH_3, H_2O	Polymer giving several amino acids upon hydrolysis.

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Electrical Discharge Sparking	Sugars	E. El'piner and A.V. Sokolskaya	1959	(54)	Methane, ammonia, water.	Formaldehyde, ribose, 2-deoxyribose.
		C. Ponnamperuma	1965	(149)	Methane, ammonia, water.	Formaldehyde, ribose, 2-deoxyribose.
		G. Steinman	1963	(176)	Aqueous solution of methanol.	Formaldehyde.
	Organic acids	C. Ponnamperuma	1963	(177)	Methane Elect. spark Semi corona arc.	Aromatics Saturated hydrocarbons
		C. Ponnamperuma et al	1966	(178)	CH_4 (semi-corona)	Even number C-atoms mainly homogeneous mixture of aliphatics, mono-polycyclic compounds.
		S.L. Miller	1953 1955	(163) (4)	CH_4,NH_3,H_2O_2,H_2	Acetic, propionic, lactic glycollic, formic, suucinic, α-hydroxybutyric
		W.A. Allen et al	1966	(179)	CH_4,H_2O (semi corona)	mono-carboxylic acids C_2-C_{12}
	Nitrogenous	R.A. Sanchez et al	1966	(180)	CH_4,N_2	Cyanoacetylene.
	Simple compounds	S.L. Miller	1953	(163)	CH_4,NH_3,H_2O,H_2	HCN
		L. Hough et al	1956	(168)	" " " "	HCN
		T.E. Pavloskaya et al	1959	(127)	" " " "	HCN
		E. El'piner et al	1959	(54)	Methane, ammonia, water.	Formaldehyde.
		C. Ponnamperuma	1965	(149)	" " " "	Formaldehyde.
		G. Steinman	1963	(176)	Aq. sol. of methanol	Formaldehyde.
Ionizing Radiations	A.A. α-part.	Garrison et al	1951 1952 1953	(3) (181) (196)	CO_2,H_2O	Formic acid, formaldehyde, malonic, malic succinic, citric acids
	β-part.	T. Hasselstrom and M.C. Henry	1956	(182)	Aq. sol. of calcium carbonate (CO_3Ca)	Oxalic acid $\begin{matrix}COOH\\ \vert \\ COOH\end{matrix}$
		M.C. Henry et al	1957	(183)	Ammonium carbonate ($CO_3(NH_4)_2$)	

Table I - (continued)

Energy Source	General Compound Synthesized		Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Ionizing Radiations	A.A.	γ-part.	R. Paschke *et al*	1957	(184)	CO_3Ca solid	Glycine, alanine
			K. Dose and K. Ettre	1958	(185)	CH_4,NH_3,H_2O,H_2,N_2	A.A., amine, (ninhydrin +ive material)
		x-rays	K. Dose and B. Rajewsky	1957	(186)	" " " " "	A.A., amine, (ninhydrin +ive material)
		e^- 2mev	T. Hasselstrom and	1956	(182)	Ammonium acetate	Glycine, aspartic acid
			M.C. Henry *et al*	1957	(183)	(CH_3COONH_4)	
		5mev	C. Palm and M. Calvin	1962	(187)	CH_4,NH_3,H_2O,H_2	Glycine, alanine, urea lactic acid, fat acids.
			J. Oro'	1963	(173)	CH_4,NH_3,H_2O	Glycine, glyciamide,
				1965	(188)	solid at azote liquid temperature	alanine, aspartic
		2mev	S.L. Miller	1959	(50)	CH_4,NH_3,H_2O	A.A.
	Purines Pyrimidines	e^-	J. Oro'	1960	(189)	Primitive earth atmosphere	Adenine
	Derivatives		C. Palm and M. Calvin	1961	(190)	" " " "	Adenine
	Nucleotides			1962	(187)		
			C. Ponnamperuma *et al*	1963	(191)	CH_4,NH_3,H_2O	Adenine
		β-part.	C. Ponnamperuma	1966	(151)	CH_4,NH_3,H_2O or HCN	Adenine, guanine.
	Organic acids	protons 60" cycl.	R. Berger	1961	(192)	CH_4,NH_3,H_2O (temp: -383°F)	Urea, acetamide, acetone
	Hydro-carbons	e^-5mev	B.C. Palm *et al*	1962	(187)	CH_4,NH_3,H_2O,H_2	Urea, CO, CO_2, ethane, ethylene, cyanide, C_3-C_7 hydrocarbons.
	Nitrogen Compounds		S.L. Miller	1957	(164)	Methane, ammonia, water	Simple organic acids.
		low energy	J.E. Manton *et al*	1960	(193)	CH_4	Ethane, ethylene, acetylene.
		high energy	F.W. Lampe	1957	(194)	CH_4	Ethane, ethylene, propane, butane.

Table I - (continued)

Energy Source	General Compound Synthesized		Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Ionizing Radiations	Organic acids	e^- 5mev	J.A. Glasel	1961	(195)	CH_4,NH_3 (6°C)	Acethylene, ethane, propane.
	Hydro-carbons	α-part	W.M. Garrison et al	1953	(196)	Aq. acetic acid	Succinic acid, tricarballylic, malonic, malic, cipric, acid.
	Nitrogen compounds	γ-part	C.E. Stoops et al	1961	(197)	CO_2 and ethylene mixture	Long chain of carboxylic acids (up to 40-C atoms), fatty acids, lipids.
	Aldehydes Ketones Sugars	γ-part Co-60	C. Ponnamperuma	1963	(155)	Formaldehyde or	Ribose, deoxyribose
				1966	(151)	HCN.	
				1965	(149)	Formaldehyde	Ribose, 2-deoxyribose
		e^-4.5mev	C. Ponnamperuma	1965	(149)	CH_4,NH_3,H_2O	Ribose, deoxyribose.
	Simple Compounds	part	W.M. Garrison et al	1951	(3)	CO_2,H_2O, H_2, Fe^{++}	Formaldehyde, formic acid.
				1952	(181)	Aq. formic acid	Formaldehyde, oxalic acid.
			T. Hasselstrom et al	1956	(182)	CO_3Ca,$CO_3(NH_4)_2$	Oxalic acid.
		e^-	A. Schimpel et al	1965	(159)	Cyanide solution or CH_4,NH_3,H_2O	Dicyandiamide.
			S.L. Miller	1957	(164)	CH_4,NH_3,H_2O	HCN
		γ-part	N. Getoff	1966	(160)	H_2,H_2O	NH_3
			N. Getoff et al	1960	(198)	CO_2	Formaldehyde, acetaldehydes, oxalic acid
		β-part	C. Ponnamperuma	1966	(151)	CH_4,NH_3,H_2O	HCN, formaldehyde.
	Porphyrins	γCo-60 x-rays	Szutka	1963	(162)	Pyrroles, aldehydes	Porphyrins

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Hypersonic Vibrations	Organic Compounds	E. El'piner et al	1959	(54)	N_2,CO,H_2O	HCN, hydrocyanic acid formaldehyde.
Thermal Energy	Amino Acids	S.W. Fox et al	1955 1957 1961	(199) (53) (200)	Malic acid, urea	Aspartic acid, α-alanine β-alanine.
		S.W. Fox	1963	(201)	Urea, α-hydroxy-glutamic	Glutamic acid.
					Glucose, urea	Glycine.
		K. Heyns and K. Pavel	1957	(202)	Glycine	Alanine, aspargine, oxalic acid, furmaric acid.
		J. Oro' et al	1959	(203)	Aq. sol. of formaldehyde chlorhydrate hydroxylamine. HCHO+NH_2OH+CHl 80-100°C 50 h.	Glycine, alanine, β-alanine, serine, threonine, aspartic acid glycinamide
		J. Oro' and J.S. Kamat	1961	(204)	Sol. of ammonium cyanide CN (NH_4) 70°C 25 j.	Glycine, alanine, aspartic acid.
		C.U. Lowe and M.W. Rees C.U. Lowe et al	1963 1963	(205) (206)	Cyanhydric acid + ammoniac + water. 90°C 18 h.	Alanine, glycine, serine threonine, β-alanine, leucine, isoleucine, aspartic acid, glutamic acid, α-aminobutyric αβ-diaminopropionic acids.
		M. Labadie et al	1967	(207)	Ammonium cyanide 90 °C 4 h.	Glycine, alanine, aspartic acid, lysine, serine threonine, histine, ormithine, γ-aminobutyric acid, glutamic acid, amide
		K. Harada and S.N. Fox	1964 1965	(208) (209)	CH_4,NH_3,H_2O (silicates 900-1100°C)	Amino acids found in proteins.

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Thermal Energy	Amino Acids	J. Oro' and Skeves	1965	(188)	CH_4,NH_3,H_2O (silicates 900-1100°C)	Amino acids found in proteins
		R.A. Sanchez _et al_	1966	(180)	Cyanoacethylene, NH_3, HCN (100°C)	Amino acids
		J. Oro'	1963	(210)	Aldehydes, HCN, NH_3,H_2O (70-100°C)	Amino nitriles, amides
			1961	(211)	NH_3,H_2O, HCN (30-100°C)	Formamidine, glycinamide.
		J.P. Ferris _et al_	1965	(212)	HCN	Amino nitriles.
	Small Peptides	H. Hanafusa _et al_	1959	(213)	Aminoacetonitrile sulfate, kaolin. (120-140°C)	Glycyl-glycine, glycyl-diglycine.
		R.R. Becker _et al_	1953	(214)	N-carboxyamine acid, anhydrides.	Peptides
		K. Heyns and K. Pavel	1957	(202)	Glycine.	Glycyl-glycine.
		C.U. Lowe _et al_	1963	(206)	Cyanhydric acid +	Peptides.
		C.U. Lowe and M.W. Rees	1963	(205)	ammoniac + H_2O.	
	Polypeptides	J. Oro' and C.L. Guidry	1960	(215)	Glyciamide	Polyglycine.
			1961	(216)	(140 C with NH_4OH)	
		J. Kovac and H. Nagy	1961	(217)	Aspargine	Polypeptides.
		C.U. Lowe and M.W. Rees	1963	(205)	Cyanhydric acid + NH_3 + H_2O	Polypeptides.
		G. Schramm _et al_	1958	(218)	Amino acids + ethylmetaphosphate (EMP)	Alanyl-glycyl-glycine, poly-alanyl-glycyl-glycine.
			1962	(219)	Arginine + EMP.	Polyariginine.
		S. Akabori	1955	(220)	Kaolinite + H_2O +	Polyglycine.
		S. Akabori _et al_	1956	(221)	Formaldehyde +	
		S. Akabori _et al_	1959	(222)	acetaldehyde. (60-100°C)	
		G. Krampitz	1959	(223)	Amino acids	Polypeptides

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Thermal Energy	Proteinoids	S.W. Fox and K. Harada	1958 1959 1960 1966	(224-225) (226) (227) (228)	L-glutamic acid + D-aspartic acid + L-aspartic acid.	Protenoids
		A. Vegotsky et al	1959	(229)	Aspartic acid. glutamic acid + + 18 amino acids. (170°C)	Protenoids.
	Purines Pyrimidines Derivatives	J. Oro'	1960 1961	(189) (230)	NH_4OH,CHN (90°C 24h) NH_3,HCN,H_2O. (70°C 1 j.)	Adenine 4-amino-imidazole-5-carboxamidine (AICAI) 4-amino-imidazole-5-carboxamide (AICA)
		J. Oro' and A.P. Kimball	1961 1962	(231) (232)	Same	Same
		C.U. Lowe et al	1963	(206)	HCN,NH_3 (90°C)	Adenine, guanine, hypoxanthine and xanthine
		J. Oro'	1963	(233)	Acrylonitrile (AN) ammonium chloride, urea. (135°C 48 h)	Uracil (guanine, xanthine)
		S.W. Fox and K. Harada	1961	(200)	Malic acid, urea (120°C 1 h. with APP)	Uracil.
		C. Ponnamperuma et al	1963 1964	(234) (235)	18 amino acids (190°C 6 h.	Guanine.
		J.P. Ferris et al	1965	(212)	HCN,NH_3 (-22°C)	Adenine, guanine, xanthine, hypoxanthine.
		J.P. Ferris et L.E. Orgel	1966	(236)		
		R. Sanchez et al	1966	(237).	Amino malononitrile	

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Thermal Energy	Phosphorus and Nucleotides	S.L. Miller and M. Parris	1964	(238)	Potassium cyanate (35°C 20 j. with apatite + $CaCl_2$)	Pyrophosphate.
		A. Beck and L.E. Orgel	1965	(239)	Same	Same
		G. Schramm et al	1961 1962 1965	(240 (219) (241)	Purines, pyrimidines pentoses (50-60°C with EMP)	Nucleotides.
		C. Ponnamperuma and R. Mack	1965	(242) (242)	Adenosine, guanosine, cytidine, uridine, thymidine. (160 °C 2h. with inorganic phosphate)	Nucleosides phosphates AMP, GMP, CMP, UMP, TMP, UpU, UpUp.
	Polynucleotides	G. Schramm et al	1961 1962 1965	(240) (219) (241)	Nucleotides, phosphate nucleotides (50-60 °C with EMP)	Polyribonucleotides (poly-A, poly-U, poly-C copolymers), polyphenylalanine.
		G. Schramm	1966	(244)	Uridylic acid, PPE	Polyuridylic acid.
		A. Schwartz et al	1964 1965	(243) (243)	Cytidine 2' (3')-phosphate. (65 °C 2 h.)	Poly-C (polycytidylic acid).
		N.K. Kochetkov et al	1964	(245)	Uridine 2' (3')-phosphate	Poly-U (polyuridylic acid)
	Nitrogenous Compounds	M. Labadie et al	1967	(207)	NH_4CN (90 °C 4 h.)	Urea, γ-amino-butyric acid.
		C.U. Lowe et al	1963	(205) (206)	Cyanhydric acid + NH_3 + H_2O (90 °C 18 h.)	α-amino-butyric acid. αβ-diaminopropionic acids, urea.
	Polymers	G. Schramm et al	1962	(219)	Nucleosides, phosphate nucleosides. (50-60°C with EMP)	Co-polymers

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Thermal Energy	Polymers	J. Oro' et al	1961	(215)	Amino acid amides (100°C)	Polymers.
		C.U. Lowe et al	1963	(205) (206)	NH_3,HCN,H_2O (90°C)	Unidentified polymers
		C. Ponnamperuma et al	1963	(234)	Various monomers	Co-polymers.
	Aldehydes Ketones	J. Oro'	1961	(211)	HCN,NH_3,H_2O (30-100°C)	C_3-C_6 Sugars
	Sugars	J. Oro' et al	1962	(246)	Acetaldehyde + glyceraldehyde (or formaldehyde) (50°C with CaO)	2-desoxyribose.
	Polysaccharides	G. Schramm et al	1962	(219)	Glucose 1-phosphate (50-60°C with polyphosphoric ester PPE)	Polyglycoside (50,000 w)
					Methyl glucose (same conditions)	Cellibiose
					Ribose (same conditions)	Polyribose
					Fructose (same conditions)	Polyfructose
		P.T. Mora P.T. Mora and J.W. Wood	1965 1958	(247) (248)	Glucose, phosphoric acid (140-170°C)	Polyglucose
		A. Thompson et al	1954	(249)	Glucose + hydrochloric acid (90°C 10 h.)	Gentiobiose, isomaltose cellobiose, maltose, sophorose, trehalose.
	Organic Acids	J. Oro' et al	1959	(203)	CH_2,NH_2OH,H_2O (70-100 °C)	Formic, glycollic, lactic.
		S.W. Fox et al	1961	(200)	CH_4,NH_3,H_2O	Acetic, malic.

Table I - (continued)

Energy Source	General Compound Synthesized	Authors	Year	Ref.	Starting Materials	Synthesized Compounds
Thermal Energy	Simple Compounds	M. Kotake <u>et al</u>	1956	(250)	CH_4 + NH_3 (heat with $Al_2O_3SiO_2$)	HCN + $3H_2$ -60 cal.
	Hydrocarbons	J. Oro' <u>et al</u>	1966	(251)	CH_4,NH_3,H_2O (1000°C with silica gel)	Arenes of 1, 2, 3, 4, and more rings, aliphatics as well as aromatic. Even number C-atoms predominate.
					CH_4	Same, less aliphatic.
	Porphyrins	A. Szutka	1965	(252)	Pyrrole + benzaldehyde (180°C)	α,β,γ,μ -tetraphenylporphins.

AR: adenosine and ribose; AN: acrylonitrile; AMP: adenosine monophosphate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; GMP: guanosine monophosphate; A4P: adenosine tetraphosphate; CMP: cytidine monophosphate; UMP: uridine monophosphate; TMP: thymidine monophosphate; EtMtP: ethylmetaphosphate (EMP); PPA: polyphosphoric acid; PPE: polyphosphoric ester; AICA: 4-amino-imidazole 5-carboxamide; AICAI: 4-amino-imidazole 5-carboxamidine.

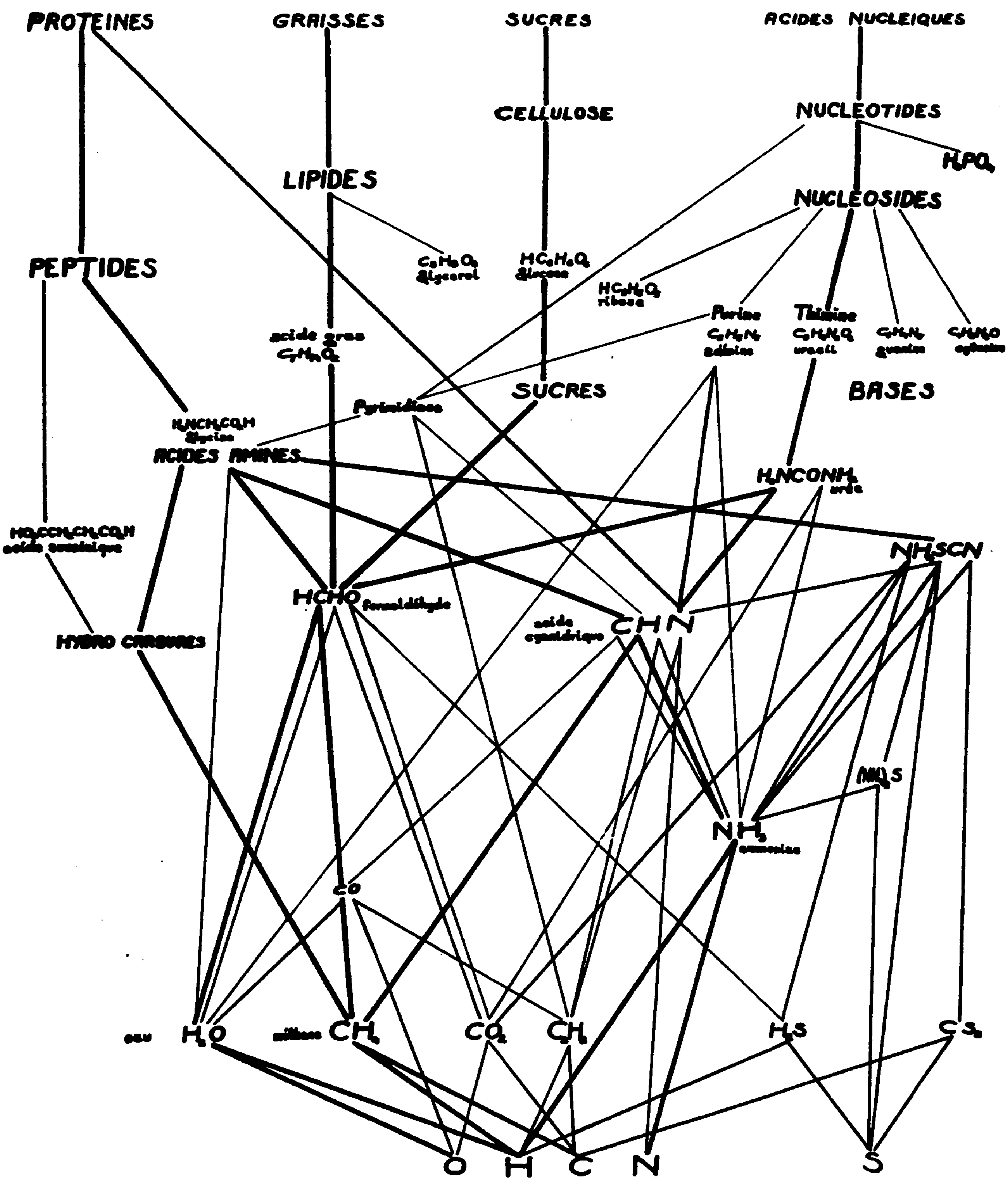


Figure 2: General scheme for the molecular evolution and abiotic synthesis.

EXPERIMENTAL PART:

Production of the Starting Material

We saw previously that the presence of carbon gases and sulfur gases were very likely, as well as nitrogen and ammonia, in volcanic gases. Hence if all those gases were available, it is, therefore, not too big a step to assume the presence of ammonium thiocyanate (NH_4SCN) as a possible constituent of volcanic exhalations. In fact, Gauthier(49) reported the presence of sulfo cyanates ($SCN.NH_4$), according to the following process:

$$CO_2 + H_{2S} + 2NH_3 \longrightarrow SCN.NH_4 + 2H_2O$$

This is not the only process which yields to the formation of the ammonium thiocyanate. We can summarize the others as:(253-254-137)

$$NH_3 + CS_2 \longrightarrow H_2S + HSCN \rightleftharpoons NH_4SCN$$

$$HCN + NH_3 + (NH_4)_2 S + S \longrightarrow NH_4SCN$$

$$NH_3 + NH_4 + H_2S + H_2O \longrightarrow NH_4SCN$$

The following organigram (Figure 3) indicates how from very simple starting chemicals we can obtain the ammonium thiocyanate.

Cell-Like Structures from NH_4SCN under Simulated Primitive Earth Conditions without U.V. Irradiation

Over 2 decades ago a group of experiments on the origin of life was performed by Herrera using ammonium thiocyanate and formaldehyde.(111)

An experiment was chosen(255) in which it was claimed that simple mixing of 7 ml 37% formaldehyde and 3 gr. ammonium thiocyanate resulted in "life-like forms".

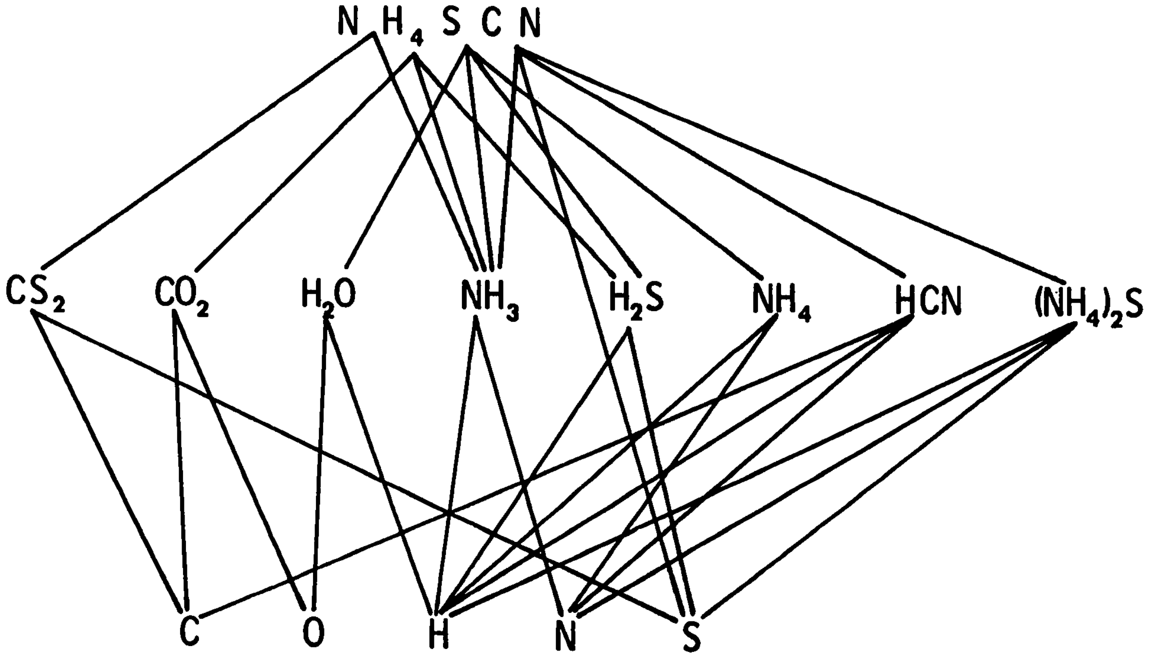


Figure 3: Formation of the ammonium thiocyanate.

After mixing the materials, the colorless liquid became slightly red in a few seconds, and finally a golden yellow after 1 hour. Microscopic examination (without a cover glass) revealed a high density of spheres 1-5 μ in diameter. (Figure 4). A drop of water was applied so it sightly overlapped onto the drying mixture. Vigorous streaming of the reaction mixture into the water resulted. The spheres darkened considerably and structures of greater size and complexity, 10-100 μ in diameter were seen (Figure 4). When the drying spheres were rehydrated with 1% solutions of methylene blue, trypan blue or Ponceau S they concentrated the stain.

Internal fluid regulation is one of the primary properties of life. The contractile vacuole is used by some unicellular organisms for this purpose.(256) In many cases we observed motion of an internal vacuole-like structure toward the boundary and its subsequent extrusion. The boundary then closed, reformed, and resumed its circular shape.

Effect of Different Parameters

Because the UV-radiation was one of the most important source of energy for chemical evolution,(257) we considered its effect on the reaction. We used diluted reactant solution to test for the catalytic effect of UV. on concentrations more likely to have occured in a primitive Earth environment. A typical starting mixture consisted of equivolume of 0.5M NH_4SCN and 1.4M fromaldehyde. Samples were subjected to darkness, room light (fluorescent), and UV-radiation (Pen-ray lamp model SC-1 with the strongest wave-length at 2537Å (Figure 5), from Ultraviolet products). The dark reaction and the second one did not show any sphere, even after 24 hours. Whereas the irradiated sample displayed spheres after 15 minutes.

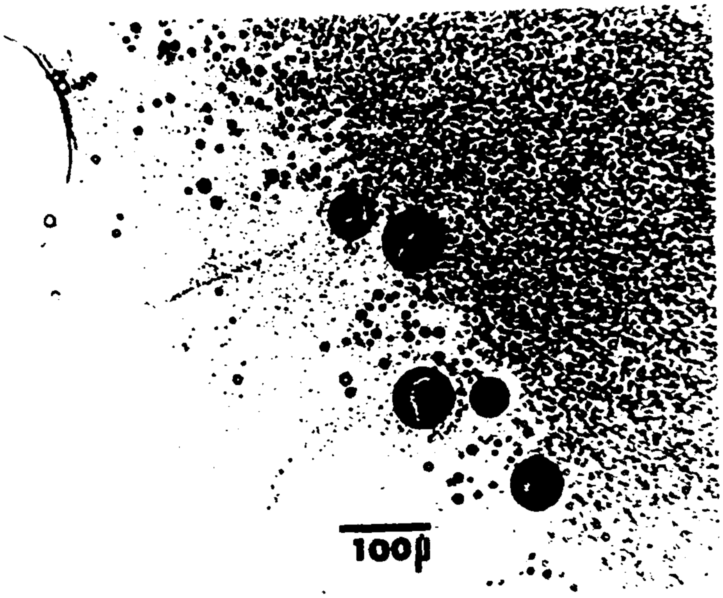


Figure 4: Multitude of small spheres from a 7 ml 37% formaldehyde. 3 g NH_4SCN mixture. The dark vacuolated spheres were formed by rehydration of the drying drop.

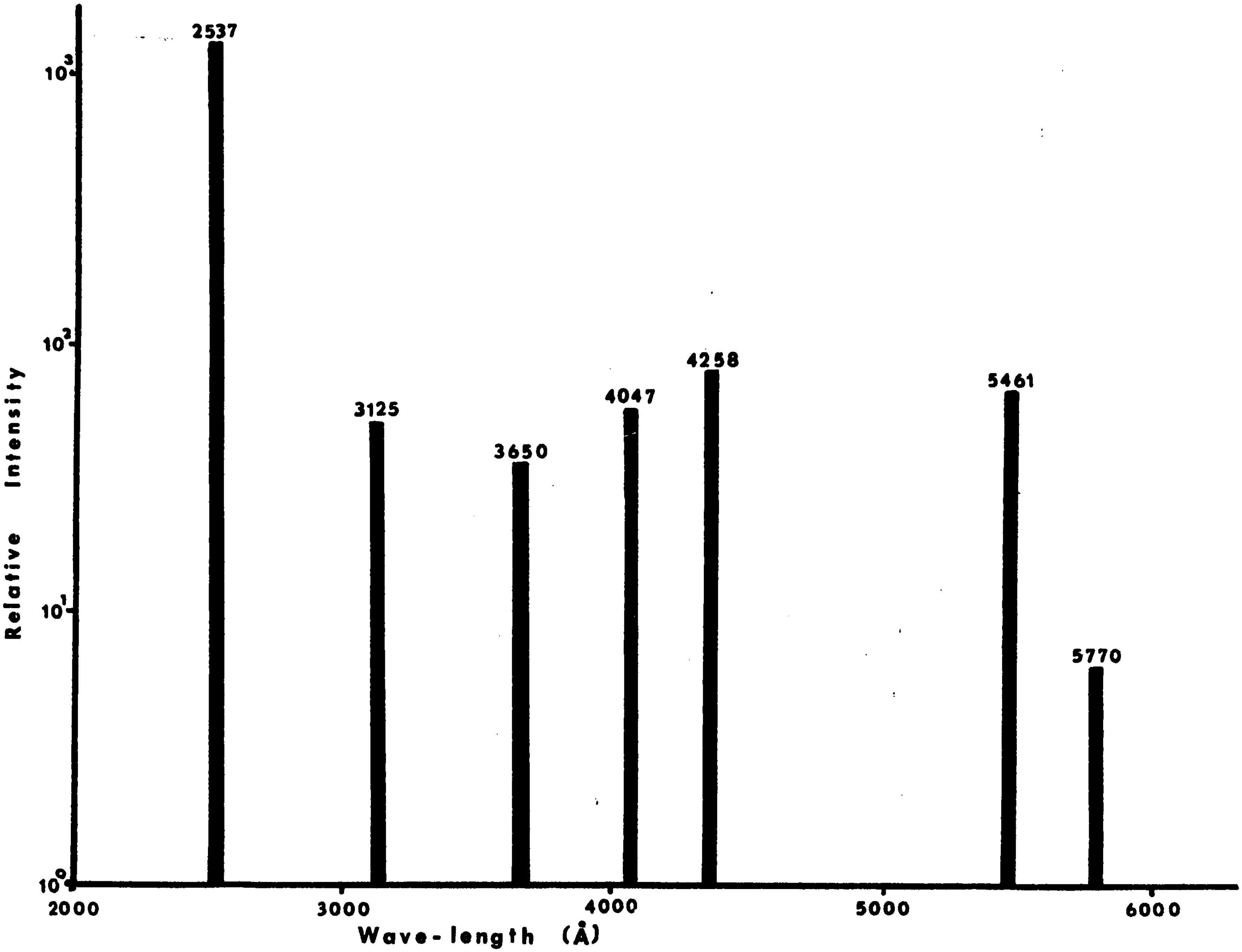


Figure 5: Typical spectrogram of Pen-Ray quartz lamp (Ultra-violet product)

The mixture of (NH_4SCN + CH_2O) with 7 c.c. of 37% CH_2O plus 3 gm. of NH_4SCN represents a system in which the ratio of AT. and CH_2O is: $N_f/N_{At} = \frac{1}{2}$ on a molecular scale. The Herrera system (10 ml. of 10% formaldehyde and 10 ml. 0.1M AT.) has a ratio of N_F/N_{AT} = 33/1. This ratio was calculated in order to see how the concentration of AT. yielded "spheres". Since irradiated formaldehyde does not give any sphere and ammonium thiocyanate does, the reaction depends on AT. The formaldehyde itself works like a catalyser. With ratios of 33/1,100/1, 200/1,300/1, a white cloud indicative of spheres appeared after 5 minutes of U.V. For 400/1 we had spheres after 10 minutes. Any more U.V. seems to have no affect, but we were able to observe coacervates about 5-15μ on the surface of the mixture. The ratio 500/1 did not reveal very much even after 15-30 minutes of irradiation. Using equivolume mixtures of 0.05 M NH_4SCN with 0.5, 1.5, 3.0, 6.0 M formaldehyde solutions and subjected to UV-radiation (up to 24 hours). The microscopic phenomena were essentially the same in all cases.

The time of irradiation was also another important parameter. Therefore 4mls. of 0.1 M of AT. was placed in a photometric test tube and irradiated with UV. The percentage of transmission was observed after 1, 3, 5, 10, 15, 20, 25, 45, 60 and 90 minutes. (Table 2)

However, it is obvious that even if the turbidity has reached a maximum around 30 minutes (Figure 6), we did not obtain the maximum yield at that time.

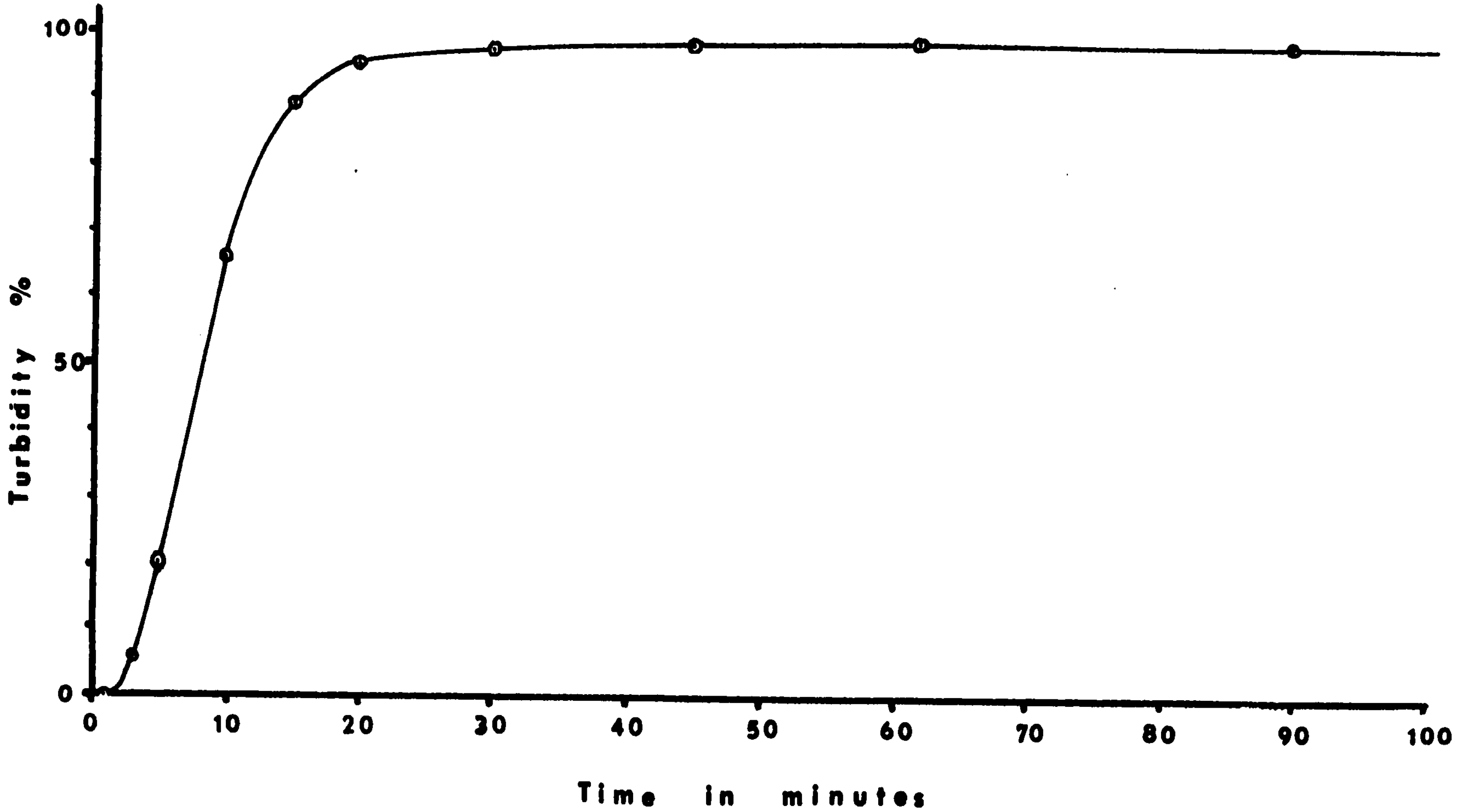


Figure 6: Turbidity saturation curve.

Table II - Transmission percentage of irradiated AT.

Time (min.)	% transmission
1	100
3	93
5	80
10	34
15	11
20	5
30	2.5
45	2.0
60	1.5
90	1.5

Ph Variation

It was interesting to see how the Ph of the irradiated solution (NH_4SCN, 0.1M) was changing as function of UV. exposition time. For this observation we used a Corning Ph-meter (model 7). Figure 7 shows the experimental Ph variations listed in Table III. We can notice an increase of the Ph at the beginning of the irradiation and a few minutes later a state of equilibrium. At the same time an increase of the temperature was recorded. Then, both the Ph and the temperature were a direct function of the irradiation time.

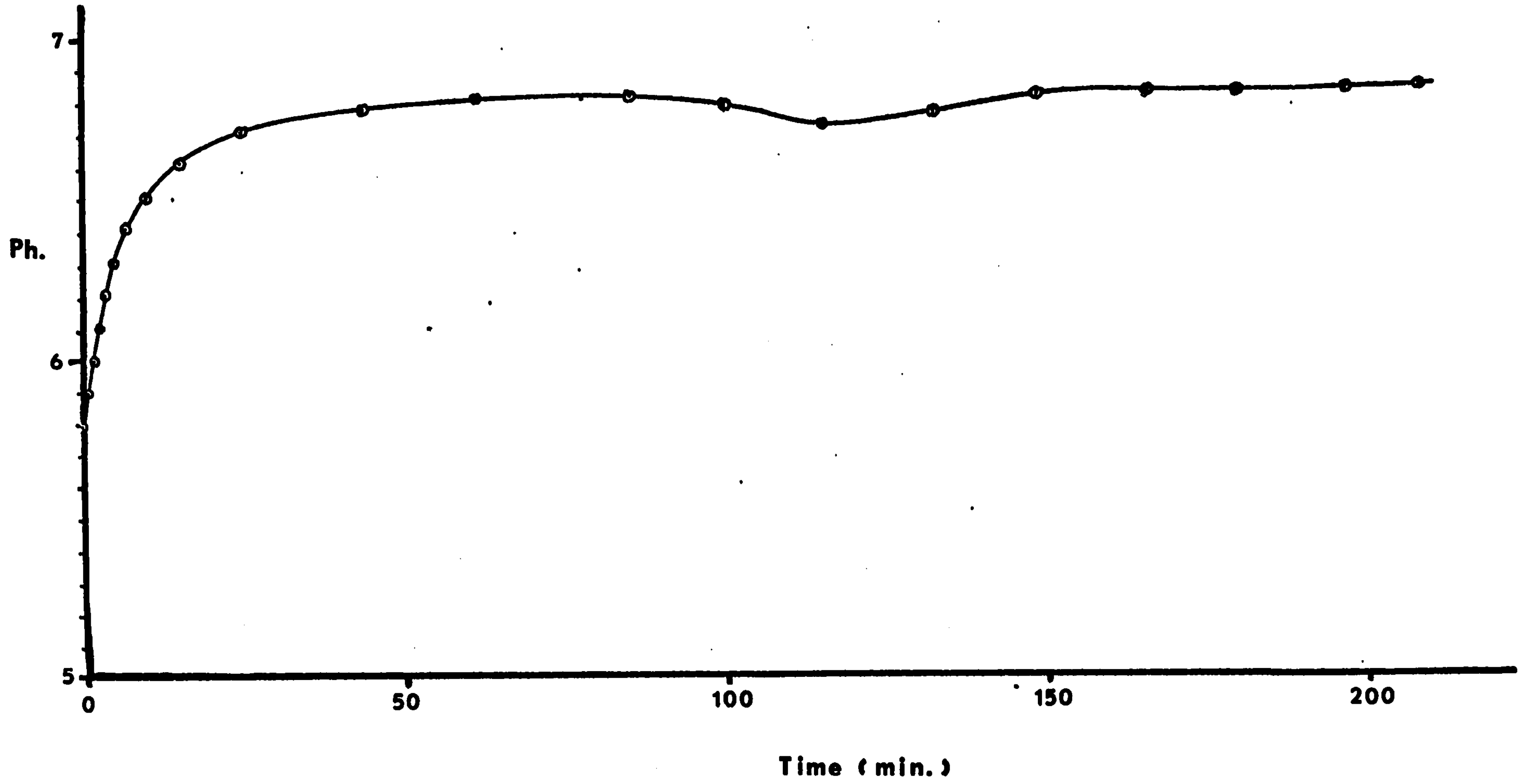


Figure 7: Ph variation curve.as function of time.

Table III - Ph variation as function of the irradiation time.

Time (min.)	Ph ± 0.01	Temp. ± 0.1
0.	5.79	21.7
2.	6.0	22.2
3.	6.1	22.6
4.	6.2	23.0
5.	6.3	23.5
7.	6.4	23.9
10.	6.5	24.3
15.	6.6	25.2
26.	6.7	26.8
41.	6.75	28.2
56.	6.8	29.0
86.	6.8	30.8
101.	6.77	30.8
116.	6.71	30.8
127.	6.75	31.1
132.	6.76	31.1
147.	6.82	31.2
162.	6.82	31.4
177.	6.82	31.4
192.	6.84	31.5
207.	6.85	31.6
222.	6.88	31.8
237.	6.90	31.8
242.	7.01	31.8
357.	6.95	31.8
21h02	7.10	29.1
25h02	7.19	29.5
26h17	7.19	30.0

Since at 90 minutes, the Ph and the temperature reached approximately the saturated point, we used this period of irradiation as the standard one, most of time. It was found also, that this time of UV. yielded to more complex microspheres. A longer time had a destructive effect and less amino acids were synthesized.

Solubility of the Microspheres under Different Chemicals.

In order to see how the integrity of the microspheres was affected in another medium, a certain amount of spheres was made from (0.1M NH_4SCN) and placed in a beaker in which various acids and bases were added. Observations were carried out after 5, 10, 30, 60 minutes and 100 hours:

1. AT and Acetic acid: No appreciable difference was noted macroscopically as well as microscopically, but up to 90 minutes we had an abundance of spheres, after 4 days, the solution was clear. The drop left on the slide showed, after 18 hours upon rehyderation, hydrophobic tendency and no sphere.

2. AT and NH_4OH: No difference was observable either way after 10 minutes still many spheres were apparent. 30 minutes later we had spheres plus greenish circular forms. After 18 hours, the spheres were less abundant and smaller. After 100 hours no more sphere were seen.

3. AT and Chlorophyl: In addition to the normal AT spheres some others were seen on the surface of the solution, but they were quickly brushed away. After 24 hours, the phenomena was still existing, after 100 hours, the spheres were apparently accompanied by some crystals.

4. AT and Metaphosphate: Spheres and crystals were present after 100 hours.

5. AT and Dimethylacetamide: Same pattern was observed, except after 100 hours where we had only crystals.

6. AT and H_2O_2: Many spheres were immediately apparent as well as many coacervates, but after drying, they retained their morphology for a few minutes longer until their "internal" water evaporated. On rehydration, not all the spheres reformed. Crystals were the only things present after 100 hours.

7. AT and CH_2O: Spheres remain the same, but after 100 hours we had crystals only.

8. AT and Propanol: After 100 hours crystals, and spheres were observed.

9. AT and H_2SO_4: Spheres seem to have dissolved. After 24 hours, no sphere was apparent.

10. AT and KCL: Spheres remained normal. As slide almost dried, rehydrated again, the spheres reappeared, agglomerated in a layer form and embedded in a gel like mass. They were still there after 1 day.

11. AT and Urea: After 72 hours, we had crystals only.

If we used those substances as solvant with AT. under UV, we would be able to determine which substance inhibits the reaction:

1. HAC,AT,UV: It was clear at the beginning, but after 30 minutes of irradiation it was full of spheres, even after 100 hours. But they do not take up Janus green B.

2. NH_4OH,AT,UV: Clear at the beginning, slightly cloudy after 15 minutes, 24 hours later the solution was clearer with only a small deposit at the bottom. (Spheres present but less abundant).

3. Chlorophyl AT,UV: These two components do not mix, A light green layer of the chlorophyl rests on the AT. At the beginning, without UV. we were able to see some coacervates at each level of the solution. After 90 minutes of UV. it was full of spheres, coacervates, and crystals.

4. NaOH,AT,UV: No spheres were formed even after 4 hours of irradiation.

5. Ethylmetaphosphate,AT,UV: Again we had two distinct layers, the AT on the top. On mixing the two, heat was generated. After 2 hours of UV. many spheres, coacervates etc. were present.

6. Di-methylacetamide AT UV: Stays colorless for about 10 minutes. then flaky white crystals appeared and finally a white cloud of spheres. After about 2 hours, it seems that the spheres are being replaced by a clear pale green solution.

7. H_2O_2 AT UV: It was clear at the start and after 15 minutes some cloudiness appeared at the bottom (not like the others) formed of crystals and spheres. After one hour the solution appeared clear microscopically; some crystal particles were present. (ph $\leqslant 2$)

8. CH_2O AT UV: We had spheres after 15 minutes.

9. Propanol AT. UV.: Appeared cloudy after 30 minutes in the upper half, and completely cloudy after 1 hour.

10. Sulfuric acid AT. UV.: It was clear at the beginning. The Pen-ray lamp became a bright yellow, coming from the sulfur after 15 minutes and the solution started to be cloudy. After 30 minutes of examination showed spheres and coacervates at all levels. (ph = 2)

11. HCl AT UV: (at ph 1.5) It became cloudy in minutes, and after 30 minutes clumps of spheres, forms, crystals, etc. were seen.

12. KCl AT. UV.: The irradiated solution was looked at after 20 minutes. The top was a mucky yellow while at the bottom it was a cloudy white. Examination from the top showed a multitude of

spheres both alone and in clumps. An additional 20 minutes of UV resulted in a somewhat lessening of the spherical form although they were still multitudinous.

13. Urea AT. UV.: After 20 minutes UV. it was cloudy and upper half, many spheres and crystal particles were seen.

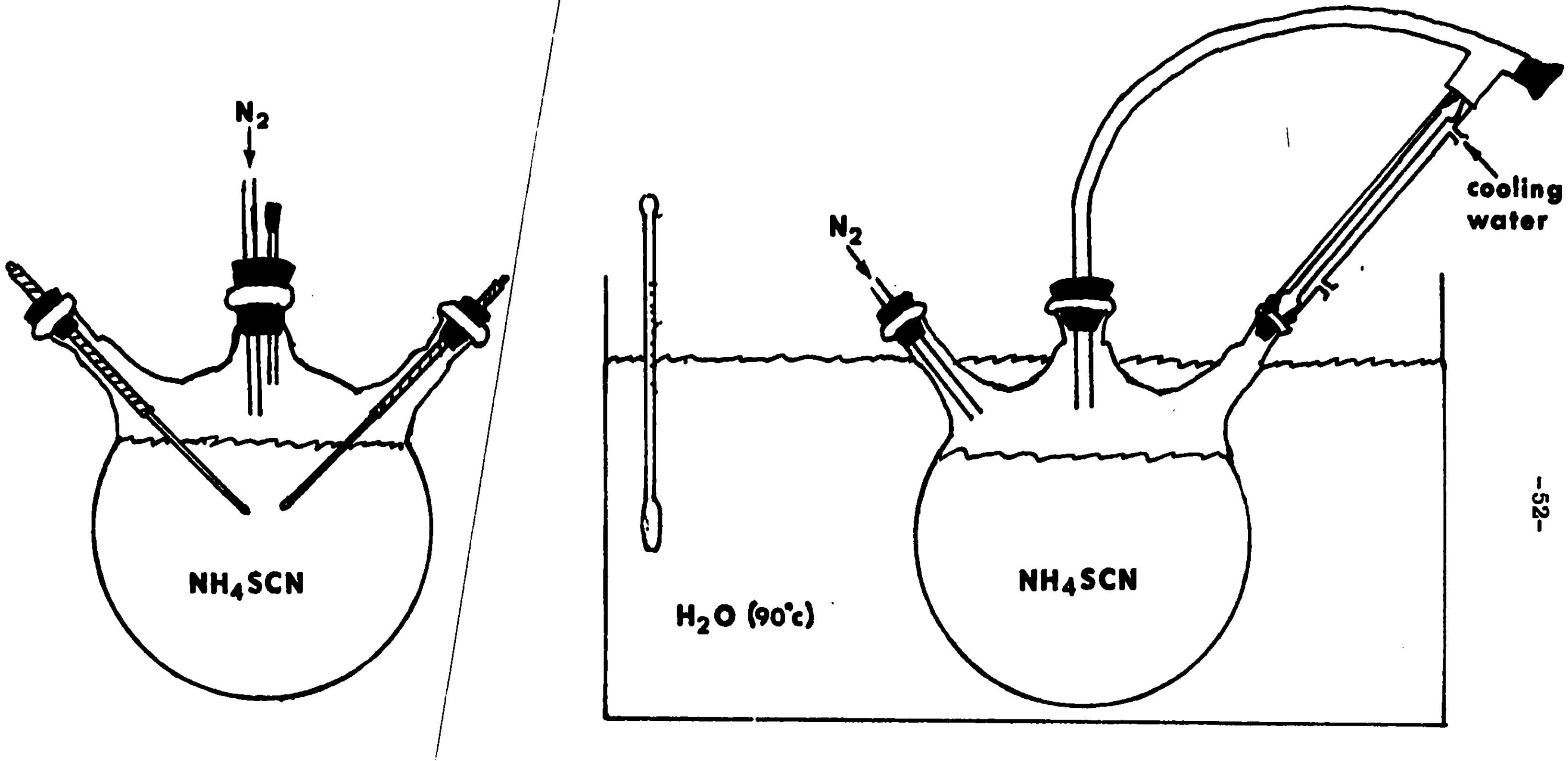
Formation of Spheres Using Heat and UV under Nitrogen Atmosphere

As all the previous results were obtained with UV irradiation under room atmosphere (oxidizing), it was important to see if we were able to produce spheres under nitrogen atmosphere (reducing), with either UV irradiation or heat. Therefore, we put 100 ml. of NH_4SCN 1 M and 20% formaldehyde in equivolume in a three-neck flask. It was mounted in such a way that we had a closed system with reflux set-up. (Figure 8) With UV irradiation we had previously many spheres after few minutes only, with no obvious difference under microscopic observation after 6 hours of heat (90°C), many small spheres were seen. Rehydration resulted in much streaming, coacervates and motion. After 5 days the spheres were still there.

Ultra-Violet Spectrophotometry

At this stage it was necessary to establish the UV spectrum of ammonium thiocyanate, in order to see if the characteristic curves of different amino acids were possibly included in the AT one.

An aqueous solution of 0.1M ammonium thiocyanate was placed in a fused silica cuvette and was measured against a water reference in a Unicam-800-UV-spectrophotometer. Also, an identical solution was irradiated up to 3 hours with a submerged Pen-ray quart UV lamp (model SC-1,



UV

Heat

Figure 8: Set-up for reducing atmosphere.

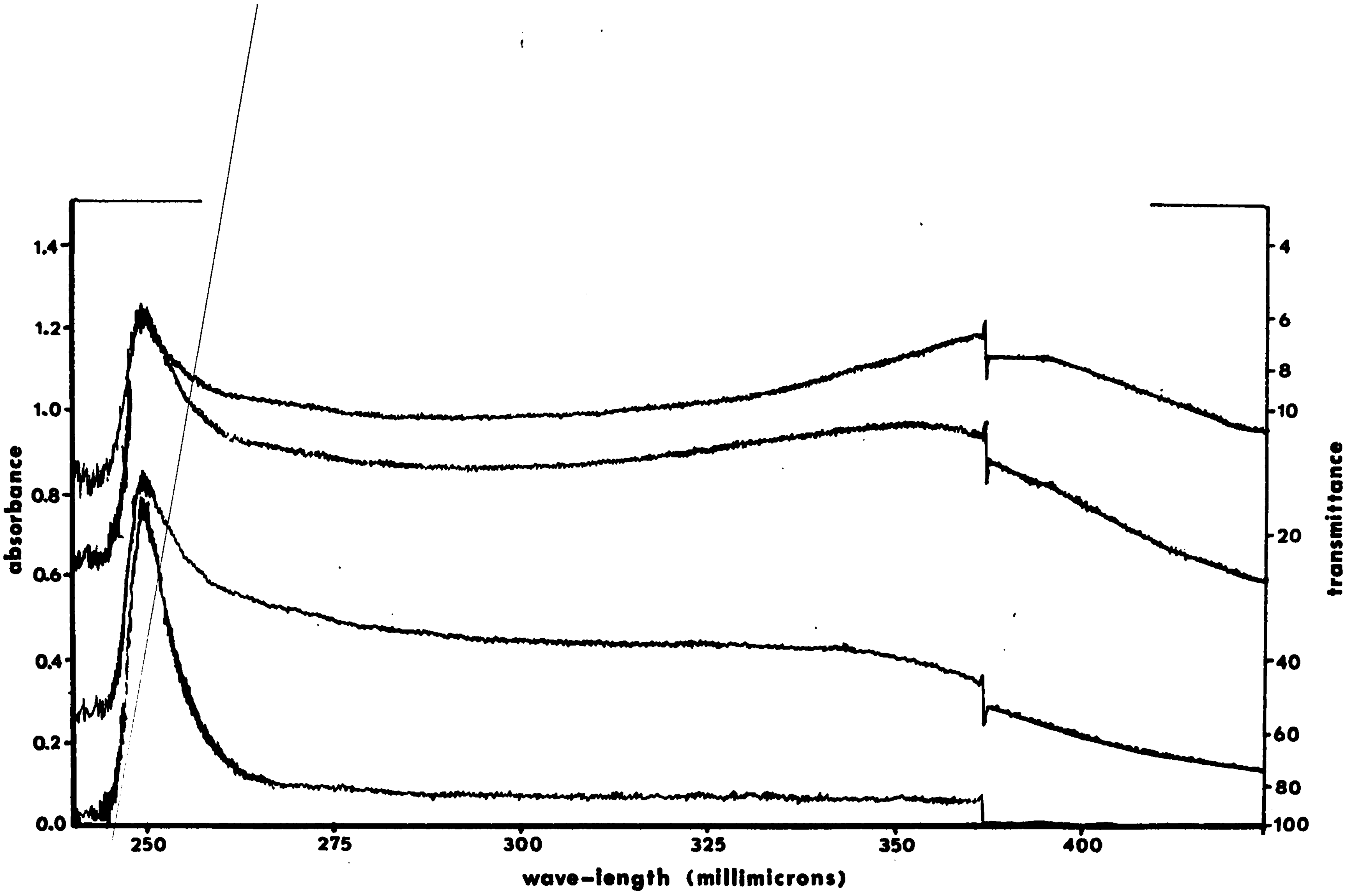


Figure 9: Characteristic UV-spectrum of irradiated AT.

Ultra-violet products), and was observed in the same manner with different standing time (Figure 9). However, even if this work was necessary, it was not relevant because the UV-spectroanalysis does not give enough information and resolution to permit any definite identification. The comparison of the guanine plus alanine and methionine spectrum to the AT one, is very inconclusive. Obviously an amino-acids analyser would have been of a great help at this point. This kind of study was, therefore, performed by a biochemist(143) who reported in particular the presence of the methionine (a sulfur amino-acid).

Note: Synthesis of this class of biocompounds fills another important gap in the development of an overall picture of how prebiological chemistry may have evolved on primitive Earth.

Morphological Investigation

We have already seen that ammonium thiocyanate and formaldehyde led to multitude of small spheres of 1-5 μ in diameter (Figure 4). The spheres darkened considerably and structures of a greater size and complexity, 10-100 μ in diameter were seen. In many cases we observed motion of an internal vacuole-like structure toward the boundary and its subsequent extraction. The ammonium thiocyanate itself led, after irradiation, to microspheres of the same aspect as AT and CH_2O (Figure 10) but if we let stand the irradiated solution for a few hours we observed first a formation of bigger spheres and chains (Figure 11). Furthermore the size of the aggregates continued to increase and fibrillar agglomeration was observed (Figure 12). In order to study these structures, we used an electron microscope (Jem-50) and chromium shadowing technique with a carbon evaporator (Edwards Type E-12E4). The chain

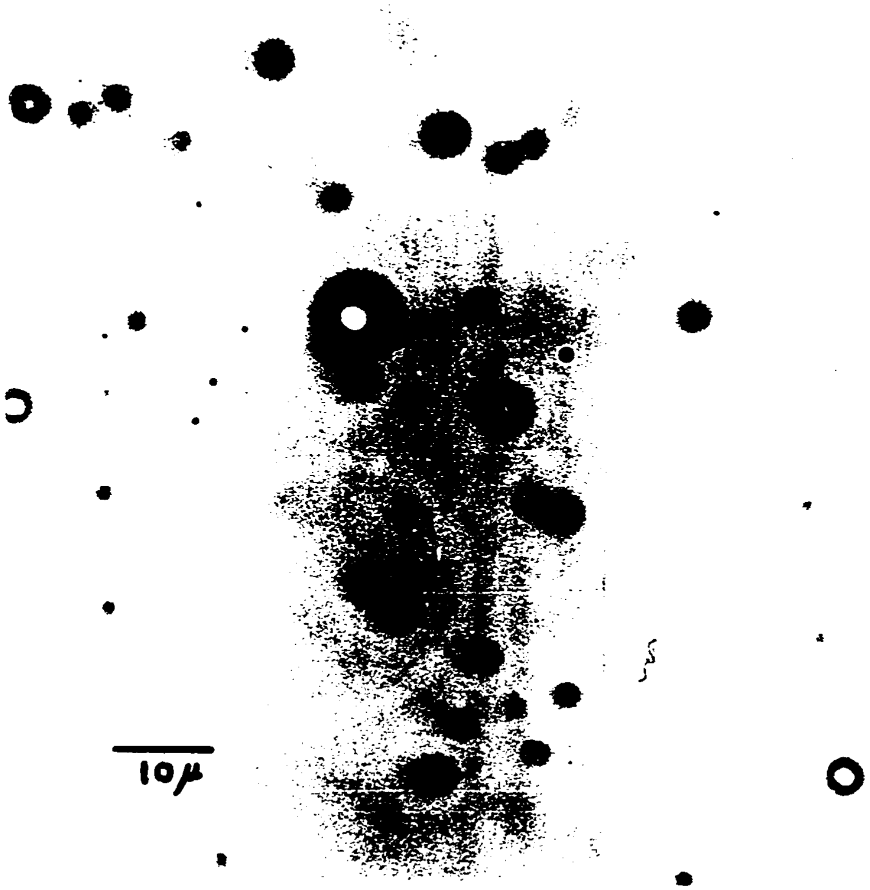


Figure 10: NH_4SCN microspheres formed after 30 minutes irradiation of a 1 M solution.

type or fibrillar aggregate seems to follow the general properties of the long chain molecules. (Possible contractility, mechano-chemical contractive forces, solvent-dependent or reaction-dependent, reduction of entropy factor, possible "wrap-around").(258)

The essential fact is that chain folding tends to occur in a precise form, probably to within atomic dimension. The regularity of this chain folding is usually sufficiently high. However the significant point is that even at simplest level of organization in single macromolecules there is the possibility of precise, three dimensional positioning of all the monomeric units without breaking the linear chain, holding the monomers together; (without this property it would be difficult to imagine molecular storage). Nevertheless, chain macromolecules are difficult to interpret, and there is no fundamental understanding of their polymerisation.

While it is likely that these organized structures contain no genetic information, and may not be precursors of life, we actually have too little detailed molecular knowledge of how genetic information determines the structure in living systems to judge the significance of morphological similarities. These particles (spheres) often show simple internal structure, and occasionally divide in two.(259) These phenomena are a valuable guide to our concepts of the possible order which we can expect from primitive macromolecules.(1)

Inevitable Appearance of Protocells on the Primitive Earth

During the past decade, the synthesis of many biochemical compounds under various hypothetical primitive earth conditions starting from various simple compounds has been accomplished. (Table I). However, Abelson(260) has stated that many of these experiments are not realistic from a geological point of view. It is our purpose to point out that the formation of cell-like structures containing biochemicals may be considered an inevitable event given any one of the presently conceived primitive earth geological conditions.

The primitive earth atmosphere is generally considered to have been reducing but a number of workers in recent years have not accepted this hypothesis.(261) The different compositions can be summarized in their extremes as (a) CH_4,NH_3,H_2O,H_2 -reducing(262) (b) CO,CO_2,N_2,H_2S H_2O - non reducing.(43) The evolution of the atmosphere, has taken the following stages: very reducing, about neutral, actually oxidizing.(45)

Starting from any one of the above compositions, experimental observations showed that biochemical microspheres can be produced. The transition from model (a) to microspheres containing amino acids and peptides has been shown by Grossenbacher and Knight.(174) These authors found that electrical discharge in their hypothetical atmosphere produced microspheres 50 - 800 Å in diameter containing organic material.

If model (b) is used, there exist similar observations which lead to the microspheres. Heating of NH_4CN, a likely compound in this model, produced microspheres containing amino acids and peptides.(207) Starting from an atmosphere of CO_2,H_2S,N_2, and H_2, electric discharge produced NH_4SCN.(137) Subsequently, it was shown that this compound under the influence of ultraviolet radiation would yield microspheres containing amino acids.(143-144)

Carbon dioxide and water, when bombarded by alpha particles, produced formaldehyde, acetic acid, an oxalic acid although the atmosphere was non-reducing.(3) A polymer of formaldehyde, paraformaldehyde, and water with inorganic catalysts, produced biochemical microstructures under the influence of sunlight.(112-113) There, it seems that carbon dioxide and water, via formaldehyde, in an oxidizing atmosphere can lead to microspheres.

It is also possible that the microspheres can be produced from cosmochemical compounds. Hydrogen cyanide, ammonia, and water are abundant in cometary atmospheres, and these molecules justify the use of ammonium cyanide as a starting material for prebiotic experiments.(188) The newlyformed earth could have had a substantial amount of complex organic compounds which were formed in the solar nebula under thermodynamic equilibrium conditions.(263)

It is of interest to note that the presence or absence of a reducing atmosphere does not appear to be essential to the cell-precursor formation once the initial material has been formed. The synthesis of the biopolymers could take place in any of the three primary stages in the evolution of the earth's atmosphere. Thus it appears that using all presently conceived notions of the earth's atmosphere as well as chemicals found in comets, protocells containing biochemicals are inevitable.

The chief outstanding problem in origin of life work is now shifted from the synthesis of single molecules to study of how these primitive micro-structures interacted with the environment and the origin of metabolic processes.

Table IV

Synthesis of Microspheres under Different Atmosphere

Starting Materials	Treatment	Atmosphere
CH_4,NH_3,H_2,H_2O	Electric Discharge	Reducing(174)
Paraformaldehyde, inorganic catalysts H_2O	Sunlight	Oxidizing(112-113)
NH_4CN, H_2C	Heat	Oxidizing(207)
NH_4SCN, H_2O	Ultraviolet radiation	Oxidizing(143-144)

Possible Abiotic Origin of Precambrian Microfossils

Biochemical and electron microscope investigations of selected precambrian rocks have shown evidence for the existence of microfossils believed to be contemporary with the rocks.(264-265) Related findings have revealed indigenous amino acids and alkanes in the samples.

Over the past decade it has been shown that a large variety of biochemicals can be formed from simple gases and liquids under the action of high energy sources using hypothetical primitive earth conditions (for reviews see Table I). Recent results have also demonstrated that the transition from simple molecules to macromolecules is often accompanied by a separation of microstructures from the medium.(266-267-174) These findings and those to be presented here support the suggestion of a possible abiotic origin of the microfossil forms found in the precambrian rocks.

As a starting material for our experiments we used ammonium thiocyanate (NH_4SCN) which is a known product of juvenile volcanic gases(49) and has been produced under simulated primitive earth conditions.(137) Previously it has been showed that a small amount of methionine is synthesized by UV-irradiation of NH_4SCN(143) and that cell-like structures are produced in the presence of formaldehyde. In the present section we report evidence demonstrating a resemblance between these abiotically produced microspheres and the microfossils on the basis of (a) morphology, (b) chemical composition and (c) physical properties.

We irradiated aqueous solutions of NH_4SCN (0.01-2M) with a submerged Pen-Ray quartz lamp (Ultraviolet Products SC-1) for various times up to 315 minutes. All reaction solutions were first filtered with a 0.22 μ Millipore filter. After a few minutes of irradiation, the solutions showed a white turbidity. We placed a drop of the products on a slide and examined it with the light microscope. The size varied up to 10 μ in diameter and was an increasing function of initial reactant concentration and irradiation time.

About 10 minutes after the irradiation had ceased (and a heavy white suspension was obtained), the microspheres began to form aggregates of larger spheres and chains. After standing for 2 hours, the size of the aggregates continued to increase (up to 200 μ) and fibrillar aggregates were observed (Figure 12). After 100 hours nearly all the microspheres had formed large aggregates. Higher concentrations and aging increased the aggregating tendencies. Our observations are similar to those reported by other workers investigating the behavior of polypeptides precipitated from solution.(268) In order to see how the microspheres would behave in the presence of adenosinetriphosphate (ATP), which has been synthesized abiotically,(242) we added microsphere

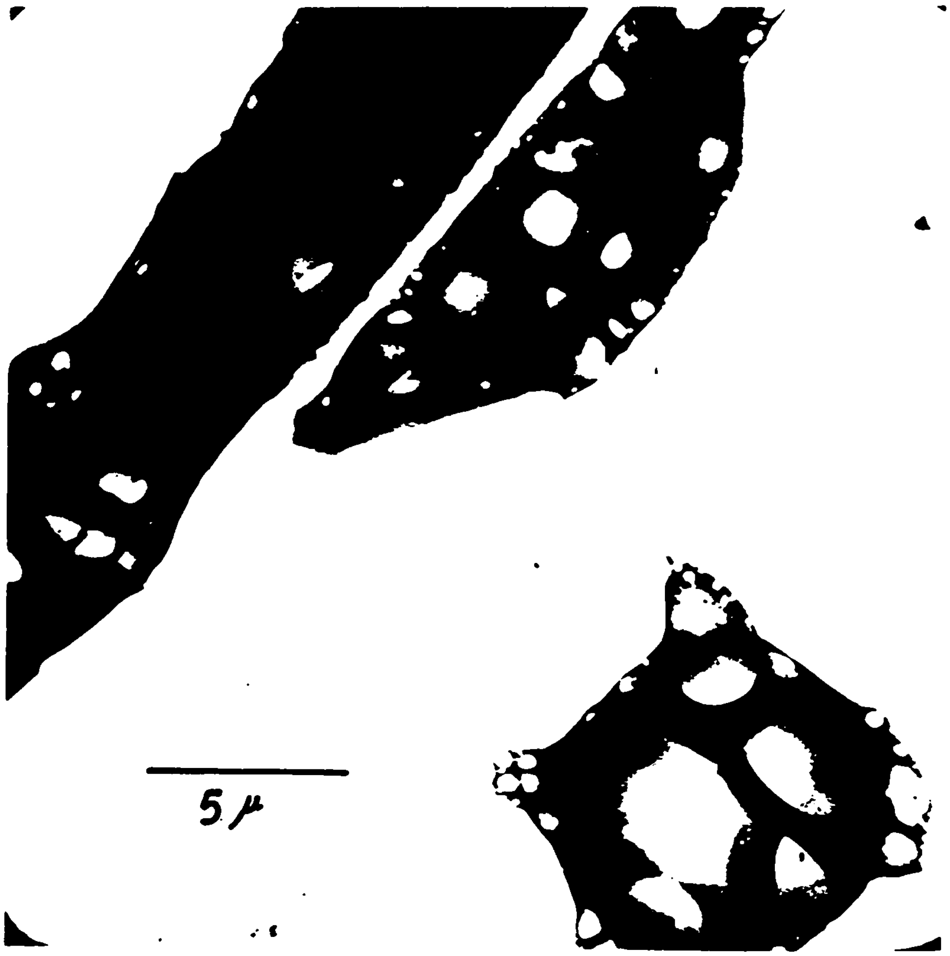


Figure 13: Electron micrograph of microsphere aggregates formed by incubation with 0.001 M ATP for 24 hours.

suspensions to 0.001M(242) ATP solutions. The aggregation proceeded more rapidly and there was more surface contact between the microspheres forming the aggregates than previously. Figure 13 shows electron micrographs of these aggregates.

The resemblance of the microsphere aggregates to precambrian microfossils is shown in Figure 14. The interior structure and dimensions appear to be similar. Since all reactant solutions were sterilized by filtration, irradiated with UV-light, and kept in closed tubes, and since these forms appeared consistently, the possibility of non-specific contamination is effectively ruled out.

Since our microstructures resembled microfossils we next investigated their stability. In tests for solubility, the microspheres were found to be insoluble in water, dilute HCl, dilute KOH, methanol, propanol, and benzene. We placed a drop of the product suspension on a microscope slide and allowed it to dry. Then water was added to the dried matrix. The microspheres appeared to be unaffected by this process which was carried out several times on the same drop. We also placed a drop of microsphere suspension on a slide placed in an oven at 100°C for 1 hour, and rehydrated it afterwards. Another drop was held at - 20°C for 1 hour. In both cases, the microspheres appeared to be unaffected with regard to gross morphology.

It seems that under common natural conditions, the microspheres maintain their integrity and thus could easily have become fossilized. Their stability with respect to heat and drying shows that their survival would be quite plausible.

Studies have been reported concerning the biochemical composition of ancient rocks which contain microfossils. To consider the possible relation of this to our own investigation, we placed 0.264mM NH_4SCN in 5 ml H_2O irradiated with a Pen-Ray lamp for various times under a nitrogen atmosphere in an ice water bath. The product was hydrolyzed in 6 N HCl under nitrogen for 20 hours at 105°C. Then it was evaporated, dissolved in H_2O, and $AgNO_3$ was added until no further precipitate appeared. The supernatant was collected and dilute HCl was added until no more precipitate was formed. The final supernatant was collected, evaporated to dryness, and analyzed on a Technicon Amino Acid Analyser (Table V). These results may be compared to those of Schoff et al(264) who considered the amino acid composition of selected precambrian rocks. This part was performed at the Biochemistry Department, Pennsylvania State University, University Park, Pennsylvania 16802, U.S.A., because of lack of equipment (Amino Acid Analyser) at Sir Georges Williams University.

Abelson(269) has found that glycine and alanine have considered the amino acid content of several bacterial and viral proteins for their glycine/alanine ratio.(270) When taken together, the overall glycine-to-alanine ratio of cytochrome C (Neurospora), ferridoxin (Clostridium), tobacco mosaic virus, ribonuclease (Aspergillus), tryptophan synthesase (E. coli), and azurin (Pseudomonas) is 1.0. This ratio may be compared to the reported ratio of 60 for precambrian samples. The large differrence between contemporary organisms and the microfossils cannot be explained by a difference in amino acid degradation rates. It is of interest to note that our synthetic (abiotic) glycine/alanine ratio of 14 (Table V) is significantly closer to the fossil ratio.

Table V - Amino Acid Analysis of Irradiated NH_4SCN Microspheres Following Hydrolysis

Time of Irradiation (minutes)	Glycine µM/mM NH_4SCN	Alanine µM/mM NH_4SCN	$\frac{\text{Glycine}}{\text{Alanine}}$
75	0.14	0	-
195	3.22	0.23	14
315	0.38	0.17	4.23

The molecular mechanism involved in the formation of the complex thiocyanate structures described here is unclear. However, we have demonstrated that under plausible primitive earth conditions such formation is a spontaneous occurrence. Furthermore, we have observed morphology, chemical composition, and stability also found in geological microfossils. These results suggest that the microfossils may actually be remnants of cell precursors or that the earliest cells possessed a biochemistry significantly different from that found in present day organisms. In any case, the need for more careful evaluation of the significance of these microfossils is clearly pointed out.

Catalytic Activity

To study the possibility of localizing catalytic activity into the spheres, an aqueous solution of 0.5M AT and 0.5M $ZnCl_2$ plus 3.33M formaldehyde was prepared. (Previous results in the pyrocondensation of amino acids to spheres have indicated that zinc incorporation can lead to a localized "ATPase"-like activity). The solution was irradiated by a rack of germicidal lamps for 1.5 hour. After drying, various amounts of the resultant solid (compound A) were added to 1 ml aliquots of 0.002M ATP and stirred for 17 hours at room temperature. Insoluble material was removed and analysis of the supernatant by the molybdate test for free phosphate and the luciferase test for ATP revealed that a small but definite amount of hydrolysis could be due to the amount of compound A. We tried to see if we had an ATPase activity for irradiated 1M aqueous solution of ammonium thiocyanate. The Fiske and subbarow test was perfomed either for ATP and AMPase. We used a Unicam-800 spectrophotometer set at 660 mμ as analytical instrument. We investigated the solution just after the irradiation and after standing overnight. For the AMPase the results were not definite; negative just after the irradiation and positive after overnight. However for the ATPase activity the results were both positive, 5% just after irradiation and 10% after overnight. Therefore, even if the results are a little low (near the experimental accuracy), we can say that our microspheres have a definite but small catalytic activity for the ATPase.

THEORETICAL INVESTIGATION

Thermodynamical Consideration

We pointed out the appearance of organization in the NH_4SCN system upon irradiation with ultraviolet. It is therefore of interest to see how thermodynamics can explain this emergence of order and the resulting decrease of entropy.

The increase in order through chemical evolution seems to be in opposition to the laws of thermodynamics, which imply that any process taking place in nature must go toward a more random state.(271) Classical thermodynamics is concerned with equilibrium state and closed systems, that is system with no exchange of matter with the environment. However, living cells as well as our microspheres are open systems by their interaction with the environment through mass and energy exchange. Our system cannot be considered closed because it receives energy from the environment in the form of ultraviolet, and is dissipative due to irreversible heat developed and finally it exchanges matter with the surrounding medium by uptake of dyes and water. Our system is also in a non-equilibrium state because chemical processes are proceeding, but the microspheres are stable under different surrounding, and thus appear to approach a steady state.

Our system (irradiated NH_4SCN) can be considered as a stationary non-equilibrium state. A stationary state may, to a good approximation,(272) be related to a state of minimum entropy production per unit time. We have shown in the morphological section the stability of the microspheres against different environments. This property of being stable under external pertubation is one of the striking characteristics of stationary states corresponding to a minimum entropy production.

The thermodynamics of irreversible processes therefore would appear more appropriate for treatment of the system. Irreversible thermodynamics gives us a general method for the macroscopic treatment of irreversible processes. This form of thermodynamics is mainly concerned with the analysis of entropy production. The modern version of this theory due to notably Prigogine, de Groot and others, led to a new realization, namely closed systems strive to reach a state of maximum entropy; open systems try to reach a state in which minimum changes of entropy occur at some particular level. The new approach deals directly with the change of entropy with time, dS/dt. Therefore the balance equation for entropy has a basic role. This balance equation expresses the fact that the entropy of a volume element is a function of time, for two reasons: 1) entropy flows into the volume element, 2) there is an entropy source within the volume element. For open systems the change in entropy can be decomposed into two parts.

$$dS = d_eS + d_iS \qquad (= 0 \text{ for steady state})$$

The first term d_eS corresponds to the exchange of entropy with the external world, therefore d_eS may be positive or negative. The second term d_iS is related to the entropy production due to irreversible processes itself in the system. The second principle tells us that d_iS is positive definite.

$$d_iS > 0$$

Then dS could be greater, equal or lower than zero, dependent on the sign of d_eS. It is evident that non-equilibrium steady states cannot occur in isolated systems which do not permit exchange with the surroundings.

In our system the ultraviolet radiation corresponds to an entropy exchange with the external world. In this case it is negative corresponding to $d_eS \leq 0$. While the local chemical and physical phenomena in the system are all characterized by $d_iS \geq 0$, the ultraviolet radiation supplies the energy for organization with the final result of $dS \leq 0$. This corresponds with the condition of emergence of order, as an increase of entropy had led to a more random state. Furthermore, the fact that the organization of the microspheres increased during their evolution corresponds also to a decrease of entropy.

In the past few years, the possibility of such organization has been studied by Prigogine and his collaborators. He proved that irreversible processes taking place inside a thermodynamic system always lower the value of the entropy production per unit time. For this he considered the time variation of the entropy production per unit time in the particular case of a system which undergoes two simultaneous chemical changes. In this case the time variation of the entropy production per unit time is given by an internal term which is always negative(272)

$$\frac{1}{2}\frac{d_iP}{dt} = \frac{1}{T}\left(\frac{\partial A_1}{\partial \xi_1}v_1^2 + 2\frac{\partial A_1}{\partial \xi_2}v_1v_2 + \frac{\partial A_2}{\partial \xi_2}v_2^2\right) < 0$$

Note: See Appendix

and an external term with no definite sign:

$$\frac{1}{2}\frac{d_eP}{dt} = \frac{1}{T}\sum\left(v_1\frac{\partial A_1}{\partial \eta_r} + v_2\frac{\partial A_2}{\partial \eta_r}\right)\frac{d_e\eta_r}{dt}$$

From this he concluded that the internal irreversible processes always operate in such a way that their effect is to lower the value of the entropy production per unit time. Therefore a system in a state of minimum entropy production cannot leave this state by a spontaneous irreversible change. That means, if, as a result of some fluctuation it deviates from this state, internal changes will take place and bring it back to its initial state, which may be called a stable state. It is found also that the entropy of the stationary state is smaller than that of the initial one. In the case of a Knudsen gas (two phases communicating by an opening whose diameter is small compared to the mean free path) when the two phases are kept at different temperatures, one finds easily that the entropy of the steady state thus established is smaller than that of the initial uniform state. The difference is then proportional to $(\Delta T)^2$. (272) In other terms the energy flow necessary to maintain the steady state leads to an increase of order which varies continuously with ΔT.

In a recent paper, Prigogine and Nicolis(273) studied the possibility of instability in purely dissipative systems, and in particular, steady states involving open systems with chemical autocatalytic reactions, and transport processes such as diffusion, but no hydrodynamic motion. They found that it is possible to have steady state concentrations although the system is far from equilibrium. The authors refer to this phenomenon as "symmetry-breaking instabilities in dissipative systems". An interesting analogy is made with the famous Bénard problem (horizontal layer of fluid in which a temperature gradient is maintained by heating it from below in the gravitational field. At a well defined critical value of this gradient, instability occurs and convection begins).(274) This problem is characterized by a generalized entropy production which can only decrease in time.

$$\Delta S = -\frac{\text{kinetic macroscopic energy}}{T} < 0$$

As a result, instability occurs when the generalized entropy production takes the same value in the system at rest and in the system perturbed by the cellular convection motion. That is, the loss of entropy at the transition is related to the maintenance of an organization in space. It is also the case in our system where the loss of entropy corresponds to an organization in space in the form of cell-like structure. Prigogine and Nicolis write "it is impossible to avoid feeling that such instabilities should play an essential role in biological processes and especially in the first biogenetic steps. It is indeed clear that biological structures can only emerge in a dissipative medium and be maintained by a continuous supply of energy". This work of Prigogine provided a link between thermodynamics and our observations of organization in the ammonium thiocyanate system.

A microscopic and atomic explanation of this organization does not exist at this time according to Blais(268) who has studied similar problems in the field of polymer physics. As yet we know almost nothing about the mechanism by which the exclusion of randomness is achieved.

Although for certain purposes we can consider life as a stationary state, it never is in thermodynamic equilibrium, and conventional diagram of state has little meaning for it. Possibly the most generalized statement characterizing life is to say it opposes randomness. We must therefore look for order pattern. The fact that our microspheres show catalytic activity and lead to aggregation (opposite of random), suggests some degree of preferred conformation or order.

CONCLUSION

These experiments have demonstrated how simple compounds likely to have been on the primitive earth can lead to the formation of cell-like structures which could have served as matrices for subsequent stages of biogenetic evolution. The spheres, capable of interacting with the environment (since they absorb dyes...) are thus possible precursors to a higher level of organization. However, no suggestion is made that these spherules are in fact "alive". Rather these experiments demonstrate one means whereby a delimited, localized could have arisen bearing a number of the characteristics from which a biodynamic system might evolve.

It is also worth noting that the microspheres have a strong tendency to form aggregates and that chain folding tends to occur. Nevertheless, chain macromolecules are difficult to interpret, and there is no fundamental understanding of their polymerisation. These phenomena, however, are a valuable guide to our concepts of the possible order which we can expect from primitive macromolecules.

Another interesting fact is that the kind of atmosphere does not appear to be essential to the cell-precursor formation once the initial material has been formed. Thus it appears that using all presently conceived notions of the earth's atmosphere as well as chemicals found in comets, protocells containing biochemicals are inevitable. It seems also that the microfossils may actually be remnants of cell-precursors or that the earliest cells possessed a biochemistry significantly different from that found in present day organisms.

The increase in order through chemical evolution seems to have been clearly pointed out. However, a microscopic and atomic explanation of this organization does not exist at this time, even in the field of polymer physics. As the most generalized statement characterizing life is to say it opposes randomness, the fact that our microspheres show catalytic activity and lead to aggregation and chain folding (opposite of random), suggests some degree of preferred conformation or order, and is in good agreement with the previous statement.

As Professor Oparin has pointed out, it may be essential to understand how life arose in order to comprehend its present nature. Therefore, future experiments in chemical evolution and biogenesis must be based upon a multidisciplinary approach.

APPENDIX

Entropy Production Due to Chemical Reactions(272)

The entropy production originated by chemical reactions in closed systems is

$$dS = \frac{dQ}{T} + \frac{A\,d\xi}{T}$$

where A is the "affinity" of the chemical reaction related to the chemical potentials (according to Th. De Donder) by

$$A = -\sum_{\gamma} \nu_{\gamma} \mu_{\gamma} \qquad (= 0 \text{ for equilibrium state})$$

μ_{γ}: its chemical potential

ν_{γ}: stoichiometric coefficient of component γ , ν_{γ} is related to the mole numbers n_{γ} $\quad dn_{\gamma} = \nu_{\gamma}\, d\xi$

ξ : is the degree of advancement or extent of reaction, introduced by Th. De Donder

the chemical reaction rate will be defined by:

$$v = \frac{d\xi}{dt}$$

Therefore the increases of the number of moles n_{γ} will be

$$\frac{dn_{\gamma}}{dt} = \nu_{\gamma} v$$

However, the entropy production in open system, starting with Gibbs formula

$$\left(dS = \frac{dE}{T} + \frac{P}{T}\, dV - \sum_{\gamma} \frac{\mu_{\gamma}}{T} dn_{\gamma} \right)$$

is given by

$$dS = \frac{d\Phi}{T} - \sum_{\gamma} \frac{\mu_{\gamma}}{T} d_e n_{\gamma} + \frac{A\,d\xi}{T}$$

Only the term due to the exchange of entropy with the surroundings is modified.

REFERENCES

1. Pattee, H.H. Experimental approaches to the origin of Life problem, Advances in Enzymology and Related Subjects of Biochemistry, F.F. Nord ed., Fordham Univ., New York, (Interscience Publishers, 1965), Vol. XXVII, p. 381-415.
2. Crick, F.H.C. Growth in living Systems Basic Books, in MX. Zarrow ed, (New York, 1961), p. 3.
3. Garrison, W.M., Morrison, D.H., Hamilton, J.G., Benson, A.A. and Calvin, M. Science 114, 416 (1951)
4. Miller, S.L. J. Am. Chem. Soc. 77, 2351 (1955)
5. Calvin, M. Am. Scientist 44, 248 (1956).
6. Blum, H.F. Am. Scientist 49, 474 (1961).
7. Fox, S.W. Nature 201, 336 (1964).
8. Fox, S.W. and Yuyama, S. Comp. Biochem. Physiol. 11, 317 (1964).
9. Dauvillier, A. The Photochemical Origin of Life (Academic Press, New York, 1965) p. 57.
10. Krauskopf, K.B. International Series in the Earth and Planetary Sciences, Introduction to Geochemistry (Mc. Graw-Hill Book Comp., New York, 1967) p. 607.
11. J.de Rosnay, Evolution chimique et systèmes prébiologiques, Ann. Chim. t. 2 (1967) p. 62.
12. Kant, I. Allgemeine Naturgeschiche Und Theorie des Himmels, Koenigsberg, (1755)
13. Laplace, P.S. Exposition du système du monde, Paris (1796)
14. Von Weizsacker, C.F. Z.Astrophys 22, 319 (1944)
15. Ter Haar, D. Rev. Mod. Phys. 22, 119 (1950)

16. Chandrasekhar, Rev. Mod. Phys. 18, 94 (1946)

17. Urey, H.C. Geochim. Cosmochim. Acta 1, 209 (1951)

18. Urey, H.C. The planets, their origin and development, (Yale Univ. Press, New Haven, Connecticut, 1952)

19. Urey, H.C. Scientific American October, 53 (1952)

20. Urey, H.C. Progr. Phys. Chem. Earth 2, 46 (1957)

21. Urey, H.C. Geochim. Cosmochim. Acta 26, 1 (1962)

22. Brown, H. Rare Gases and the Formation of the Earth's Atmosphere, in the Atmosphere of the Earth's and Planets, G. Kuiper ed., (Univ. Chicago Press, 1947)

23. Latimer, W.M. Science 112, 101 (1950)

24. Hoyle, F. Quart. J. Roy, Astron, Soc. 1, 28 (1960)

25. Hoyle, F. Frontiers of Astronomy Mentor, New York (1957)

26. Levin, B.Yu. in the Origin of Life on the Earth, Oparin ed., (Pergamon Press, New York, 1959) p. 67

27. Kuiper, G.P. Astrophys. J. 109, 308 (1949)

28. Kuiper, G.P. On the Origin of the Solar System, Astrophysics, Hyneck J.A. ed., (McGraw Hill, New York, 1951)

29. Alfvén, H. On the Origin of the Solar System, (Oxford Univ. Press, London, 1954)

30. Mc Crea, W.H. The Origin of the Solar System, Contemporary Physics 4 (1962)

31. Schmidt, O.J. A Meteoric Theory of the Origin of the Earth and Planets Dokl. Akad, Nauk SSSR 45, 229 (1944)

32. Levin, B.J. New Scientist 13, 323 (1962)

33. Von Weizsacker, C.F. Z Astrophys 22, 319 (1944)

34. Lyttleton, R.A. Month. Not. Roy. Astron. Soc. 121, 551 (1960)

35. Mestel, L. *Month, Not. Roy. Astron. Soc.* *119*, 249 (1959)

36. Krauskopf, *Introduction to Geochemistry*, (Mc Graw-Hill International Series in the Earth Planetary Sciences, 1967) chap. 21-5

37. Rubey, W.W. Geologic History of Seawater: An Attempt to State the Problem *Geol. Soc. America* *62*, 1111 (1951)

38. Kulp, J.L. Origin of the Hydrosphere *Geol. Soc. America Bull* *62*, 326 (1951)

39. Sagan, C. Origin of the Earth, *International Dictionary of Geophysics*, S.K. Runcorn and Urey H.C. ed., (Pergamon Press, London, 1965)

40. Poole, J.H.J. The Evolution of the Atmosphere, *Royal Dublin Soc. Sci. Pr.* *22 (n-s)*, 345 (1941)

41. Oparin, A.I. *The Origin of Life* (MacMillan Co., New York, 1938) (Dover, N.Y., 1953)

42. Bernal, J.D. *The Physical Basis of Life* (Routledge and Kegan Paul, London, 1951)

43. Rubey, W.W. *Geol. Soc. Amer. Spec. Paper.* *62*, 631 (1955)

44. Revelle, R. *J. Marine Res.* *14*, 446 (1955)

45. Holland, H.D. *Petrologic Studies: a volume to Honor A.F. Buddington,* (Geological Society of America, New York, 1962) p. 447

46. Szabo, A. Tudge, A. Macnanava, A. Thode, H.G. *Science* *111*, 464 (1950)

47. Krauskopf, K.B. *Introduction to Geochemistry*, International Series in the Earth and Planetary Sciences, (Mc Graw-Hill, New York, 1967) chap. 16

48. White, D.E. Waring, G.A. Volcanic Emanations, in Data of Geochemistry, 6th ed., chap. K, *U.S. Geol. Survey Prof. Paper* *440-K* (1963)

49. Gautier, A. *Compt. Rend.* *132*, 932 (1901); *150*, 1564 (1910)

50. Miller, S.L., Urey, H.C. *Science* *130*, 3370 (1959)

51. Whipple, F.L. *Science* *153*, 54 (1966)

52. Keosian, J. *The Origin of Life*, 2nd ed., (Reinhold book corp. New York, 1968)

53. Fox, S.W., Johnson, J.E., Vegotsky, A. *Science* *124*, 923 (1956) *Ann. N.Y. Acad. Sci.* *69*, 328 (1957) *J. Chem. Educ.* *34*, 472 (1957)

54. El'piner E., Sokoskaya, A.V. *The Origin of Life on the Earth*, Oparin A.L. ed., (I.U.B. Symposium Series, Pergamon Press, New York, Vol. 1, 1959) p. 172

55. Hockstim, R.A. *Proc. Natl. Acad. Sci.* *50*, 200 (1963)

56. Dauvillier, A. *The Photochemical Origin of Life* (Academic Press, New York, 1965) p. 44

57. Aristote, *De Generatione Animalium*

58. Redi, F. *Esperienze intorno alla generatione degl' insetti* Firenze, (1668), Italia

59. Van Leeuwenhoek, A. *Naturs Verborgentheden Ontdekt, Delft*, (1697)

60. Needham, J.T. *Phil. Trans* *490*, 615 (1749)

61. Spallanzani, L. *Opuscoli di Fiscia animale et vegetabile*, Modena, Italia, (1776)

62. Pouchet, F. *Hétérogénie ou traité de la génération spontanée basé sur de nouvelles expériences*, (Paris, 1859)

63. Pouchet, F. *Compt Rend. Acad. Sci.* *47*, 979 (1858) *48*, 148, 546 (1859) *56*, 734 (1863)

64. Pasteur, L. *Compt. Rend. Acad. Sci.* *50*, 303, 675, 849 (1860) *51*, 348 (1860) *56*, 734 (1863)

65. Pasteur, L. *Ann. Sci. Nat.* *16*, 5 (1861)

66. Pasteur, L. *Ann. Chim. Phys.* *64 (3)*, 5 (1862)

67. Pasteur, L. Etudes sur la bière, Paris (1876)

68. Darwin, D. The Origin of Species by Means of Natural Selection, (London, 1859)

69. Darwin, D. L'origine des espères, Schleider, Paris (1859)

70. de Montlivault, E.J.F. Conjectures sur la réunion de la lune à la terre ..., (Paris, 1821)

71. Richter, H.E. Schmidts Jahrb. Ges. Med. 126, 243 (1865) 148, 57 (1870)

72. Lord Kelvin, (W. Thomson) Rep. Brit. Ass., CIII (1871)

73. Von Helmholz, H. Handbuch der Theoretischen Physik (Brunswick, 1874)

74. Berzelius, J.J. Ann. Phys. Chem. 33, 113 (1834)

75. Wöhler, M.f., Hörnes, M. Sitzber. Akad. Wiss. Wien, Math-naturw. kl 59, 37 (1864)

76. Daubree, Compt. Rend. Acad. Sci. 59, 1065 (1864)

77. Cloez, S. Compt. Rend. Acad. Sci. 59, 37 (1864)

78. Berthelot, H. Compt. Rend. Acad. Sci. 67, 849 (1868)

79. Arrhenius, S. Verldanas Ulveckling (Stokholm, 1906)

80. Arrhenius, S. Worlds in the Making, H. Borns (trans) (Harper and Row, Publishers, New York, 1908)

81. Arrhenius, S. L'évolution des mondes (Béranger, Paris, 1910)

82. Becquerel, P. Astronomie 38, 393 (1924)

83. Esnault-Pelterie, R. L'Astronautique (Lahure, Paris, 1930)

84. Lipman, C.B. Sci. Month 19, 357 (1924)

85. Alexander, J. Science 96, 252 (1942)

86. Muller, H.J. Proc. 4th Int. Congr. Plant. Sci. 1, 897 (1926)

87. Wald, G. Scientific American, August 45 (1954)

88. Engels, F. Dialectique de la nature (M.E.A., 1927)

89. Schäffer, E.A. Rep. Brit. Ass. 3 (1912)

90. Schäffer, E.A. "Presidential Adress to the Brit. Assoc. Adv. Sci." (1912)

91. Huxley, T.H. Rep. Brit. Ass. 1, 30, 111 (1870)

92. Spencer, H. First Principles (D.Appleton and Co., New York, 1864)

93. Spencer, H. Various Fragments (D. Appleton and Co., New York, 1898) p. 131

94. Spencer, H. The Principles of Biology (Williams and Norgate, London, 1898)

95. Tyndall, J. Fragments of Science for Unscientific People (Longmans, Green and Co. London, 1871)

96. Tyndall, J. Rep. Brit. Ass. 1, 15, 1 (1874)

97. Haeckel, E. The Wonders of Life (Harper and Brothers, N.Y., 1905)

98. Bastian, H.C. The Beginnings of Life (D. Appleton and Co., N.Y., 1872) p. VIII

99. Moore, B. The Origin and Nature of Life (Henry Holt and Co., N.Y., 1912; William and Norgate, London, 1913; Thornton, London, 1930)

100. Schrödinger, E. What is Life? (Macmillan, New York, 1947)

101. Von Bertalanffy, L. Problems of Life (J. Wiley and Sons, N.Y., 1952)

102. Von Bertalanffy, L. General System Theory, General System Year Book 1, 1 (1956)

103. Weaver, W. Science and Complexity, Main Currents in Modern Thought 6, 74 (1952)

104. Theilhard de Chardin, P. Le phénomène humain (Seuil, Paris, 1955)

105. Blum, H.F. Am. Scientist 49, 474 (1961)

106. Blum, H.F. Time's Arrow and Evolution, 2nd ed., (Harper and Row publishers, N.Y., 1962)

107. Simon, A.M. The Architecture of Complexity, Year Book of the Society for General System Research 10, 63 (1965)

108. Dobzhansky, T. Main Currents in Modern Thought 23, 3 (1965)

109. Oparin, A.I. Proisklozhdenie Zhizni (Moscov, Izd Moskovskii Rabochi, 1924)

110. Haldane, J.B.S. Rationalist Annual 148 (1928)

111. Herrera, A.L. Science 96, 14 (1942)

112. Bahadur, K. Nature 173, 1141 (1954)

113. Bahadur K. et al Nature 182, 1668 (1958)

114. Baly, E.C.C., Baudish, O., Heilbron J. Chem. Soc. 121, 1078 (1922)

115. Perti, O.N., Bahadur, K., Pathak, H.D. Proc. Natl. Acad. Sci. India 30 A, 206 (1961)

116. Waldsten, T. et al Acta Chim. Scand. 13, 1069 (1959)

117. Steinman, G. et al Nature 206, 707 (1965)

118. Steinman, G. et al Science 147, 1574 (1965)

119. Pounamperuma, C. et al Nature 203, 400 (1964)

120. Steinman, G. et al Proc. Natl. Acad. Sci. 52, 27 (1964)

121. Szutka, A. et al Zeit. Naturforschung 20 b, 217 (1961)

122. Berthelot, D. and Gaudechon, H. Compt.Rend. 150, 1169, 1327, 1517 1690; 151, 395, 478, 1349 (1910); 152, 262, 376, 522; 154, 1803 (1912); 156, 1243, 1766 (1913) J. Pharm. Chim. July (1910), Rev. Gen. Sci. 22, 322 (1911)

123. Berthelot, D. J. Phys. Jan. (1917)

124. Cattera, J.R. and Ferrari, G. Ann. Chim. (Rome) 49, 1639 (1959)

125. Ferrari G. and Cattera, I.R. Nature 190, 326 (1961)

126. Deschreider, A.R. Nature 182, 528 (1958)

127. Pavlovskaya, T.E. and Pasynskii The Origin of Life on the Earth, A.I. Oparin ed., (I.U.B. Symposium Series Vol. I, Pergamon Press, New York, 1959) p. 151

128. Pavlovskaya T.E. and Pasynskii, A.G. Intern, Congr. Biochem., 4th Congr. Abstr. Communs, (1958) p. 12

129. Pavlovskaya, T.E. and Pasynskii, A.G. Doklady Akad. Nauk. SSSR 135, 743 (1960)

130. Ellenbogen, E. Abstract. Am. Chem. Soc. Meeting (Chicago, 1958) p. 47 c.

131. Groth, W. Photochemistry in the Liquid and Solid States (John Willey, N.Y., 1960)

132. Groth, W. Angew. Chem. 69, 681 (1957)

133. Groth W. and Weyssenhoff, H.V. Naturwiss. 44, 510 (1957)

134. Groth W. and Weyssenhoff Ann. Physik. 4, 69 (1959)

135. Groth W. and Weyssenhoff, H.V. Planet Space Sci. 2, 79 (1960)

136. Franck, B. Ber. 93, 446 (1960)

137. Heyns, K., Walter, W., Mayer, E. Naturwiss. 44, 385 (1957)

138. Terenin, A.N. The Origin of Life on the Earth A.I. Oparin ed., (I.U.B. Symposium Series Vol. I, Pergamon Press, New York, 1959) p. 139

139. Dodonova, N.Y. et al Vestnik Leningrad Univ. No. 16, 14, Ser. Fiz i Khim No. 3, 33 (1959)

140. Baudish, O. Z. Angew. Chem. 26, 612 (1913)

141. Dhar, N.R. and Mukherjee, S.K. Nature 134, 499 (1934)

142. Master, F. M.S. Thesis, University of Houston, Houston, Texas (1957)

143. Steinman, G., Smith, A.E., Silver, J. Science 159, 1108 (1968)

144. Smith, A.G., Steinman, G. Galand, C. Experientia 25, 255, (1969)

145. Ponnamperuma, C. et al Science 147, 1572 (1965)

146. Bahadur, K. and Ranganayaki, S. Proc. Natl. Acad. Sci. India 27, 292 (1958)

147. Fisher, E. in The Photochemical Origin of Life, by Dauvillier (Academic Press, N.Y., 1965) p. 105

148. Ponnamperuma et al Nature 198, 119 (1963) 199, 222 (1963)

149. Ponnamperuma, C. et al in The Origin of Prebiological Systems, S.W. Fox ed., (Academic Press, N.Y., 1965) p. 221

150. Ponnamperuma, C. et al 19th Intern. Congr. Pure Appl. Chem. (London, 1963)

151. Ponnamperuma, C. Abstracts, third Intern. Congr. of Radiation Res. (Cortina d'Ampezzo, 1966)

152. Stoklasa, J. Compt. Rend. 156, 646 (1913)

153. Baly, E.C.C. Ind. Eng. Chem. 16, 1016 (1924)

154. Irvine, J.C. and Francis, G.V. Ind. Eng. Chem. 16, 1019 (1924)

155. Ponnamperuma, C. et al Radiation Res. 19, 183 (1963)

156. Taylor, H.S. and Marshall, A.I. J. Phys. Chem. 29, 1140 (1925)

157. Groth, W. Z. Physik Chem. 37, 315 (1937)

158. Sagan, C. and Miller, S. Astronomy J. 65, 499 (1960)

159. Schimpel, A. et al Science 147, 149 (1965)

160. Getoff, N. Nature 210, 940 (1966)

161. Kenyon, D.H. et al Photochem. and Photobiol. 4, 335 (1965)

162. Szutka, A. Radiation Res. 19, 183 (1963)

163. Miller, S.L. Science 117, 528 (1953)

164. Miller, S.L. Biochim. et Biophys. Acta 23, 480 (1957)

165. Löb, W. Ber. 46, 684 (1913)

166. Peters, K. and Kuster, H. Brennstof. Chem. 10, 108 (1929)

167. Brinner, E. et al Helv. Chim. Acta 2, 663 (1919) 21, 859 (1938) 23, 826 (1940)

168. Hough, L. and Rogers, A.F. J. Physiol. 132, 28 (1956)

169. Abelson, P.H. Science 124, 935 (1956)

170. Abelson, P.H. Ann. N.Y. Acad. Sci. 69, 274 (1957)

171. Woeller, F. et al Abstract of Paper for Joint Meeting of Optical Society of Amer., Amer. Chem. Soc. for Applied Spectroscopy,(San Francisco, Calif., 1966)

172. Otozai, K. et al Bull. Chem. Soc. (Japan) 27, 476 (1954)

173. Oro', J. Nature 197, 862 (1963)

174. Grossenbacker, K.A. and Knight, C.A. in Origin of Prebiological Systems, S.W. Fox ed., (Acad. Press, N.Y., 1965) p. 173

175. Ponnamperuma, C. et al Abstract of Paper for 152nd Natl. Meeting Am. Chem. Soc. 1 (N.Y., 1966)

176. Steinman, G. M.S. Thesis, Michigan State Univ., East Lansing, Michigan (1963)

177. Ponnamperuma, C. Differences in the Character of C_6 to C_8 Hydrocarbons from Gaseous CH_4 in low Frequency Electric Discharges, NASA, Moffet Field, (Calif. 1963)

178. Ponnamperuma, C. et al Nature 209, 979 (1966)

179. Allen, W.A. and Ponnamperuma, C. Personal Communication (1966) in The Origin of Life, J. Keosian ed., (Reinhold Book Corp., N.Y., 1968) p. 37

180. Sanchez, R.A. et al Science 154, 784 (1966)

181. Garrison, W.M. et al J. Amer. Chem. Soc. 74, 4216 (1952)

182. Hasselstrom, T. and Henry, M.C. Science 123, 1038 (1956)

183. Hasselstrom, T. and Henry, M.C. et al Science 125, 350 (1957)

184. Paschke, R. et al Science 125, 881 (1957)

185. Dose, K. and Ettre, K. *Z. Naturforsch* **13 b**, 784 (1958)

186. Dose, K. and Rajewsky, B. *Biochim. Biophys. Acta* **25**, 225 (1957)

187. Palm, C. and Calvin, M. *J. Amer. Chem. Soc.* **84**, 2115 (1962)

188. Oro', J. in *The Origin of Prebiological Systems and their Molecular Matrices*, S.W. Fox ed., (Acad. Press, N.Y., 1965) p. 137

189. Oro', J. *Biochim. Biophys. Res. Commun.* **2**, 407 (1960)

190. Palm, C. and Calvin, M. *Bio-Organic Chemistry Quarterly Report*, Univ. Calif. Lawrence Radiation Laboratory **9900**, 51 (1961)

191. Ponnamperuma, C. et al *Proc. Natl. Acad. Sci. U.S.* **49**, 737 (1963)

192. Berger, R. *Proc. Natl. Acad. Sci.* **47**, 1434 (1961) *Ann. N.Y. Acad. Sci.* **108**, 482 (1963)

193. Manton, J.E. and Tickner, A.W. *Canad. J. Chem.* **38**, 858 (1960)

194. Lampe, F.W. *J. Amer. Chem. Soc.* **79**, 1055 (1957)

195. Glassel, J.A. *Proc. Natl. Acad. Sci. U.S.* **47**, 174 (1961)

196. Garrison, W.M. et al *J. Amer. Chem. Soc.* **75**, 2459 (1953)

197. Stoops, C.E. and Furrow, C.L. *Science* **134**, 839 (1961)

198. Getoff, N. et al *Tetrahedron Letters* **18**, 17 (1960)

199. Fox, S.W. et al *J. Amer. Chem. Soc.* **77**, 1048 (1955)

200. Fox, S.W. et al *Science* **133**, 1923 (1961)

201. Fox, S.W. in *Organic Geochemistry*, (Pergamon Press, Oxford, 1963)

202. Heyns, K. and Pavel, K. *Naturforsch.* **12 b**, 97 (1957)

203. Oro', J. et al *Arch. Biochem. Biophys.* **85**, 115 (1959)

204. Oro', J. and Kamat, J.S. *Nature* **190**, 442 (1961)

205. Lowe, C.U. and Rees, M.W. *Federation Proc.* **22**, 479 (1963)

206. Lowe, C.U. et al *Nature* **199**, 219 (1963)

207. Labadie, M. et al *Bull. Soc. Chim. Biol.* **49**, 46 (1967)

208. Harada, K. and Fox, S.W. Nature 201, 335 (1964)

209. Harada, K. and Fox, S.W. in The Origin of Prebiological Systems and their Molecular Matrices, S.W. Fox ed., (Acad. Press, N.Y., 1965) p. 187

210. Oro', J. Ann. N.Y. Acad. Sci. 108, 464 (1963)

211. Oro', J. Nature 190, 389 (1961)

212. Ferris, J.P. et al J.Am. Chem. Soc. 87, 4976 (1965)

213. Hanafusa, H. et al Bull, Chem. Soc. Japan 32, 626 (1959)

214. Becker, R.R. et al J. Biol. Chem. 204, 737 (1953)

215. Oro', J. and Guidry, C.L. Nature 186, 156 (1960)

216. Oro', J. and Guidry, C.L. Arch. Biochim. Biophys. 93, 166 (1961)

217. Kovac, J. and Nagy, H. Nature 190, 531 (1961)

218. Schramm, G. and Wissman, H. Chim. Ber. 91, 1073 (1958)

219. Schramm, G. et al Angew. Chem. (Inter. ed.) 1, 1 (1962)

220. Akabori, S. Kagaku (Science Japan) 25, 54 (1955)

221. Akabori, S. et al Bull. Chem. Soc. Japan 29, 608 (1956)

222. Akabori, S. The Origin of Life on the Earth, A.I. Oparin ed. (Pergamon Press, N.Y., 1959) p. 189

223. Krampitz, G. Naturwiss 46,558 (1959)

224. Harada, K. and Fox, S.W. J. Amer. Chem. Soc. 80, 2694 (1958)

225. Fox, S.W. and Harada, K. Science 128, 1214 (1958)

226. Fox, S.W. et al Experientia 15, 81 (1959)

227. Fox, S.W. and Harada, K. Arch. Biochem. Biophys. 86, 281 (1960)

228. Fox, S.W. et al 2nd Annual Report of the Inst. Molec. Evol., Univ. of Miami, Coral Gables, Florida (1966)

229. Vegotsky, A. et al Federation Proc. 18, 343 (1959)

230. Oro', J. Nature 191, 1193 (1961)

231. Oro', J. and Kimball, A.P. Arch. Biochem. Biophys. 94, 217 (1961)

232. Oro', J. and Kimball, A.P. Arch. Biochem. Biophys. 96, 293 (1962)

233. Oro', J. Federation Proc. 22, 681 (1963)

234. Ponnamperuma, C. Federation Proc. 22, 479 (1963)

235. Ponnamperuma, C. et al Science 143, 1449 (1963)

236. Ferris, J.P. and Orgel, L.E. J. Amer. Chem. Soc. 88, 1074 (1966)

237. Sanchez, R. et al Science 153, 72 (1966)

238. Miller, S.L. and Parris, M. Nature 204, 1248 (1964)

239. Beck, A. and Orgel, L.E. Proc. Natl. Acad. Sci. 54, 664 (1965)

240. Schramm, G. et al Angew. Chem. 73, 619 (1961)

241. Schramm, G. et al The Origin of Prebiological Systems and their Molecular Matrices, S.W. Fox ed., (Acad. Press, N.Y., 1965) p. 229

242. Ponnamperuma, C. and Mack, R. Science 148, 1221 (1965) and Abstract of Paper for 150th Natl. Meeting Amer. Chem. Soc. Atlantic City, N.J. (1965)

243. Schwartz, A. et al Biochim. Biophys. Acta 87, 694 (1964) and The Origin of Prebiological Systems and their Molecular Matrices, S.W. Fox. ed. (Acad. Press, N.Y., 1965) p. 317

244. Schramm, G. Personal Communication (1966) in The Origin of Life, J. Keosian ed., (Reinhold Book Corp., N.Y., 1968) p. 35

245. Kochetkov, N.K. et al Biochim. Biophys. Acta 80, 145 (1964)

246. Oro', J. and Cox, A.C. Federation Proc. 21, 80 (1962)

247. Mora, P.T. The Origin of Prebiological Systems and their Molecular Matrices, S.W. Fox ed., (Acad. Press, N.Y., 1965) p. 281

248. Mora, P.T. and Wood, J.W. J. Amer. Chem. Soc. 80, 685 (1958)

249. Thompson, A. et al J. Amer. Chem. Soc. 76, 1309 (1954)

250. Kotake, M. et al *Kogyo Kogaku Zasshi* *59*, 121, 151 (J. Chem. Soc., Japan Ind. Chem. Sect.) (1956)

251. Oro', J. and Han, H. *Science* *153*, 1393 (1966)

252. Szutka, A. *The Origins of Prebiological Systems and their Molecular Matrices*, S.W. Fox ed., (Acad. Press, N.Y., 1965) p. 243

253. Dauvillier, A. ed, in *The Photochemical Origin of Life* (Acad. Press, New York, 1965) p. 75

254. Nelson, B. Russel *U.S.* *2*, 678, 871

255. Herrera, A.L. *Bull. Lab. Pasmog., Mex.* *2*, 3 (1940)

256. Storer, T.I. and Usinger, R.L. *General Zoology* (Mc Graw-Hill, New York, 1965) p. 26

257. Calvin, M. *Proc. R. Soc. A.* *288*, 441 (1965)

258. Platt, J.R. *J. Theoret. Biol.* *1*, 342 (1961)

259. Smith, A.E. et al *Experientia* *24*, 36 (1968)

260. Abelson, P.H. *Proc. Nat. Acad. Sci.* *55*, 1365 (1966)

261. Berkner, L.V. and Marshall, L.C. *J. Atmo. Sci.* *22*, 225 (1965)

262. Urey, H.C. in *The Origin of Life on the Earth* Symp. Intern. Union Biochem., Moscow 1957 (Macmillan, New York, 1959) p. 16

263. Studier, M.H., Hayatsu, R., Anders, E. *Science* *149*, 1455 (1965)

264. Schopf, J.W., Kvenvolden, K.S. and Barghoorn, E.S. *Proc. Nat. Sci. US59*, 639 (1968)

265. Oberlies, F. and Prashnowsky, A.A. *Naturwiss.* *55*, 25 (1968)

266. Bahadur, K. in *Synthesis of Jeewanu the Protocell*, (Ram Narain Lal Beni Prasad, Allahabad-2, India, 1966)

267. Labadie, M., Cohere, G. and Bechenmacher, C. *C.R. Séanc. Soc. Biol.* *161*, 1689 (1967)

268. Blais, J.J. and Geil, P.H. *J. Ultrastruct. Res.* *22*, 303 (1968)

269. Abelson, P.H. in *Researches in Geochemistry*, P.H. Abelson ed., (John Wiley, New York, 1959) p. 79

270. Eck, R.V. and Dayhoff, M.O. *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Silver Springs, Md. 1966)

271. Lewis, G.N. and Randall, M. *Thermodynamics*, rev. by K.S. Pitzer and C. Brewer, (Mc Graw-Hill book comp. New York, 1961)

272. Prigogine, in *Thermodynamics of Irreversible Processes*, (Interscience Publishers, John Wiley & Sons, New York, 1961) p. 83, 87

273. Prigogine, I. and Nicolis, G. *J. Chem. Phys.* *46*, 3542 (1967)

274. Chandrasekhar, S. *Hydrodynamic and Hydromagnetic Stability*, (Oxford, Clarendo~~m~~n Press, 1961)

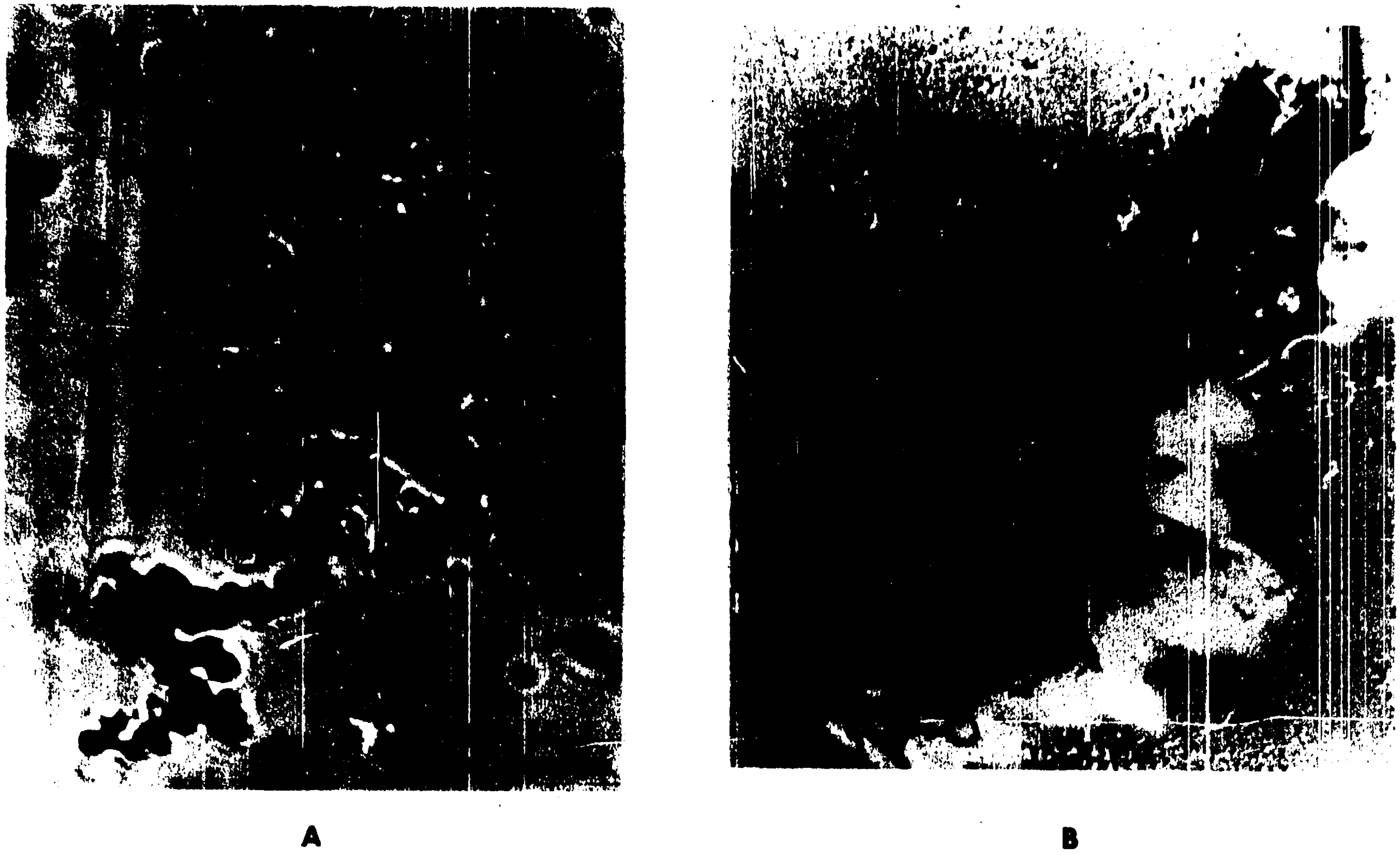


Figure 11: a) Aggregates of microspheres in the form of a large sphere and chain.

b) Aggregates of microspheres shadowed with chromium (Tan^{-1} 0.1)

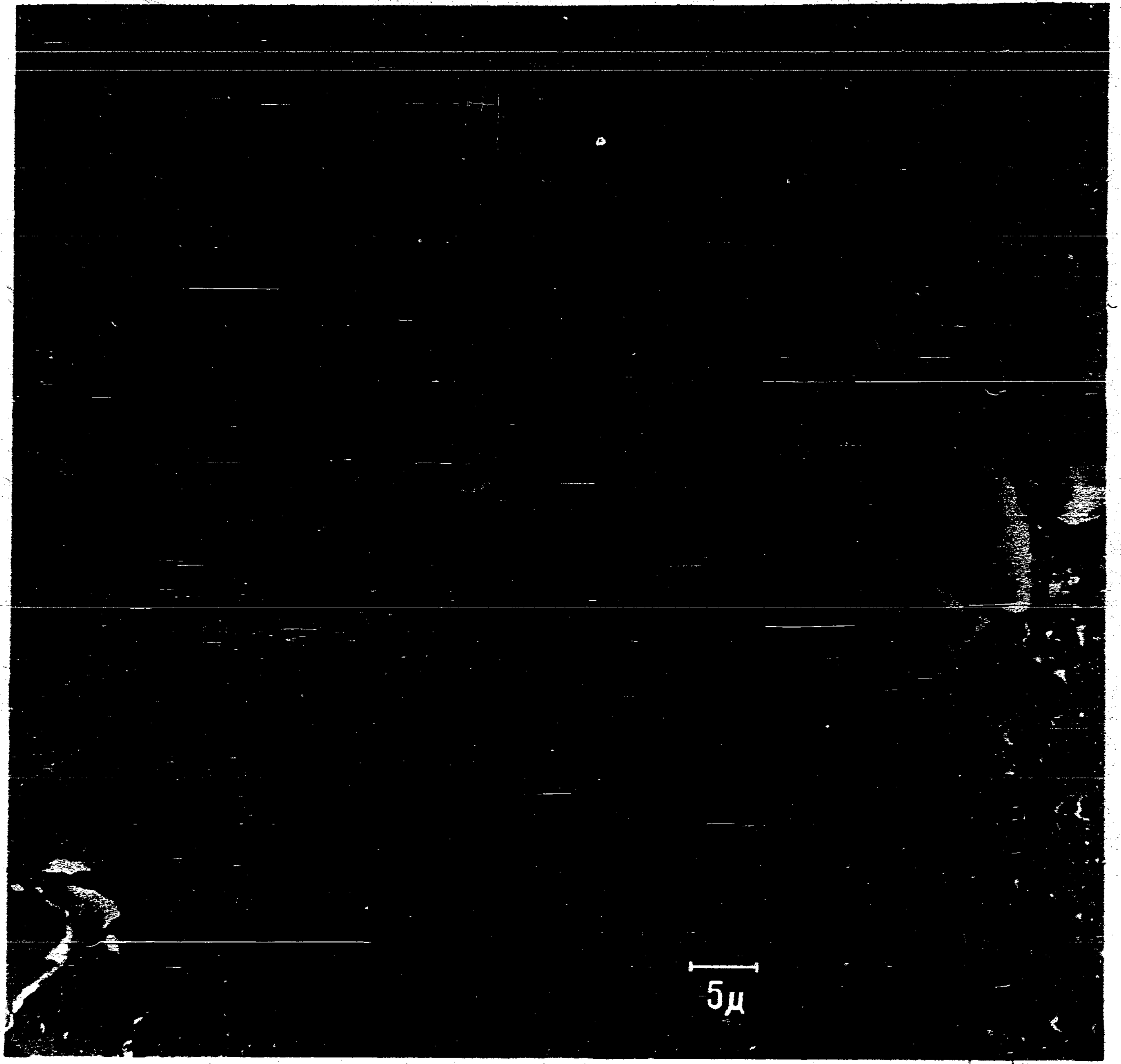


Figure 12: Shadowed electron micrograph of microspheres and fibrils. Tan^{-1} 0.I. (After 24 hours)

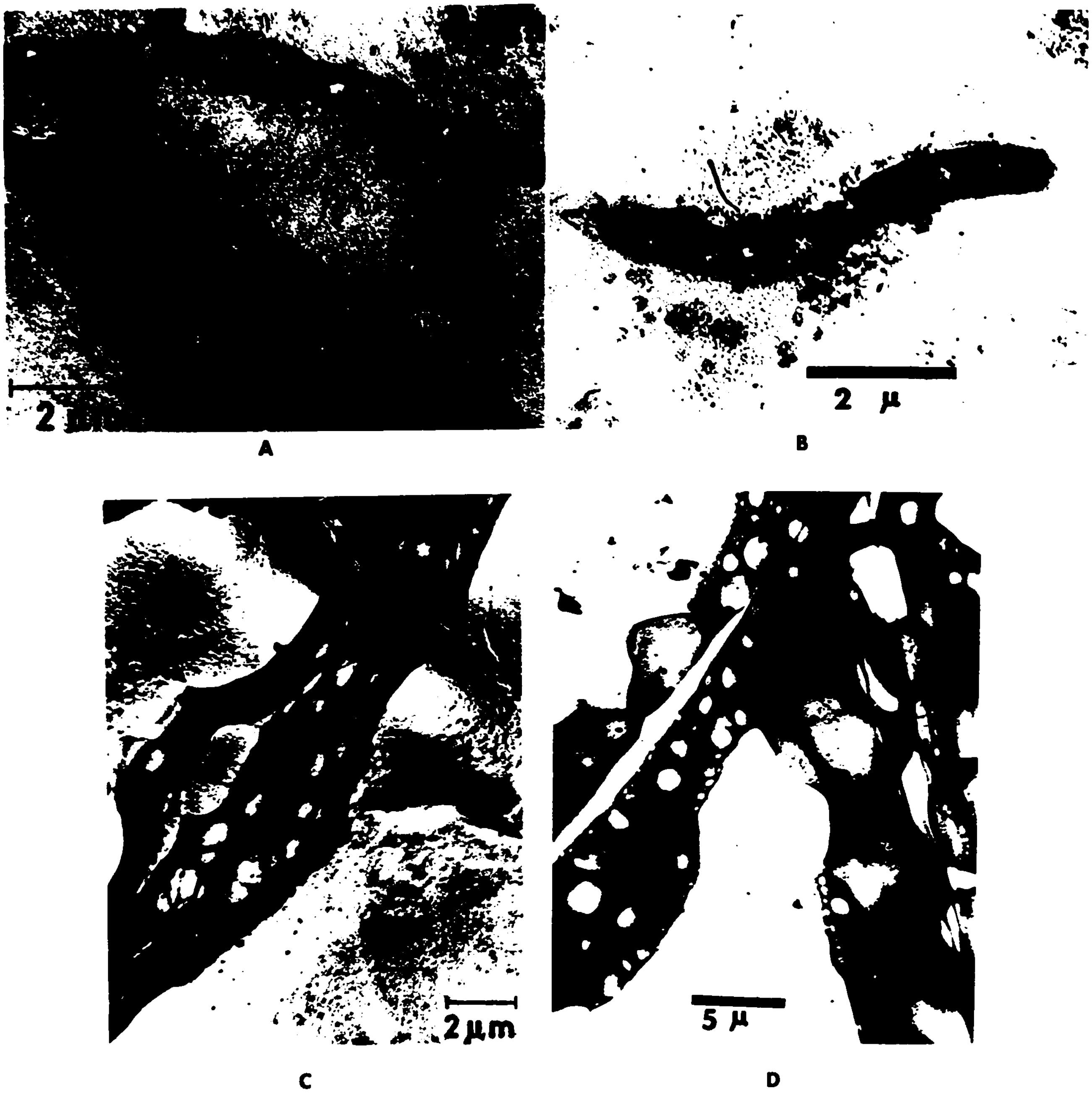


Figure 14: Morphological similarity between the microsphere aggregates (b,d) and the precambrian microfossils (a,c)