ABIOGENIC PHOTOSYNTHESIS OF PARTICLES SHOWING SOME PROPERTIES OF THE LIVING CELL

by

Pierre Desautels

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PHYSICS DEPARTMENT
FACULTY OF SCIENCE
SIR GEORGE WILLIAMS UNIVERSITY
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ABSTRACT

The object of this work is to show that under the conditions that existed on the earth before the appearance of life, the natural formation of cell-like structures is highly probable.

To reproduce the conditions that then existed, we prepared a solution intended to simulate the composition of the prebiotic hydrosphere. Since oxygen was then absent from the atmosphere, nitrogen was bubbled through the solution so as to prevent oxygen from coming into contact with the solution. The solution was irradiated for different times with ultraviolet light since before the formation of the ozone layer in the upper atmossphere this ultraviolet radiation coming from the sun could reach the sea level easily.

Under these conditions, insoluble particles were easily obtained. They look like what could be called a primitive cell. They associate with others to form large aggregates, like cells. They contain amino acids, like cells. They have the ability to concentrate ions and maybe molecules from their surrounding, like cells. They also seem to possess some catalytic activity. So, they really could be a precursor of the modern cell.

SOMMAIRE

Ce travail a pour but de montrer qu'il est fort probable que se forment, dans les conditions qui existaient sur la terre avant l'apparition de la vie, des "particules" semblables aux cellules.

Afin de simuler les conditions terrestres prébiologiques, nous avons préparé une solution aqueuse dont la composition se rapproche de celle de l'eau de mer. Nous y avons fait barboter de l'azote afin d'en retirer l'oxygène, puisque ce gaz était alors absent de l'atmosphère. Des échantillons de cette solution furent ensuite soumis à la lumière ultraviolette pour des irradiations de durée variable. Aux temps reculés qui nous intéressent, la terre n'était pas encore protégée par une couche d'ozone, ce qui permettait à la lumière ultraviolette solaire d'atteindre aisément la surface du globe.

Ainsi, grâce à la reconstitution partielle des conditions prébiologiques, nous avons obtenu des "particules" qui s'apparentent à une cellule primitive.

En effet, les particules obtenues sont à peu près de même dimension et de même forme que les cellules; comme ces dernières, elles présentent des protubérances à

leur surface, elles semblent posséder une certaine structure interne et elles s'associent facilement en aggrégats compacts et résistants. Nous avons décelé des acides aminés dans ces particules ainsi qu'une faible trace de purines et de pyrimidines. Finalement, ces particules ont manifesté une activité catalytique; c'est peut-être leur plus grand intérêt.

Toutes ces similitudes entre les particules obtenues et les cellules nous permettent de croire qu'il est peut-être possible que les premières soient les lointaines ancêtres des secondes.

INTRODUCTION

The problem of the origin of life is probably as old as humanity. But only a scientific civilization like ours could be expected to give a satisfactory answer to the question.

Aristotle (1) expressed the idea that living things are the result of the interaction of "Matter and Form". This was the birth of the "spontaneous generation" theory. This theory survived almost two thousands years of history. Flies, insects, animalcules could come spontaneously into existence from non living substance such as decaying matter.

It was not until the discovery of sterilization by Spallanzani in 1765 and, more than all, to the experiments of Pasteur in 1860 (2) that the true origin of the living organisms that were found in decaying matter was explained: it was shown without doubt that air carried "germs". These germs are themselves living organisms in a more or a less latent form. Starting with that assumption, Pasteur showed that if one sterilized, by boiling, a solution susceptible to fermentation (that is capable of supporting the life of bacteria) and, if only well

filtered air is admitted in contact with the solution, there is no fermentation. Sterilization killed the bacteria that were already in the solution and filtering of the air retained all those carried by the air. So no living matter could be expected to arise spontaneously from non living matter.

The spontaneous generation theory was dead and, for everyone, only life could give birth to life. This was the state of mind that prevailed until more knowledge of living organisms and in particular of the living cell, its morphology, its metabolism and the function of enzymes, led to our present attitude towards the problem.

The general opinion today is that life originated from non living matter as the result of a kind of molecular evolution, somewhat similar to the biological evolution that started with the one cell algea and finally produced the homo sapiens.

In the light of our present knowledge, living organisms as we know them can generally be characterized by the presence of proteins, of enzymes, of nucleic acids and by the ability to reproduce themselves. Viruses are one exception: they do not contain enzymes(3). However, they cannot reproduce without the enzymes of another

living organism, usually the bacteria they infect.

The experimental approach to the problem of how these products could be synthesized from inorganic substance without the help of a living organism was to simulate the prebiological conditions that existed on earth and to look for the formation of these products. The results were positive.

Trying to simulate the original atmosphere and the electrical discharges between the clouds and the earth, S.L. Miller had a spark discharge run in an atmosphere of H_2, CH_4, NH_3 (4), (5). In his experiments, he used the spark discharge produced by a high frequency Tesla coil. The peak voltage was 60,000 volts. The pressures of H_2, CH_4 and NH_3 were 10, 20 and 20 cm. of Hg respectively. After a few days, a large number of organic compounds could be found in the reaction vessels: large amounts of HCN, formic acid, acetic acid, succinic acid, urea and some amino acids, glycine, alanine and aspartic acid. Amino acids are the building blocks of proteins and they have been shown to polymerize under the action of ultraviolet radiation to form polypeptides(6). Miller's experiment was repeated by a large number of workers among which Grossenbacher and Knight who obtained amino acids, peptides, HCN from a mixture of NH_3, CH_4, H_2O (7).

In Russia, Pavlovskaya and Pasynskii synthesized amino acids by ultraviolet irradiation of a water solution containing 2.5% of formaldehyde and 1.5% of ammonium nitrate (8). Ammonium nitrate can be found in the sea (9) and formaldehyde is easily formed by the action of ultraviolet light on an atmosphere containing H_2O and a source of carbon like CH_4 (10). The solution was irradiated 20 hours at 40°C and chromatography revealed the presence of serine, glycine, glutamic acid, alanine, valine and phenylalanine in the irradiated solution.

Also in Russia, Kolomiychenko (11) irradiated with ultraviolet light (200-400 nm.) a solution of CH_2O (2.5%), NH_4NO_3 (1.5%) and KSCN (5%) in water, for a period of 30 hours. He obtained a large number of amino acids: alanine, glutamic acid, leucine, proline and histidine. The experiment was repeated with solutions of CH_2O (2.5%) and ammonium nitrate (1.5%). Glycine, serine and aspartic acid were detected after irradiation. A 50 hours ultraviolet (253.7 nm.) irradiation of a solution of CH_2O (5%), NH_4NO_3 (5%) yielded alanine, lysine, proline, threonine, ornithine, serine, histidine, aspartic acid and glutamic acid. Kolomiychenko also used infrared radiation (1.5-2.5 microns) and obtained glycine, asparagine, alanine, serine, glutamic acid after a 45 hours irradiation of a solution .1M in CH_2O and .1M in NH_4NO_3.

Experiments were also performed using other sources of energy. Solutions of simple compounds or mixture of simple gases were irradiated with X-rays, γ-rays, high energy electrons and constantly, amino acids were produced. Dose and Rajewsky (12) irradiated with X-rays and with 2 Mev γ-rays gaseous mixtures of methane, ammonia, water vapor, hydrogen, carbon dioxide and nitrogen. They obtained amino acids: glycine, alanine. Palm and Calvin (13) used 5 Mev electrons to irradiate a mixture of methane, ammonia, water vapor and traces of hydrogen. They also obtained many amino acids: glycine, alanine, aspartic acid and large amounts of urea.

Less energetic sources also yielded amino acids. Bahadur (14), (15) irradiated with a 500W incandescent lamp a solution of paraformaldehyde in the presence of molybdenum oxide (MnO_3) and obtained glycine, alanine and valine.

The building blocks of nucleic acids, purines and pyrimidines have also been synthesized from simple starting compounds. Ponnamperuma obtained adenine from the bombardment of a mixture of CH_4, NH_3, H_2O by 4.5 Mev electrons (16), (17). A mixture of C^{14} labeled methane (300 mm. of Hg) and ammonium hydroxide (20 ml. of 4N solution) was irradiated with 4.5 Mev electrons obtained from a linear accelerator. The irradiation lasted 45 mi-

nutes at a beam current of 18 microamperes. Adenine could be detected by chromatography and by autoradiography since it had incorporated the C^{14} from the methane.

Even ATP, the "energy currency" of all living systems, has been synthesized by irradiation with ultraviolet light of a dilute aequous solution of adenine, ribose and ethyl-metaphosphate (18), (19). Ponnamperuma irradiated for 1 hour with ultraviolet light (235.7 nm.) a solution containing 1.5×10^{-6} moles of adenine (200 µl) and 3×10^{-6} moles of ribose (50 µl). The solution was kept at 40°C. Chromatography revealed the presence of AMP, ADP, ATP and A4P after irradiation.

So, we have experimental proof that some of the most important chemical compounds that enter in the composition of living things can be synthesized in conditions simulating those that existed on the prebiological earth.

But today's living organisms need enzymes for growth and multiplication. These are complex, efficient and labile organic catalysts. Enzymes are usually high molecular weight proteins which incorporate other chemical groups and sometimes metallic ions (20). They are very active under optimum conditions and can increase the speed of a reaction by a factor of more than a million. Their properties are associated with their ter-

tiary structure. If this structure is modified by even a moderate heating or by ultraviolet irradiation, the enzyme loses most of its properties. Enzymes are also very much affected by external conditions such as pH (21). For example, pepsin can operate only between pH 1 and 4. Another characteristic of enzymes is that they are very selective. One enzyme can catalyze only certain very specific reactions and no others. So, these complex molecules probably could not operate under pre-biological earth conditions.

But there are other catalysts, less sensitive to these conditions; they are however also much less effective. The presence of some inorganic ions can catalyze many chemical reactions. Absorption on a surface (quartz, clay, ...) can also affect the speed of certain chemical reactions (22). But all these inorganic catalysts are usually thousands of times less effective than the enzymes that the modern cell uses.

Less effective catalysts means less active metabolism. So, the growth rate of the organism that uses them must be very slow. However, budding has already been reported by Bahadur (23) and also by Fox (24).

Some of essential biochemicals have already been produced under a large number of different conditions trying to simulate the situation that existed on prebiological earth. The study of systems obtained under these conditions is a very interesting and very promising field of research.

OBJECT OF THESIS

The object of this thesis is to show that under the conditions prevailing on the prebiological earth, the formation of particles showing to a certain degree some properties of the living cell was highly probable.

The origin of the earth goes back to about 4.5 billion years (25), (26). During the millions of years that preceded the appearance of life on our planet, its surface is believed to have been covered only by rocks and seas containing in solution ions of all kinds (9) Na^+, K^+, Cl^-, NH_4^+, $SO_4^=$, $PO_4^{\equiv}$, ... Volcanic eruptions were frequent and produced locally higher concentrations of these ions (26). Oxygen was then absent in the atmosphere (27), (28) so that no ozone in the higher regions of the atmosphere could filter out the ultraviolet radiation coming from the sun.

These are the conditions that we tried to simulate in the laboratory: water containing some simple ions, absence of oxygen, ultraviolet radiation. Formaldehyde was added because its formation from the action of ultraviolet radiation on a mixture of H_2O and a source of carbon like CO_2 or CH_4 (10) is well known and because these

gases are believed to have been present in these days (26), (29).

Solutions irradiated with ultraviolet light yielded insoluble particles whose morphology was studied under both light and electron microscope. These particles were tested for the presence of some organic compounds: amino acids, peptides, sugars, purines and pyrimidines. Experiments were also performed to see if they showed any catalytic activity in the decomposition of H_2O_2, ATP and urea.

MATERIAL AND METHOD

Preparation and irradiation of the solutions

Every solution was prepared in the same way by dissolving 0.7 gr. (2.2×10^{-3} mole) of potassium thiocyanate (KSCN), 0.21 gr. (1.8×10^{-3} mole) of ammonium nitrate (NH_4NO_3) and 0.14 gr. (5.5×10^{-4} mole) of monobasic calcium phosphate ($CaH_4(PO_4)_2.H_2O$) in 7 ml. of a mineral solution. The latter was prepared by dissolving 0.2 gr. of sodium chloride (NaCl), 0.2 gr. of potassium chloride (KCl), 0.2 gr. of calcium acetate ($Ca(CH_3COO)_2.H_2O$), 0.2 gr. of magnesium sulfate ($MgSO_4.7H_2O$) and 0.2 gr. of monobasic potassium phosphate (KH_2PO_4) in one liter of distilled water (30). By using this mineral solution, we intended to simulate the ion content of sea water.

To these 7 ml. of solution, 0.28 ml. of 36% formaldehyde was added so that the final molarity of the different compounds present in each sample was as follow: KSCN: 1 Molar, NH_4NO_3: 0.36 Molar, $CaH_4(PO_4)_2$: 0.08 Molar, CHOH: 0.46 Molar. Each sample was irradiated in a 15 mm. diameter test tube wrapped in aluminium foil so that no ultraviolet radiation could escape. (Figures 1, 2, 3).

The radiation was produced by a quartz ultravio-

let penlight (Model 11 SC-1 made by U.V. Prod. Inc., San Gabriel, Calif.) that was immersed in the solution. (See spectrum on Figure 4). To reduce heating and to increase the life of the lamp, the ultraviolet source was operated at reduced voltage: 70 volts were applied to the primary of the transformer (Mineralight Model SCT1 made by U.V. Prod. Inc., San Gabriel, Calif.) that was driving the lamp. The ultraviolet energy density in the solution was of the order of 444 μ watts/cm^2 as measured with an ultraviolet light meter (U.V. Meter made by U.V. Prod. Inc., San Gabriel, Calif.).

Nitrogen was bubbled through the solution for a period of 15 minutes preceding the irradiation, so as to remove most of the dissolved oxygen. Care was also taken so that no air could enter the tube. Nitrogen was also bubbled through the solution during the whole period of irradiation.

Irradiation times of 30, 45, 60 and 120 minutes were used for solutions prepared for chemical analysis and a 60 minutes irradiation time was used for those solutions prepared for catalytic activity tests.

Pre-analysis Treatments

After irradiation, the particles were removed

from the solution by sedimentation (most of the insoluble material had settled down under its own weight after 5 minutes) and washed three times with 10 ml. of distilled water so as to remove most of the water soluble material.

The supernatant was kept, filtered with a 5 μ micropore filter and desalted with a Baird & Tatlock Electrolytic Desalter, Model CD-1. Adequate desalting, as indicated by a rapid drop of the current going through the solution, was completed only after about three hours. The 0.2N NaOH and 0.2N H_2SO_4 solutions in the anode and cathode compartments respectively were renewed every 10 minutes until completion of the desalting. After desalting, the supernatant was divided into two parts: one to be analysed for free amino acids and another one to be analysed for the total amino acid content.

The portion to be analysed for free amino acids was evaporated to dryness under reduced pressure. The dry residue was dissolved with 0.2 ml. of distilled water and kept frozen in a small test tube until chromatography.

The portion to be analysed for the total amino acid content was hydrolyzed in sealed glass ampules with 6N HCl at 110°C (40, p.66). After completion of hydrolysis,

the hydrolyzed samples were vacuum dried. The dry residue was again dissolved in 0.2 ml. of distilled water and kept frozen in a small test tube until chromatography.

The particles obtained by sedimentation were submitted to the same treatment (hydrolyzed, dried, redissolved) as the second portion. However, some were kept for microscopic examination; these particles were simply deposited on a glass slide for examination with the optical microscope. For observation under the electron microscope, some particles were simply deposited on a copper grid, the aggregates being large enough to provide adequate support. Some other particles were treated with a solution of silver nitrate (36) so as to coat them with a protective silver layer and then deposited on a copper grid.

Chemical Analysis

Chromatography was performed on 0.25 mm. Silica Gel TLC plates. Five microliters of each 0.2 ml. solution was spotted together with standards.

For the detection of amino acids, two solvent mixtures were used: a n-butanol, glacial acetic acid, water mixture (80:20:20 by volume) (31, p.399) and an ethanol-water mixture (70:30 by volume) (31, p.399). Glycine,

alanine, glutamic acid, histidine. proline, lysine, valine, isoleucine were spotted as standards. After around six hours, the solvent front had moved some 14 cm. The plates were then dried under a hot air stream, sprayed with a solution of 20 mg. of ninhydrin in 10 ml. of n-butanol containing 0.3 ml. glacial acetic acid (31, p.496). Development was completed in 15 minutes in an oven at 100°C. The plates were then kept in a freezer until a picture was taken. (Figures 5,6).

For the separation of the sugars, a methyl-ethyl ketone, glacial acetic acid, methanol solvent mixture (60:20:20 by volume) (31, p.364) was used. Detection was done using the aniline phtalate reagent (31, p.485). Standards used were ribose, deoxiribose, fructose, lactose, maltose and dextrose dissolved in water. About 5 μg. of each sugar was spotted. (Figure 7).

For the separation of purines and pyrimidines, a n-butanol, acetone, glacial acetic acid, water mixture (9:3:2:6 by volume) (31, p.448) was used. Detection was done by fluorescence under ultraviolet light (31, p.448). Standards used were adenine, thymine, uridine, guanosine, uracil, cytidine and adenosine dissolved in water. (Figure 11).

Catalytic Activity

As it was also our purpose to see if the particles showed any catalytic activity, tests were performed for the influence of the particles on the decomposition rate of H_2O_2, ATP and urea.

To see if the particles obtained after a 60 minutes irradiation showed catalytic activity in the decomposition of H_2O_2, these particles were separated from the initial solution and washed three times in 10 ml. of distilled water. A 0.1% solution of H_2O_2 containing 16 mg/l of $FeCl_3$ as a cocatalyst was prepared (32). 100 ml. of this solution was put in two 150 ml. beakers, one to serve as a control, the other to receive the particles. (Figure 8).

The concentration of H_2O_2 in both beakers was determined immediately before the particles were added, immediately after and at subsequent intervals over a period of more than one week.

To find the concentration of H_2O_2, 5 ml. of 10N H_2SO_4 were added to a 5 ml. aliquot of the H_2O_2 solution. Titration was done with a solution of 150 mg/l. (9.5×10^{-4} Molar) $KMnO_4$. A pale pink color indicated the

end of the titration (33). So the rate of H_2O_2 decomposition in the beaker that contained the particles may be compared with that of the spontaneous H_2O_2 decomposition. Both beakers were kept in the dark.

Ability of the particles to increase the decomposition rate of ATP was also tested. Particles obtained after a 60 minutes irradiation were washed ten times with 10 ml. of distilled water and put in a 150 ml. beaker containing 100 ml. of 5×10^{-4} Molar ATP solution. Another beaker containing also 100 ml. of the same solution was kept as a control. The free phosphate concentration in both solutions was tested immediately before the particles were added to one of the beakers, immediately after and at subsequent intervals over a period of more than a week.

A colorimetric test was used (34) involving the formation of a blue complex of phosphomolybdate. To 1.75 ml. of the solution to be tested, 1.0 ml. of acid molybdate solution and 0.25 ml. of Fiske & Subbarow reducer were added in a spectrophotometer cuvette. The absorbance at 660 mμ was compared with that of distilled water. The readings were taken on a Unicam SP. 800 B U.V. spectrophotometer. (Figure 9).

Finally, the ability of the particles to increase the decomposition rate of urea was tested. Particles obtained after a 60 minutes irradiation were washed three times with 10 ml. of distilled water and put in a 150 ml. beaker containing 100 ml. of a 1% solution of urea in a pH 4.01 potassium acid phtalate buffer. Another 150 ml. beaker also containing 100 ml. of the same solution of urea was kept as a standard. Immediately before the particles were added, immediately after and at subsequent intervals over a period of more than one week, the concentration of NH_3 in both solutions was measured. For this determination (35), 0.3 ml. of Nessler reagent was added to 2.7 ml. of the urea solution and the absorbance at 460 mμ was compared with that of pure water, using a Unicam SP 800 B U.V. spectrophotometer. (Figure 10).

RESULTS

Visible effects of irradiation

Before irradiation with ultraviolet light, the solution (see p. 11) was clear. A few minutes after the beginning of irradiation, some turbidity appears. This turbidity goes on increasing and in less than 30 minutes pale yellow particles or aggregates of particles can be seen in the solution. These particles settle down by themselves when the solution is left undisturbed.

Morphology of the particles

If the solution is examined under the optical microscope, particles of a wide variety of forms can be observed. (Figures 12, 13, 14). The size of the particles goes up to $\sim 3\,\mu$ and a few of them show some internal structure. Quite often, the particles are linked together in an irregular chain or in relatively large aggregates. (Figure 15).

Under the electron microscope, it can be seen that some of the particles, like pollen grains, show protuberances on their surface. (Figures 16, 17). It can also be observed that the particles are quite sensitive to the electron bombardment. Some of them can be seen to sud-

denly shrink to almost nothing. After a few minutes of observation, even at low beam intensity, no particle remains and the grid is covered by a black film, probably mostly made of carbon. If the particles are put in a solution of silver nitrate (36), they become coated with silver after a few minutes and they can then withstand a more intense electron bombardment, making observation easier. Figure 16 shows the untreated particles and Figure 17 the silver coated particles. As can be seen, the morphology does not seem to be affected. The shape of the particles does not differ appreciably from what can be observed under the optical microscope.

Chemical Analysis

It appears from Figures 5 and 6 that amino acids are produced by the ultraviolet irradiation of the solution (see p. 14).

In the supernatant, before hydrolysis, we can detect the presence of polypeptide(s). Their concentration appears to increase with irradiation time since the intensity of the spots increases with irradiation time. In the two hours irradiated sample, glycine can also be detected.

After hydrolysis of the supernatant, glycine ap-

pears after only 30 minutes of irradiation and its concentration appears to increase with irradiation time. Two other amino acids can also be detected: lysine after a one hour irradiation and histidine after two hours.

Hydrolysis of the particles yields glycine, whose concentration seems to increase with irradiation time. For the 30 minutes irradiation sample, the glycine spot is so faint however that it can hardly be detected.

The methods used do not reveal the presence of any sugar, purine or pyrimidine in the irradiated solutions.

Catalytic Activity

It is observed that the particles (see p. 16) can reduce the decomposition speed of the H_2O_2 in the presence of $FeCl_3$ (Table I and Figure 18). Two days after the addition of the particles to the solution of H_2O_2 (see p. 16), ten times more H_2O_2 had decomposed in the control (without particle) than in the beaker with particles. The solution of H_2O_2 in contact with the particles turns from pale yellow to colorless in a few hours. (Figures 8A, 8B). However, around the particles, a deep brown region can be observed. Upon mixing, the solution recovers its initial color and the whole process can be repeated several times.

Table II and Figure 19 show that the ATP decomposition rate seems to be increased by the presence of the particles. However, even in the presence of the particles, the phosphate ion concentration increases very slowly. 430 hours after the addition of the particles to the ATP solution, the absorbance of the solution containing the particles is about four times larger than the control's absorbance.

A similar behavior can be observed for the decomposition rate urea. (Table III and Figure 20). The decomposition rate of urea seems to be increased in the solution that contains the particles. 430 hours after the addition of the particles to the urea solution, the absorbance is three times larger in the solution containing the particles than in the control.

ANALYSIS OF RESULTS

Morphology

Most of the particles have a roughly spherical shape and many small bulges on their surface. Some of them seem to have a complex internal structure. But as their size is almost at the limit of the resolving power of the optical microscope, this observation is not entirely satisfactory. The observation with the electron microscope does not tell more. In fact, since whole particles are opaque to the electrons, no information can be obtained concerning their internal structure. Only the use of thin slices of particles could reveal more details about the internal morphology. But these could not be prepared since no ultramicrotome was available.

However, the observations made show that the particles have some morphological similarity with cells: general shape, size, bulges and the possibility of an elaborate internal structure. Another similarity with cells is the ability of the particles to associate and to strongly aggregate. Even if the suspension containing the aggregates is vigorously agitated, most of them are unaffected.

Chemical composition

Amino acids are synthesized easily and even after a relatively short irradiation time. These results confirm those of Kolomiychenko (11), Pasynskii and Pavlovskaya (8).

The amino acids obtained are probably due to ultraviolet radiation acting on the solution and not to contamination by dirt or bacteria. As a control, a solution was prepared like all the others and submitted to the same treatment with the exception of ultraviolet irradiation. No particles were obtained and the solution remained clear. After desalting, drying, redissolving in 0.2 ml. of distilled water, hydrolysis with 6N HCl and chromatography, no amino acid could be detected.

Another proof that our results are not due to contamination is the fact that the concentration of the amino acids obtained seems to increase with the irradiation time. This would not be the case if these amino acids were initially introduced in the solution with the products: their concentration would remain constant or even decrease with time since amino acids are somewhat decomposed by ultraviolet radiation (37). The same is true if the amino acids were due to bacteria since bacteria cannot withstand an intense ultraviolet irradiation (38) and so could not

multiply under these conditions.

It would have been interesting to get quantitative results concerning the increase in concentration of the amino acids as a function of irradiation time that is clearly observed on the chromatograms (Figures 5 and 6) from the intensity of the spots. But the necessary instrumentation was not available.

The simplest of all amino acids, glycine, forms most easily. Glycine was also the only one incorporated in the particles. This may be due to the fact that it is smaller than histidine or lysine, the two other amino acids that were detected.

Chromatography does not reveal the presence of any sugar. This might be due to the fact the solution has a pH of 2. Formaldehyde is known to polymerize and give sugars only when the pH is greater than 7 (39).

In the case of purines or pyrimidines, a very faint broad spot have been observed by fluorescence in the region where the standards had all gathered. The absence of any appreciable amount of purine or pyrimidine may be due to the fact that these molecules are easily broken down by ultraviolet radiation. So that as soon as

they are formed, most of them rapidly decompose. If the vessel containing the solution would have been larger, these molecules would probably have been more easily preserved, having the possibility to reach a region where the ultraviolet intensity would be less. It would have been the case if the molecules were formed at the surface of the sea by the action of ultraviolet radiation coming from the sun. They could migrate towards the bottom so that they might escape the destructive effect of the radiation.

Catalytic activity

The particles have a strong effect on the decomposition rate of H_2O_2 in the presence of $FeCl_3$. This seems to be due to the fact that the ferric ions are attracted towards the particles. The Fe^{+++} concentration near the particles is much higher than otherwhere in the solution, making it dark brown around the particles and colorless otherwhere.

This result is very important. It means that the particles can concentrate ions and maybe molecules from a dilute solution so that the reaction rates in or near the particles may be much higher than in the milieu (the sea for example). This also means that they might be able to take from a dilute solution the materials from which

they are built so that they could grow. Of course, if the concentration of the products in the solution is reduced by the presence of the particles, the reaction rate there is reduced and this is exactly what we observed in the case of the H_2O_2 decomposition. These results are similar to those obtained by Labadie (39) in the case of the decomposition of ATP in the presence of Mg^{++}.

The study of the effect of the particles on the decomposition rate of ATP leaves some doubts about what really happens. The test used measures the phosphate liberated by the reaction

$$\text{ATP} \longrightarrow \text{ADP} + \text{phosphate}$$

It can be seen from Table II and Figure 19 that the phosphate concentration increases rapidly in the first minutes after the particles are put in contact with the ATP solution. Later on, the phosphate formation is much slower (but still faster than in the control solution). Is this due to phosphate ions that might have been in the particles and that are simply released in the solution, rapidly in the beginning and more slowly afterwards? Or is it due to the decomposition of the ATP? One can hardly tell since even if the particles had been washed ten times in distilled water, it is still possible that some phosphate has been retained by them.

A test measuring the amount of ATP left in the solution would of course be better but it would be useful only if the efficiency of the reaction would be high enough. If the efficiency is only a few percent after many days, as what we got, we cannot expect to be able to measure this difference with a satisfactory accuracy.

An answer to this problem might be to introduce some Mg^{++} ions in the ATP solution (41) as a cocatalyst. The presence of this metallic ion might increase the spontaneous decomposition rate of ATP and make the effect of the particles more evident and this probably after a shorter time.

These observations with regard to the effect of the particles on the decomposition rate of ATP seems to hold also for their effect on the decomposition rate of urea.

Even after 430 hours, the amount of NH_4^+ released is not very large. And since the particles might have retained some NH_4^+ ions even after ten washings with distilled water, it is not perfectly clear that the NH_4^+ concentration measured is really due to the decompostion of urea.

A better result could probably be obtained if we would also use a metallic ion as a cocatalyst to speed up the spontaneous decomposition rate of urea so that the effect of the particles be more evident.

CONCLUSION

The results that have been obtained confirm the work of other workers on the abiogenic photosynthesis of biochemicals: amino acids and polypeptides in the occurrence.

It has also been shown that under the conditions that are believed to have existed on earth before the appearance of life, the formation of particles similar to a primitive cell in such respects as size, morphology, chemical composition and even catalytic activity is highly probable.

For the future, it would be interesting to study the organization of the systems that have been synthesized and their interaction with their environment.

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Figures 1 and 2: Irradiation set-up.

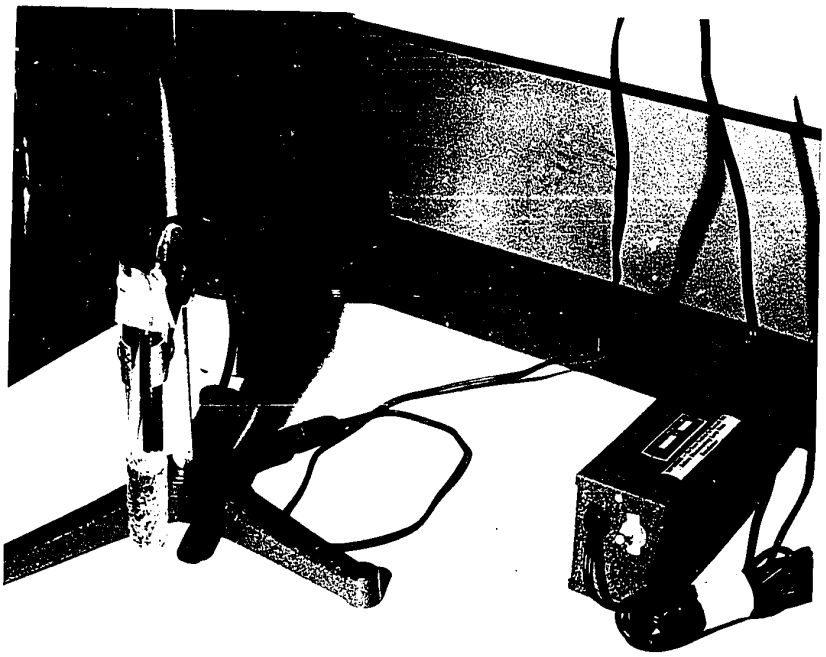


Figure 1

Figure 2

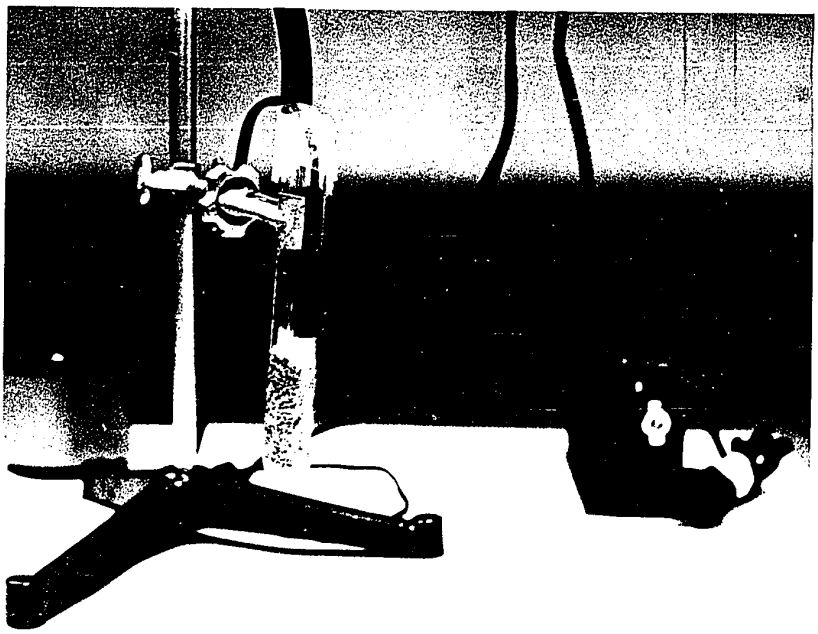
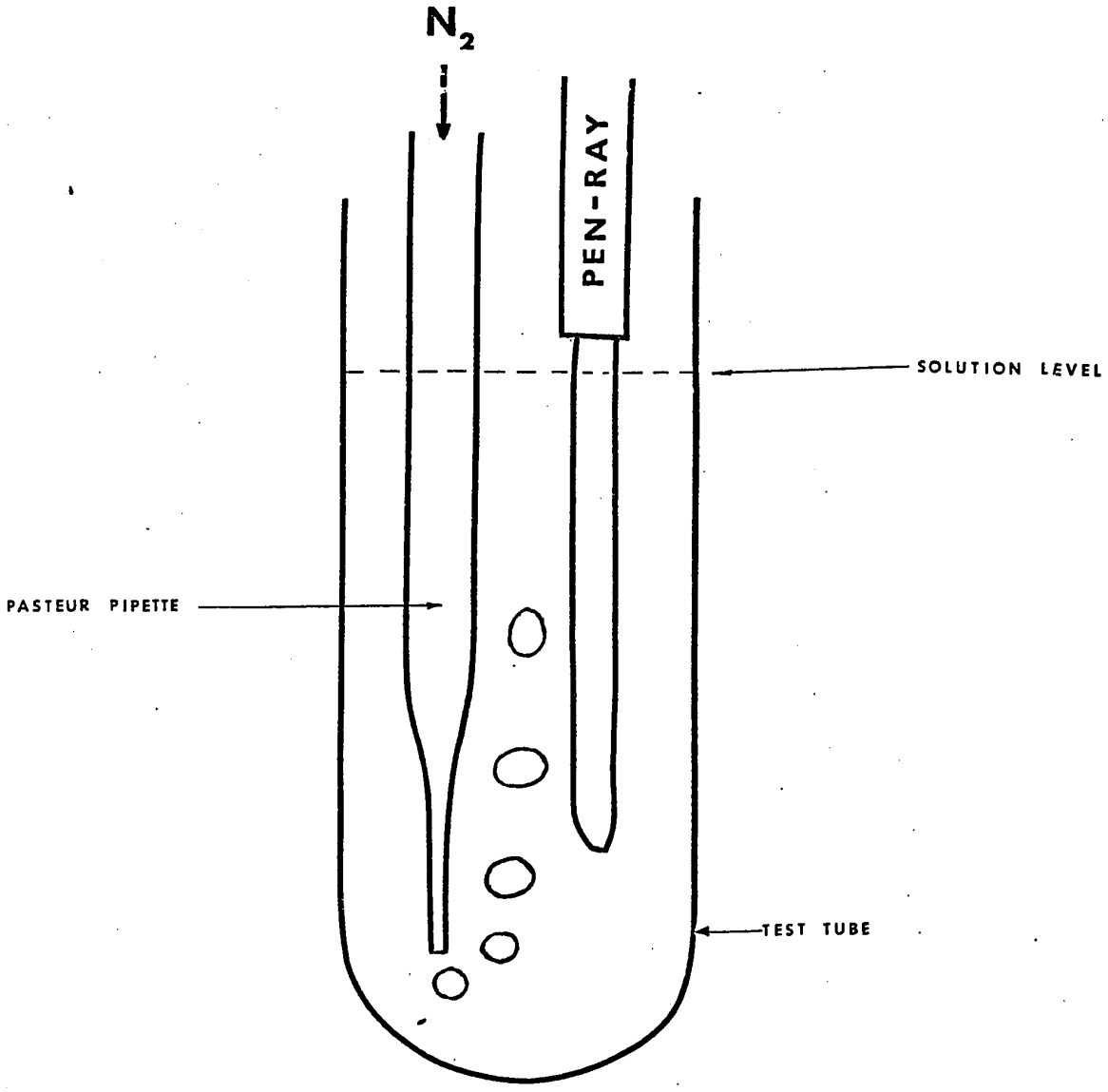


Figure 3

IRRADIATION SET-UP DIAGRAM



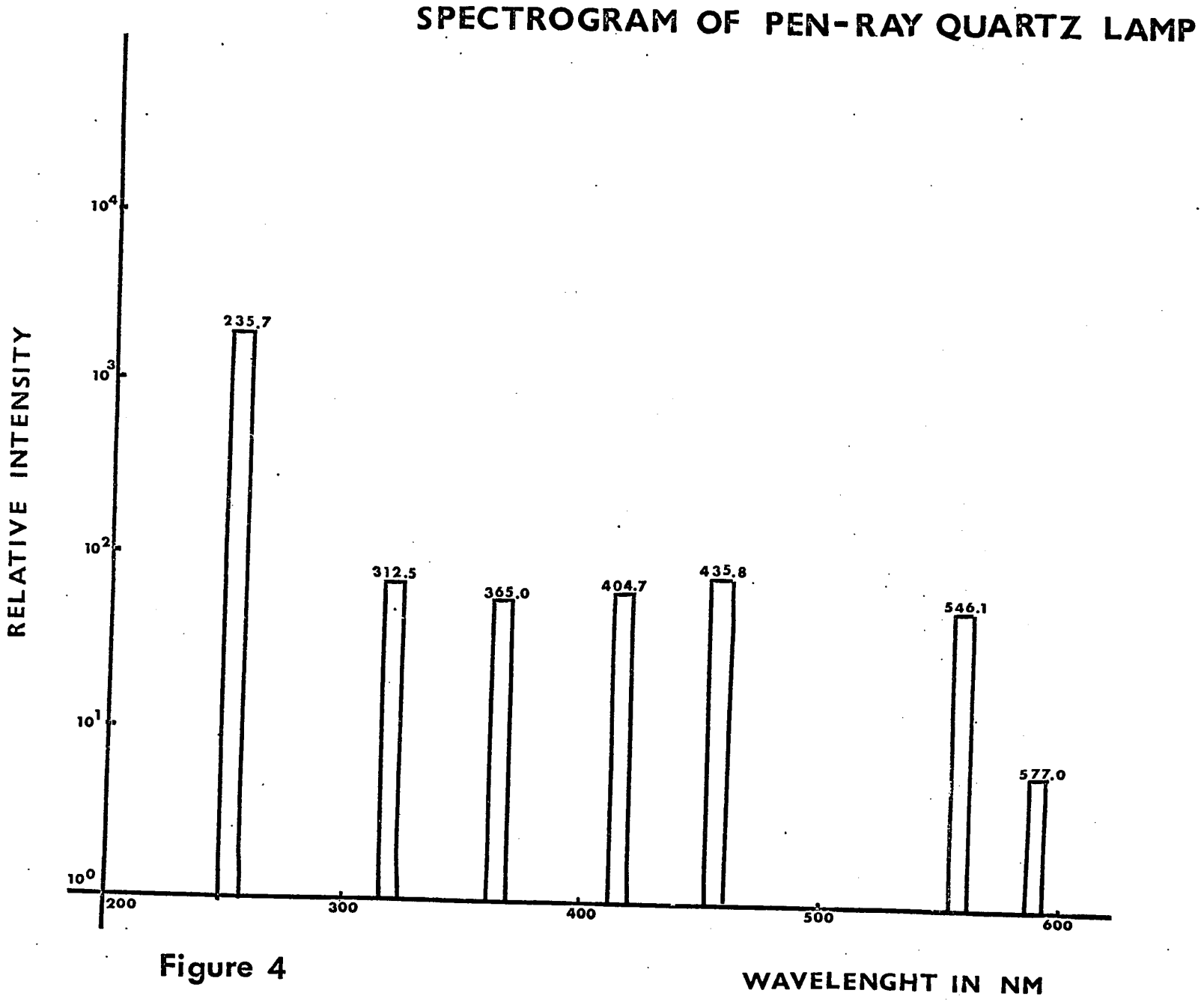

SPECTROGRAM OF PEN-RAY QUARTZ LAMP
RELATIVE INTENSITY
10^4
10^3
10^2
10^1
10^0
235.7
312.5
365.0
404.7
435.8
546.1
577.0
200
300
400
500
600
WAVELENGHT IN NM

Figure 4

Figure 5: Chromatogram for the detection of amino acids. Solvent: n-butanol, glacial acetic acid, water (80:20:20) Reagent: ninhydrin. Samples: 1, non hydrolyzed supernatant 30' irr.; 2, hydrolyzed supernatant 30' irr.; 3, hydrolyzed particles 30' irr.; 4. non hydrolyzed supernatant 45' irr.; 5, hydrolyzed supernatant 45' irr.; 6, hydrolyzed particles 45' irr.; 7, non hydrolyzed supernatant 60' irr.;8 hydrolyzed supernatant 60' irr.; 9, hydrolyzed particles 60' irr.; 10, non hydrolyzed supernatant 120' irr.; 11, hydrolyzed supernatant 120' irr.; 12, hydrolyzed particles 120' irr. Standards: 13, histidine; 14, lysine; 15, glycine; 16, proline; 17. glutamic acid; 18. alanine; 19, valine; 20, isoleucine.

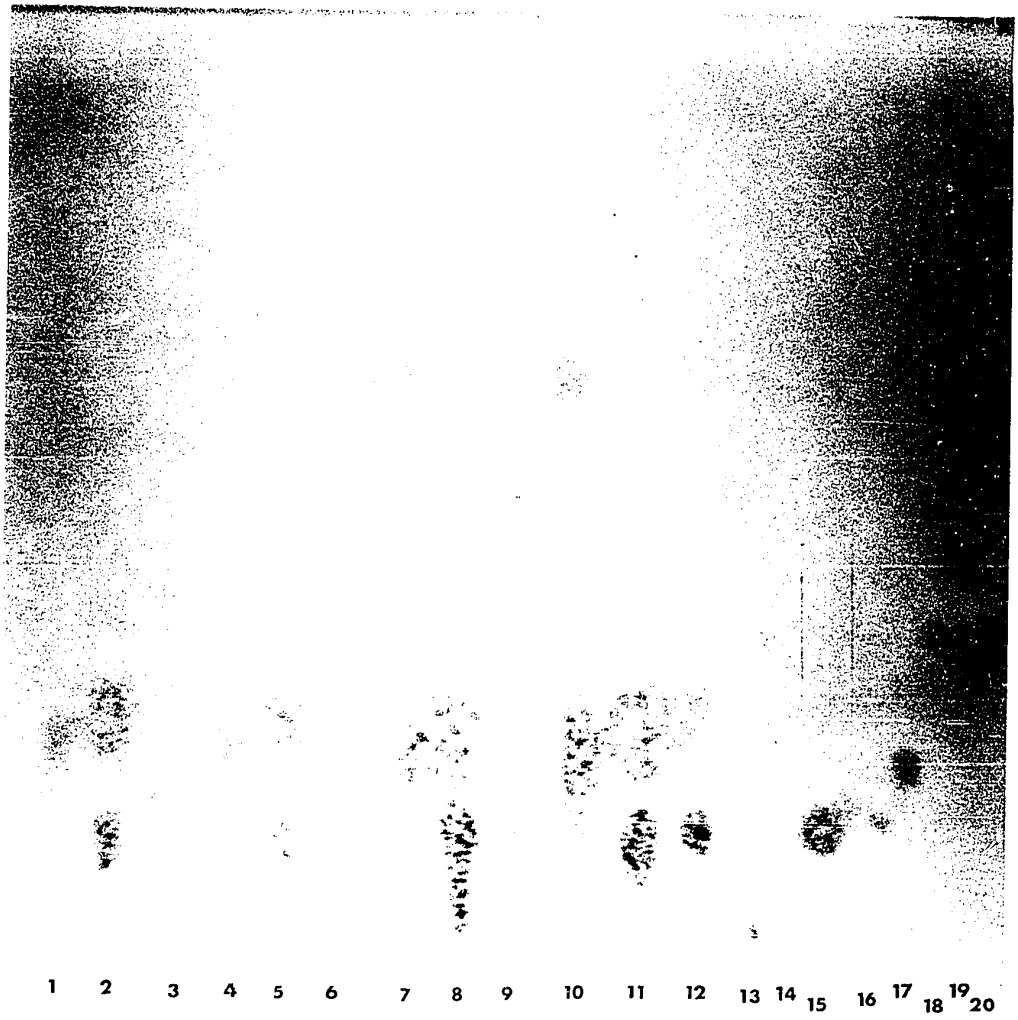


Figure 5

Figure 6: Chromatogram for the detection of amino acids. Solvent: ethanol water (70:30). Reagent: ninhydrin. Samples: 1, non hydrolyzed supernatant 30' irr.; 2, hydrolyzed supernatant 30' irr.; 3, hydrolyzed particles 30' irr.; 4, non hydrolyzed supernatant 45' irr.; 5, hydrolyzed supernatant 45' irr.; 6. hydrolyzed particles 45' irr.; 7, non hydrolyzed supernatant 60' irr.; 8, hydrolyzed supernatant 60' irr.; 9, hydrolyzed particles 60' irr.; 10, non hydrolyzed supernatant 120' irr.; 11, hydrolyzed supernatant 120' irr.; 12, hydrolyzed particles 120' irr. Standards: 13, histidine; 14, lysine; 15, glycine; 16, proline; 17, glutamic acid; 18. alanine; 19, valine; 20. isoleucine.

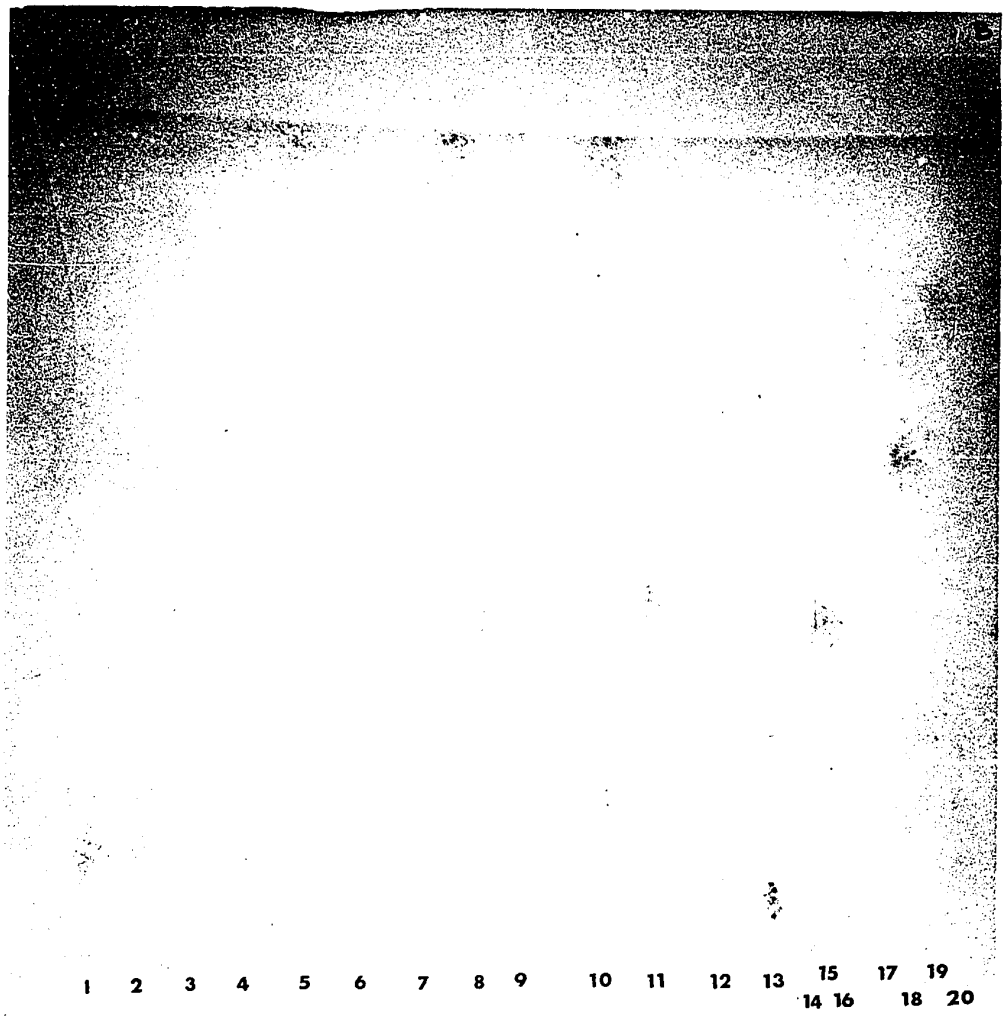


Figure 6

Figure 7: Chromatogram for the detection of sugars. Solvent: methyl-ethyl ketone, glacial acetic acid, methanol (60:20:20). Reagent: aniline phtalate. Standards: 1, ribose; 2, deoxiribose; 3, fructose; 4, lactose; 5, maltose.

Figure 7

Figure 11

TREATMENT OF SOLUTIONS DIAGRAM

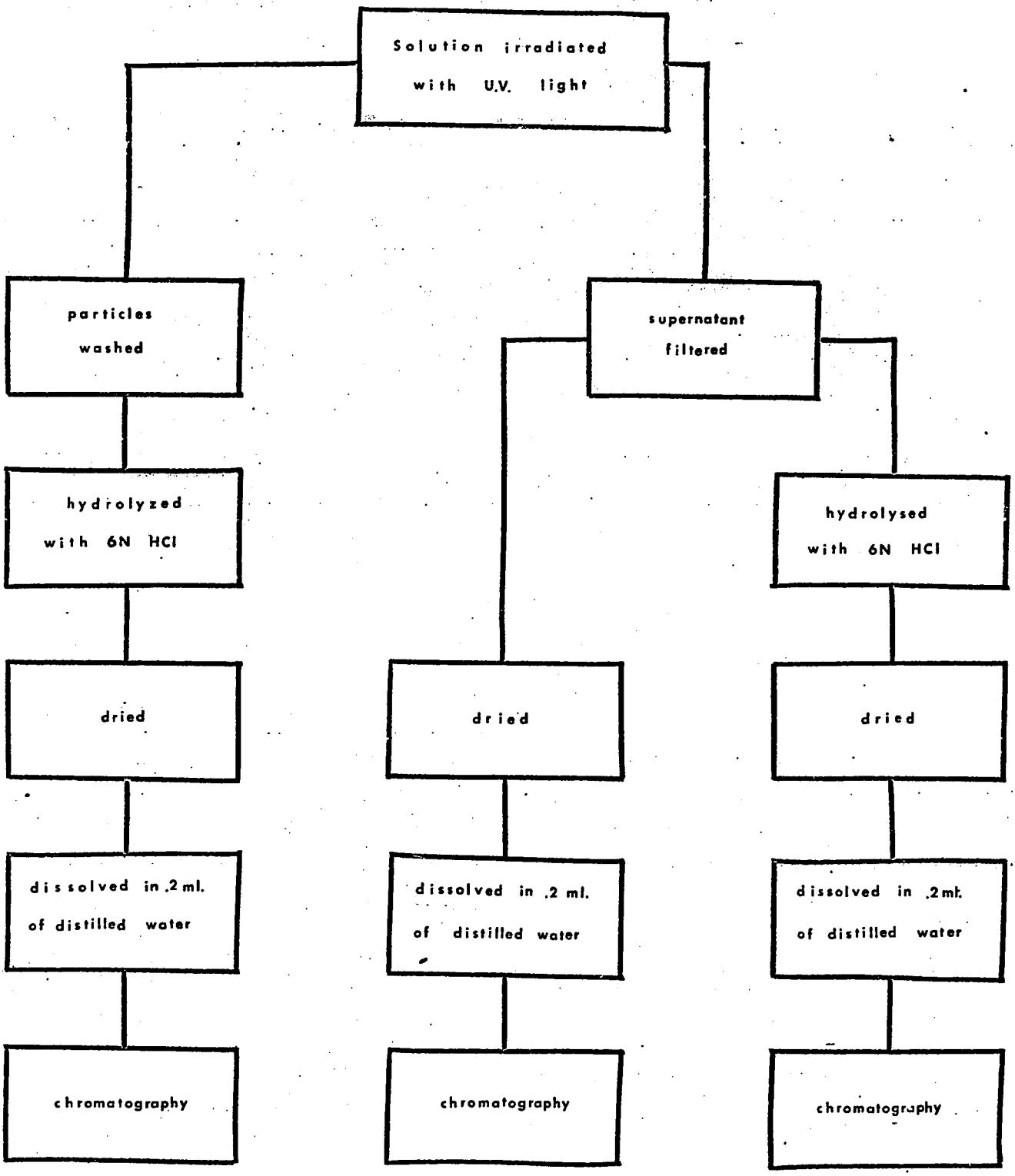


Figure 8. A: Beaker I: Control, contains H_2O_2 (0.1%) and $FeCl_3$ but no particle; Beaker II, contains H_2O_2 (0.1%) $FeCl_3$ and particles. B: close-up of Beaker II.

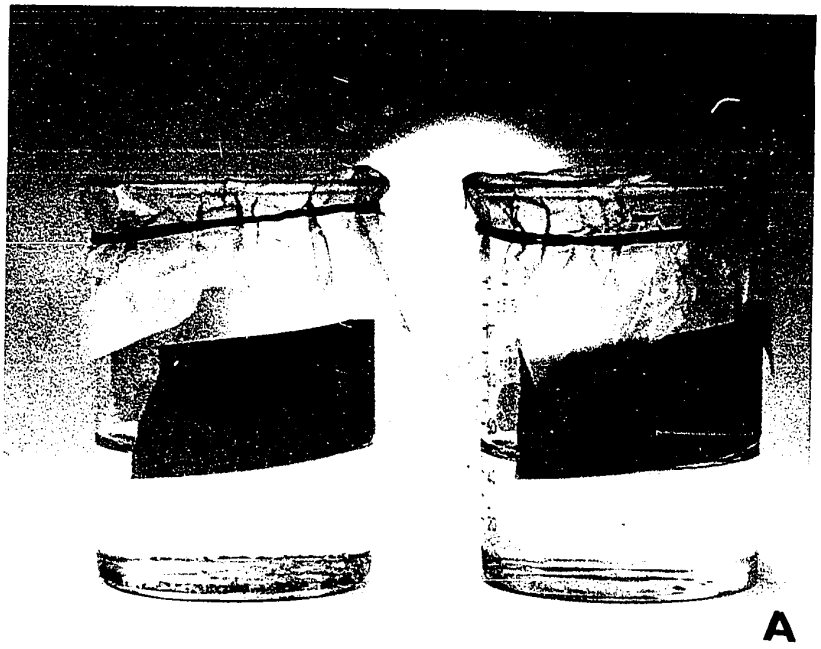


Figure 8

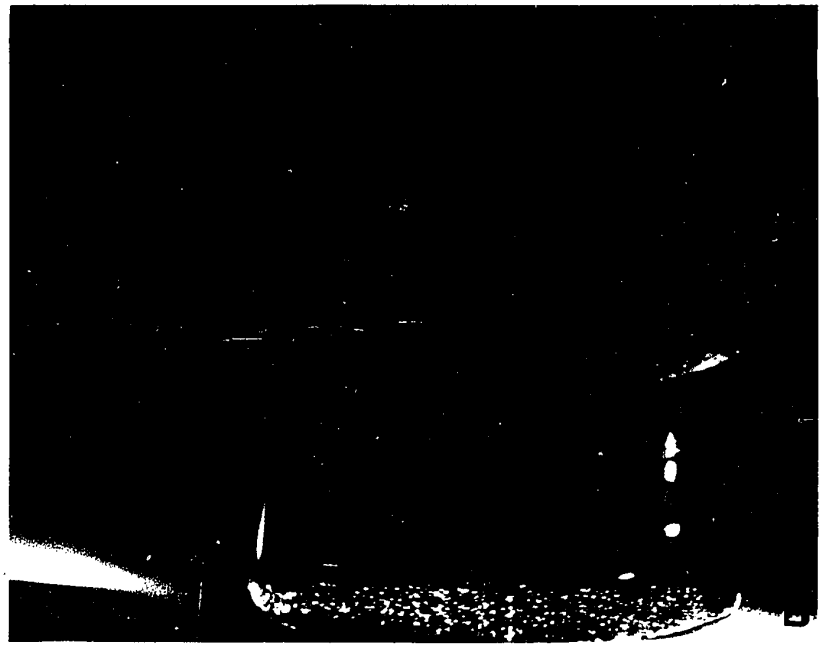
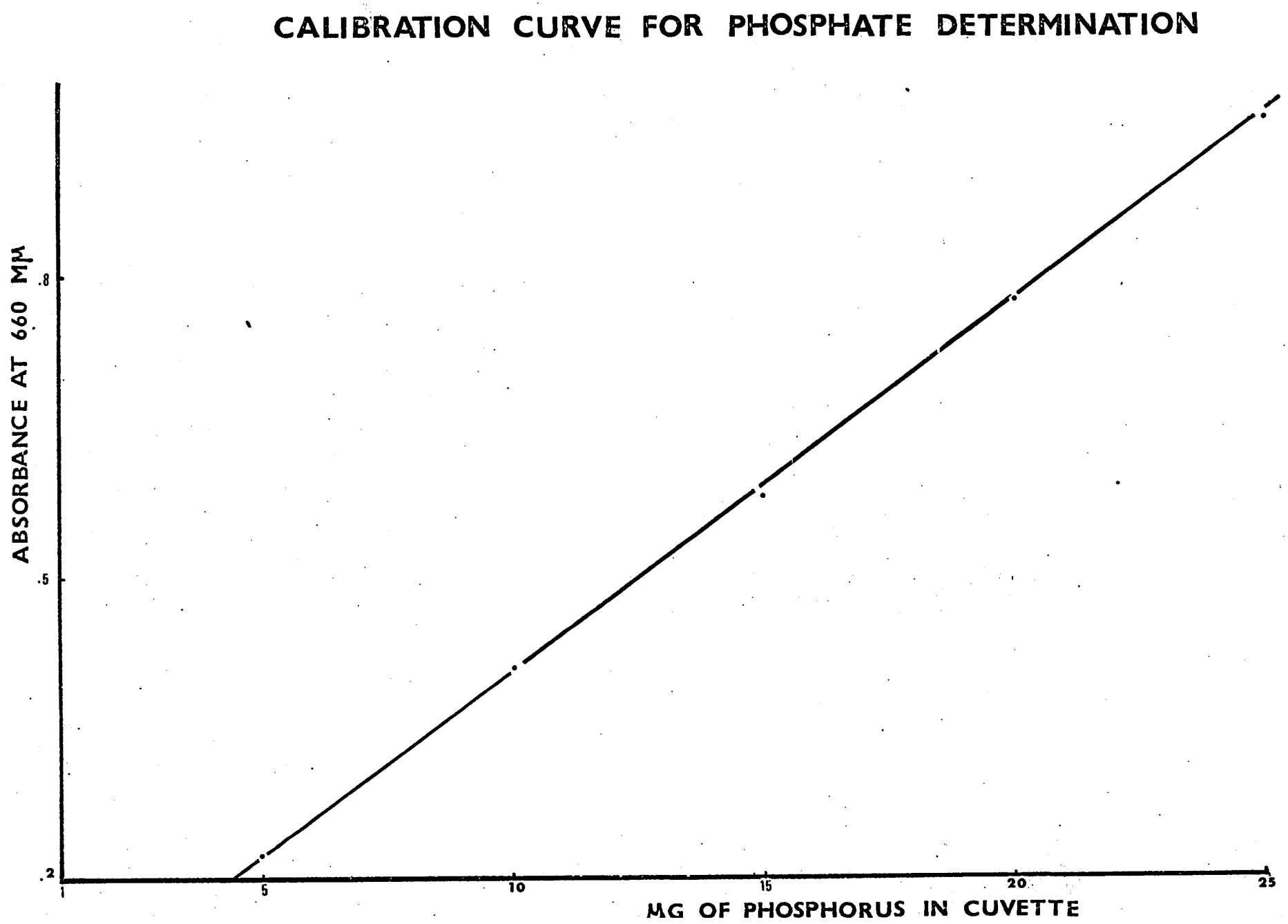


Figure 9


CALIBRATION CURVE FOR PHOSPHATE DETERMINATION
ABSORBANCE AT 660 MM
.8
.5
.2
1
5
10
15
20
25
MG OF PHOSPHORUS IN CUVETTE

CALIBRATION CURVE FOR AMMONIA DETERMINATION

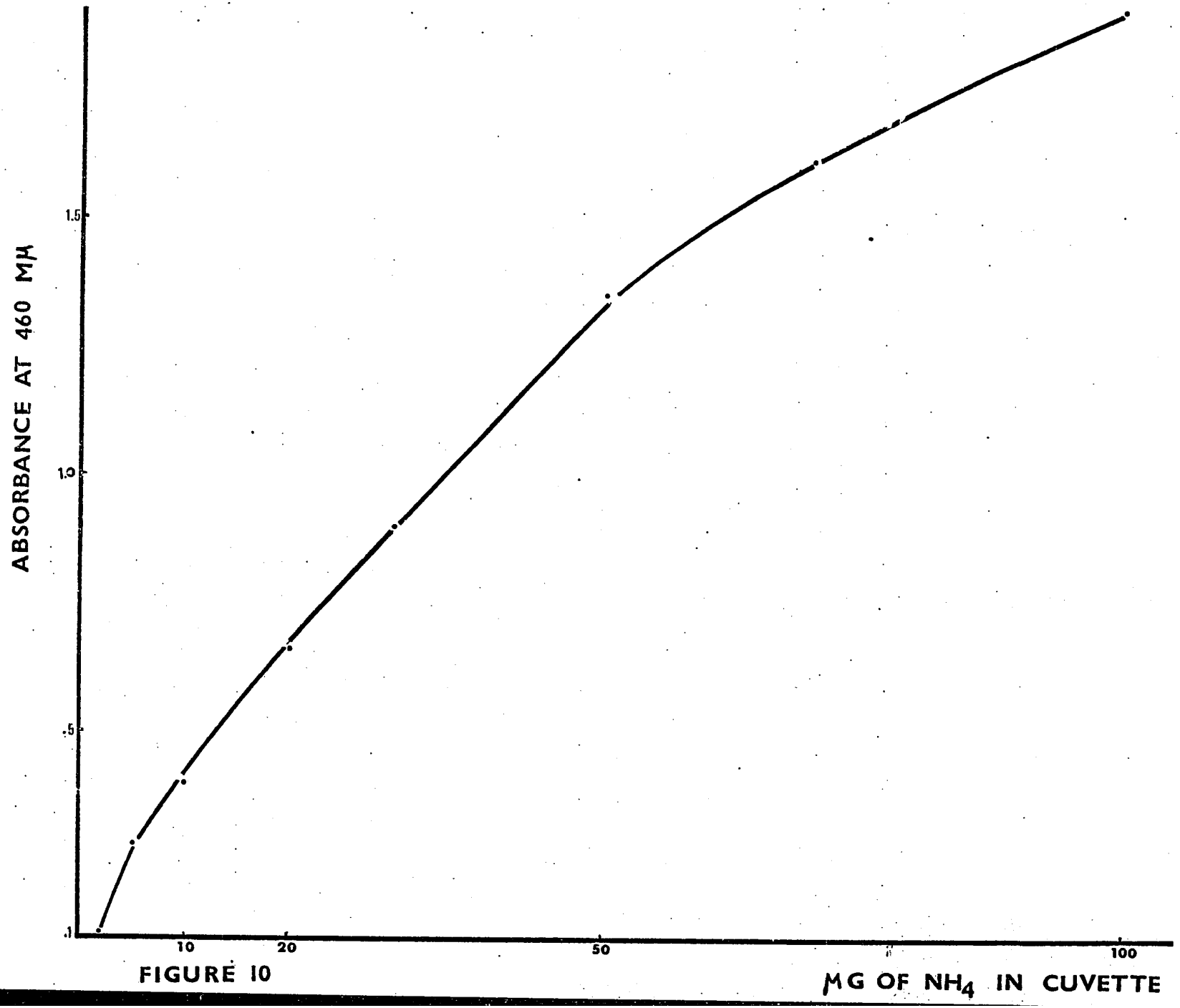


FIGURE 10

Figures 12 and 13: Particles as seen under the optical microscope.

Figure 12

10μ

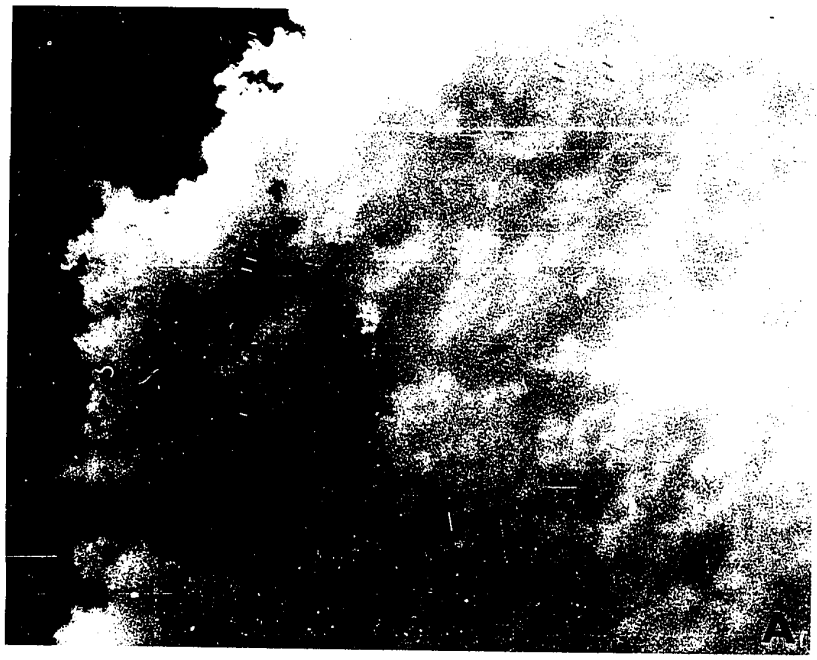
13

5μ

Figure 14: particles as seen under the optical microscope.

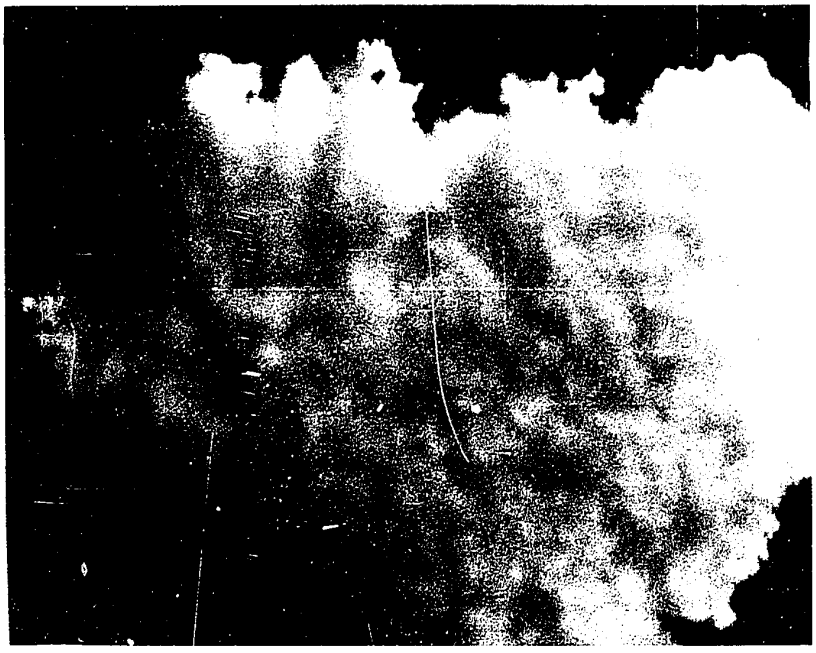
FIGURE 14

Figures 15A and 15B: aggregate of particles as seen under the optical microscope.



20μ

FIGURE 15



50μ

Particles as seen under the electron microscope.
Figure 16: untreated. Figure 17: silver coated.

FIGURE 16

FIGURE 17

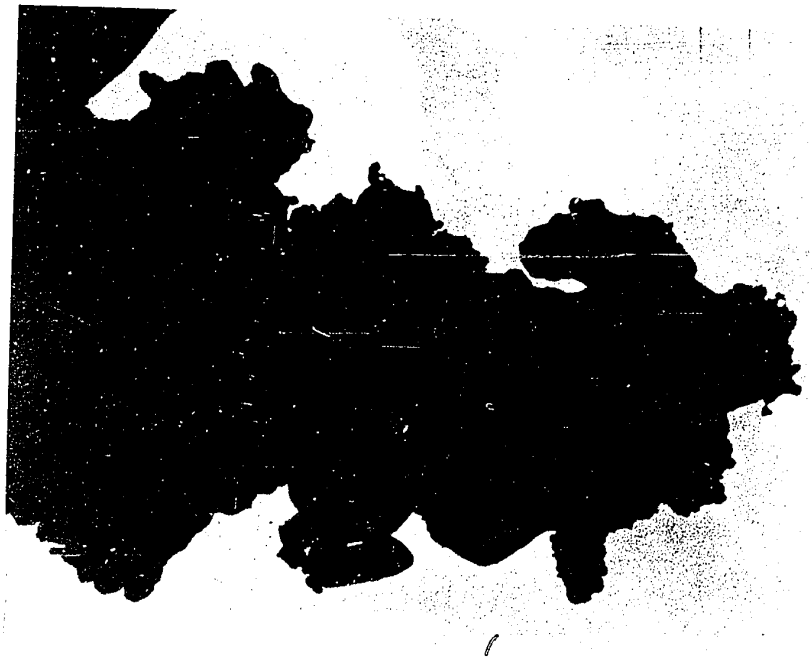


Table I: t: time between addition of the particles to the solution and titration; V_1: volume of $KMnO_4$ solution needed for titration of control; V_2: volume of $KMnO_4$ solution needed for titration of sample containing particles; ΔV_1: difference between V_1 as measured just before adding the particles and its value at time t; ΔV_2: same ΔV_1, but for V_2; ΔC_1: H_2O_2 concentration decrease in control; ΔC_2: same in sample containing particles.

TABLE I

H_2O_2 CONCENTRATION AS A FUNCTION OF TIME

t in HOURS	V_1 in ML	ΔV_1 in ML	ΔC_1 in MOLE/LITRE x 10^{-4}	V_2 in ML	ΔV_2 in ML	ΔC_2 in MOLE/LITRE x 10^{-4}
0	46.3	0	0	46.2	0	0
.25	45.8	.5	2.38	45.4	.8	3.80
1.0	45.3	1.0	4.75	45.4	.8	3.80
23.25	35.5	10.8	51.2	44.5	1.7	8.06
45.75	30.0	16.3	77.4	44.4	1.8	8.55
70.75	25.1	21.2	100	43.3	2.9	13.8
90.75	22.0	24.3	115	42.5	3.7	17.6
191.0	12.5	33.8	140	39.5	6.7	31.8
266.0	9.2	37.1	176	37.0	9.2	43.6
427.5	4.6	41.7	198	33.3	12.9	61.4

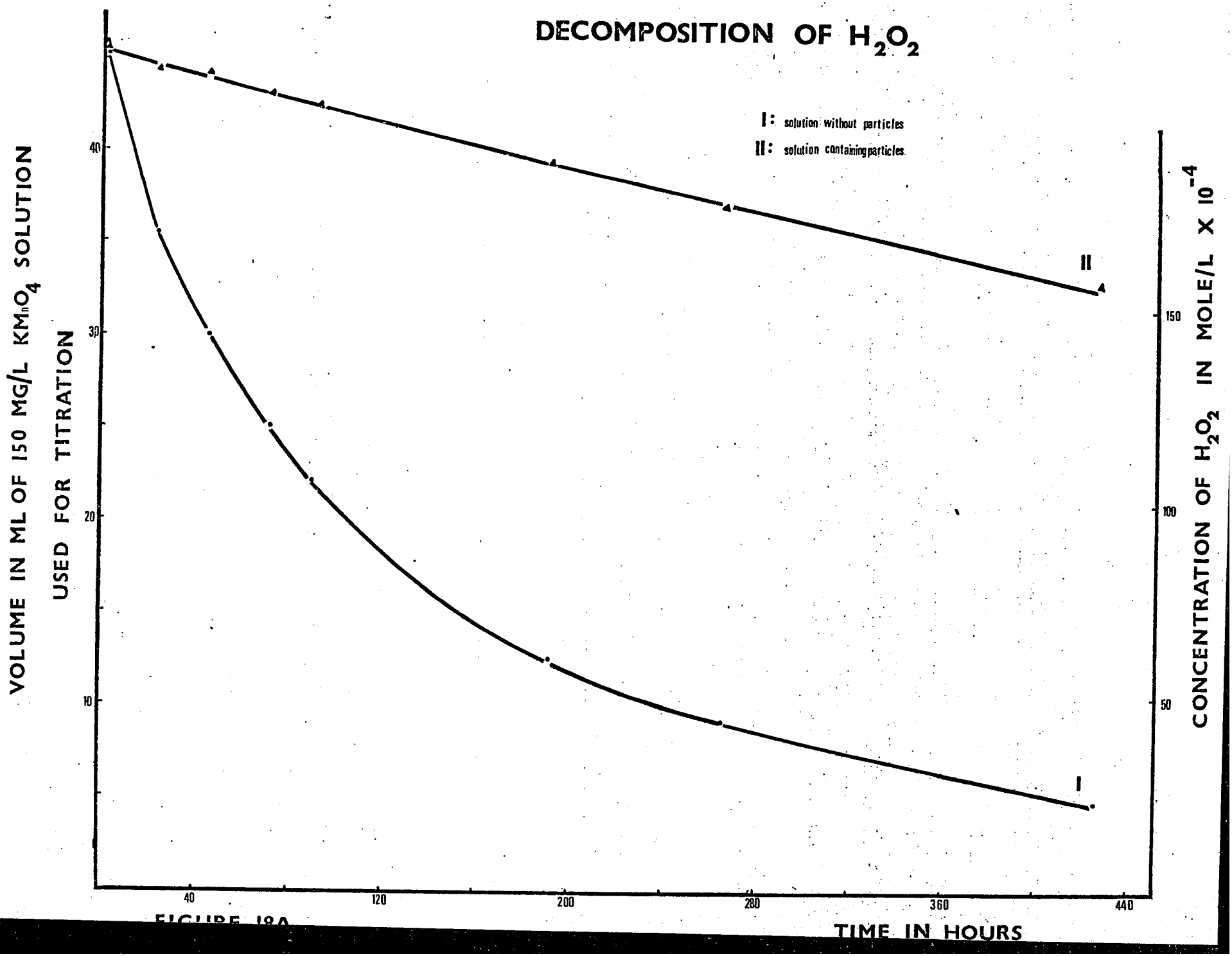

DECOMPOSITION OF H_2O_2
I: solution without particles
II: solution containing particles
VOLUME IN ML OF 150 MG/L $KMnO_4$ SOLUTION USED FOR TITRATION
CONCENTRATION OF H_2O_2 IN MOLE/L X 10^{-4}
TIME IN HOURS
10
20
30
40
50
100
150
40
120
200
280
360
440
I
II

FIGURE 18A

DECOMPOSITION OF H_2O_2

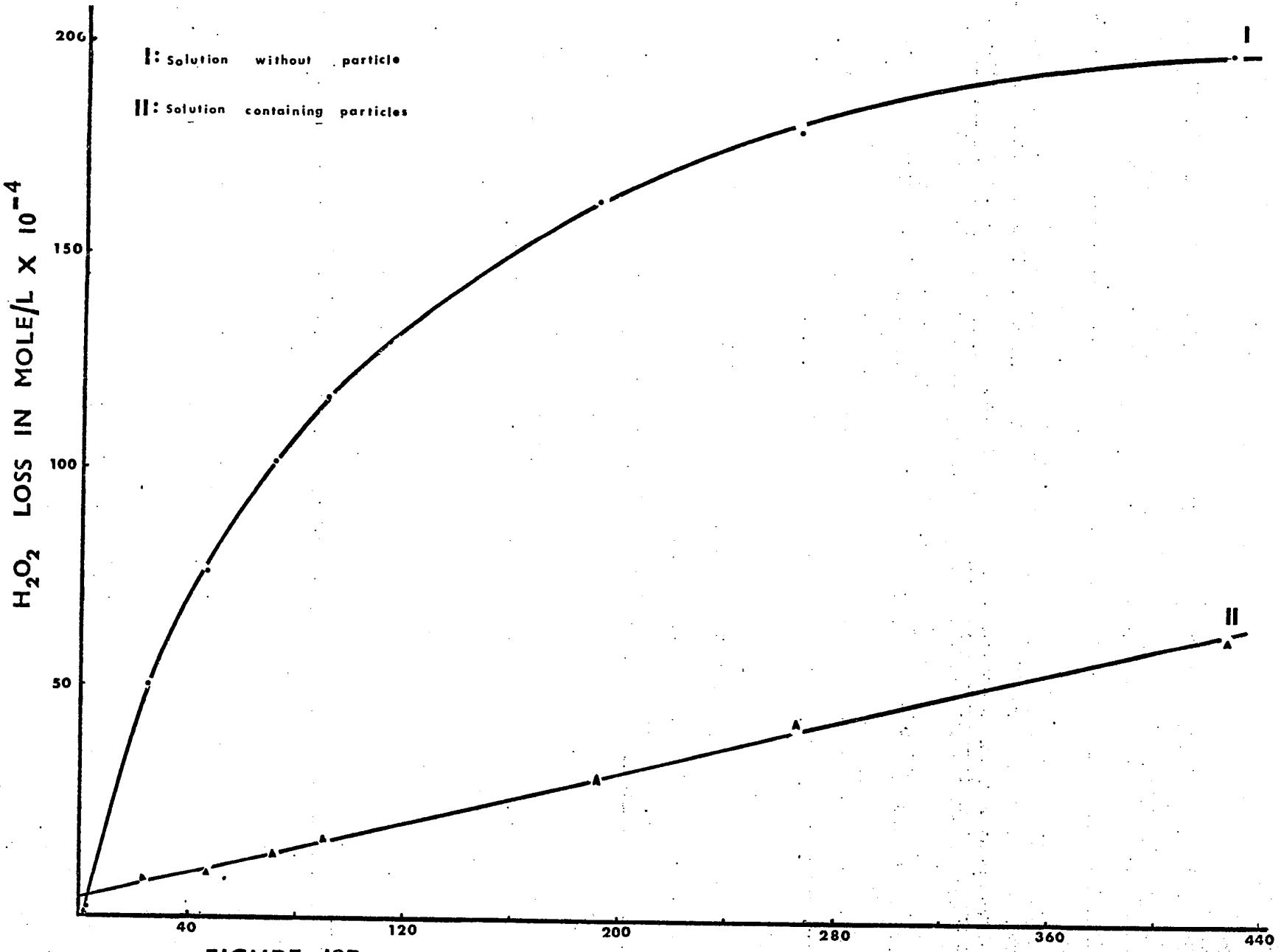


FIGURE 18B

Table II: t: time between addition of the particles to the ATP solution and determination of phosphate concentration; A_1: absorbance of control solution after colorimetric reaction; A_2: Absorbance of sample after colorimetric reaction; C_1: concentration of phosphate in control; C_2: concentration of phosphate in sample.

TABLE II

ATP CONCENTRATION AS A FUNCTION OF TIME

t in hours	A_1	C_1 in μg/ml	A_2	C_2 in μg/ml
0	.02	< .5	.01	< .5
.25	.02	< .5	.03	< .5
1.17	.02	< .5	.08	.6
1.5	.02	< .5	.08	.6
25.17	.02	< .5	.16	1.9
47.75	.02	< .5	.16	1.9
72.25	.02	< .5	.17	2.0
92.25	.02	< .5	.17	2.0
193.75	.04	< .5	.21	2.7
268	.05	< .5	.22	2.8
430	.06	~ .6	.27	3.6

DECOMPOSITION OF ATP

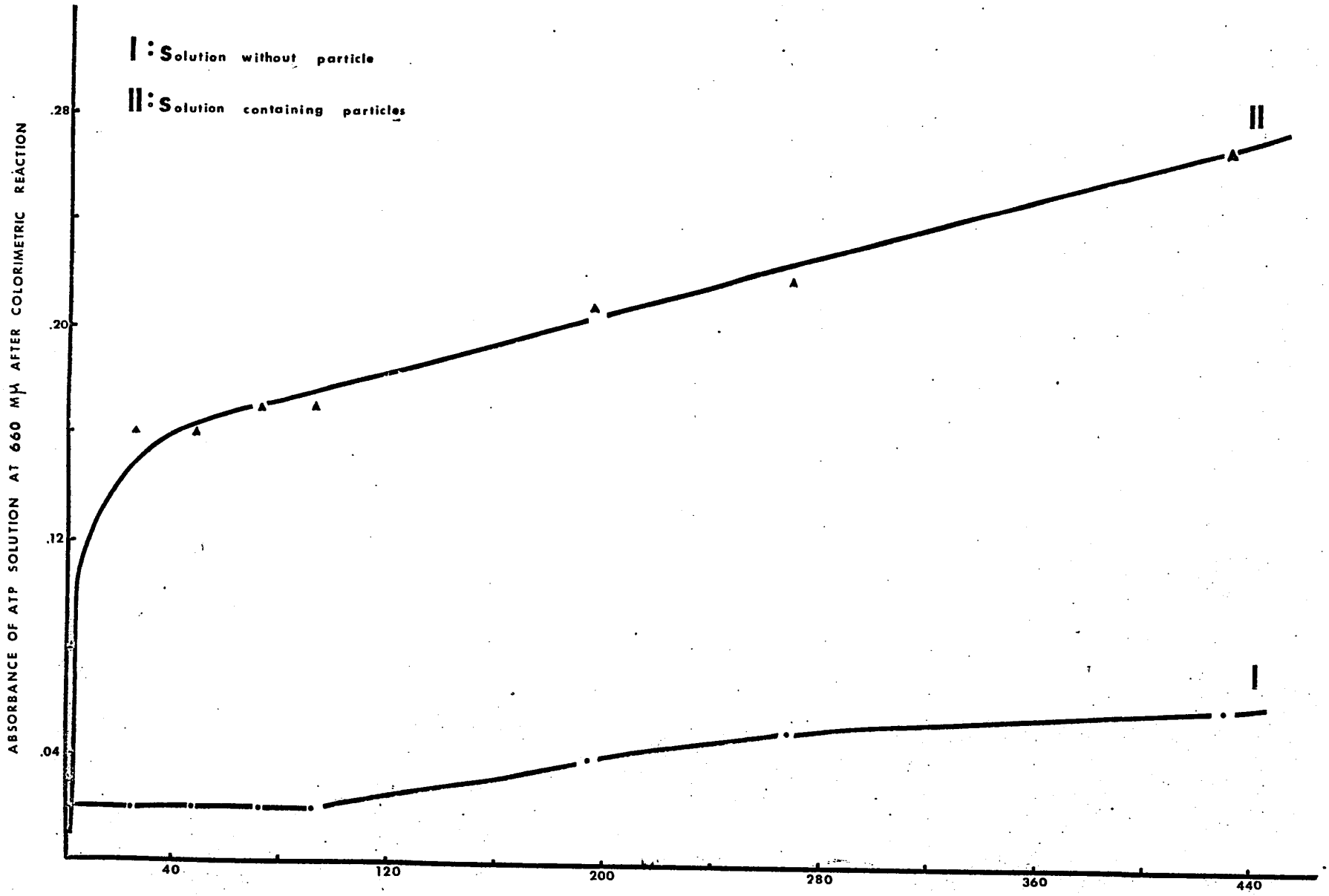


FIGURE 19

Table III: t: time between addition of the particles to the urea solution and determination of ammonia concentration; A_1: absorbance of control solution after colorimetric reaction; A_2: absorbance of sample after colorimetric reaction; C_1: concentration of ammonia in control; C_2: concentration of ammonia in sample.

TABLE III

UREA CONCENTRATION AS A FUNCTION OF TIME

t in hours	A_1	C_1 in µg/ml	A_2	C_2 in µg/ml
0	.00	< .8	.00	< .8
.25	.00	< .8	.03	< .8
1.0	.00	< .8	.03	< .8
24.6	.00	< .8	.04	< .8
47.2	.00	< .8	.04	< .8
72.0	.00	< .8	.04	< .8
91.7	.00	< .8	.06	< .8
192.7	.01	< .8	.08	< .8
266.7	.01	< .8	.16	.9
430.0	.06	< .8	.17	1.0