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THE PATH OF CARBON: FROM STRATOSPHERE TO CELL

Melvin Calvin

January 1983

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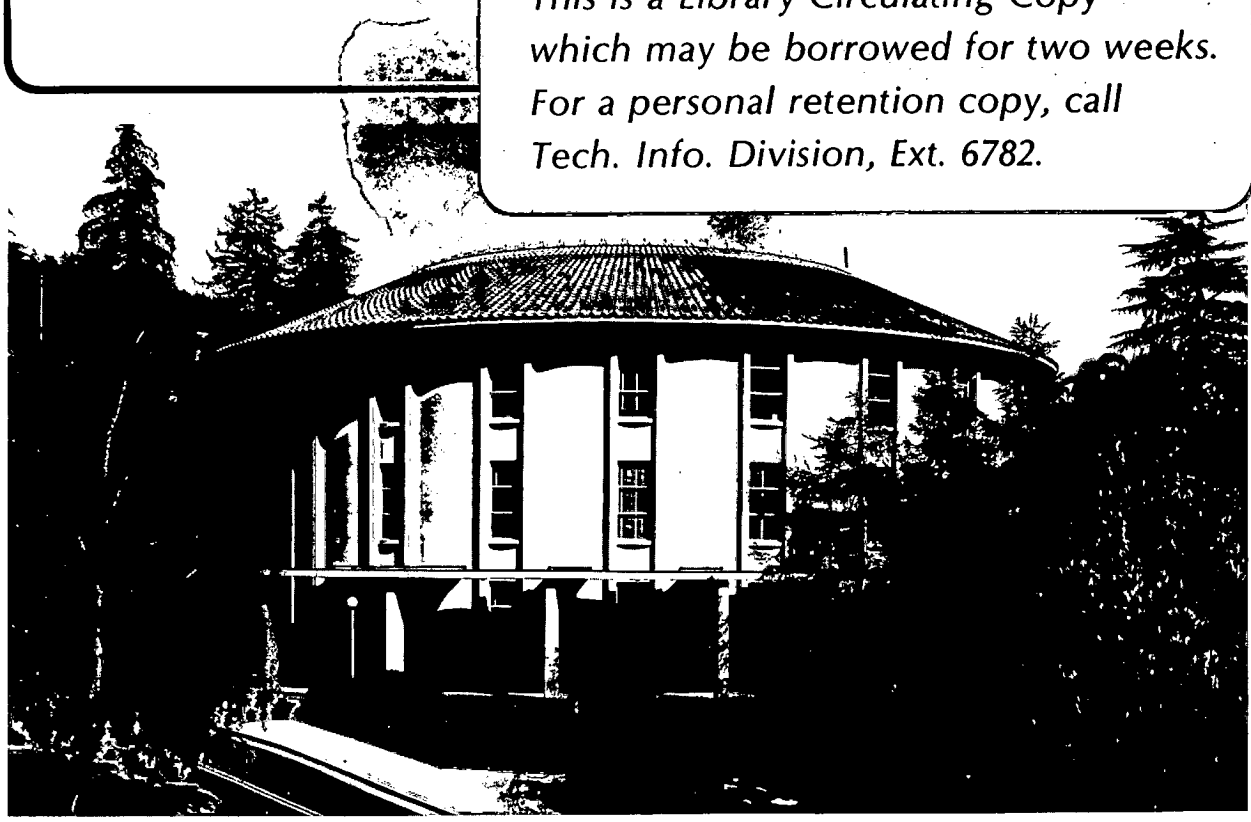

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THE PATH OF CARBON:

FROM STRATOSPHERE TO CELL

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ABSTRACT

A lifetime concern with organic reaction mechanisms turned to biological oxidation and reduction has proliferated to an understanding of chemical evolution, photosynthesis and the application of that information in alleviation of societal energy and disease problems.

Feodor Lynen Lecture, 15th Miami Winter Symposium "Advances in Gene Technology: Molecular Genetics of Plants and Animals". To be published by Academic Press. Dates of Symposium: January 17-21, 1983

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* This work was sponsored, in part, by the Office of Basic Energy Sciences, Division of Chemical Sciences; and the Office of Renewable Energy, Biomass Energy Technologies Division of the U.S. Department of Energy under Contract No. DE-AC03-76SF00098.

THE PATH OF CARBON: FROM STRATOSPHERE TO CELL

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I. INTRODUCTION

In preparing for this occasion it occurred to me that Feodor Lynen was one of the pioneers in developing our understanding of biosynthesis, particularly of hydrocarbons in animal systems. These included fatty acids, on the one hand, and isoprenoids on the other. It seemed entirely appropriate, therefore, for me to consider a brief history of the evolution of our knowledge of how plants manufacture from carbon dioxide and water all of their substances. Furthermore, in line with the title of this year's 15th Miami Winter Symposium--Advances in Gene Technology: Molecular Genetics of Plants and Animals--in order to perform any genetic manipulation of biosynthetic pathways one must first know the pathway(s) and how it is metabolically regulated and then understand in detail the genetic control of each of the steps (enzymes) in that pathway.

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[1] The work described was sponsored, in part, by the Office of Basic Energy Sciences, Division of Chemical Sciences and the Office of Renewable Energy, Biomass Energy Technologies Division of the Department of Energy under Contract Number DE-AC03-76SF00098.

II. HISTORICAL BACKGROUND

In examining the history of the relation of plants to the atmosphere it appears that the people involved in the chemical revolution which occurred at the end of the 18th century were also involved in the development of our understanding of plant nutrition and metabolism. (1) One of the first experiments designed to demonstrate that the plant is converting its nutrients into its own substance and what those nutrients are, was done by Johann Baptista van Helmont, a Belgian physician, in 1648. His famous willow tree experiment in which he followed the growth of a tree in a pot of soil which was fed only water and kept an accounting of the weight of the tree showed an increase in dry matter over and above the water added. His contribution at that time was constrained by the alchemists' view of things in which materials could be transmuted from one into the other by various agencies. The English chemist, William Boyle, repeated these experiments in 1661-1666 with the same result. There was considerable discussion at the time of "transmutation". In 1766 another English experimenter, Stephen Hales (a clergyman) took a large step forward when he learned how to use the inverted glass tube that Boyle had devised to measure gaseous absorption and evolution by plants.

However, it remained for Joseph Priestley to recognize, using the same technique that Hales devised, that plants could "revitalize" air which had been "vitiated" by either maintaining a mouse in it until it no longer survived or burning a candle in it until the candle was extinguished (Figure 1). Priestley performed his experiments between 1776 and 1781 and recognized the balance between the animal respiration and the plants' activity in reversing the effect of the animal and the candle on the spoiling of an enclosed air space. His early contributions, however, were still circumscribed by the alchemists' view of phlogiston.

The work was taken up by a Dutch physician, Jan Ingen-Housz in 1779 and he continued with his experiments until 1796. It was Ingen-Housz who pointed out the participation of at least two components in the balance of nature that Priestley saw. The difficulty that Priestley had encountered was to recognize that the green matter which appeared in the water supporting his whole plant experiments, which may have lasted from several weeks to months, was indeed plant material, that is, algae. At first he believed it wasn't so, because the experiments were done in well stoppered vessels and he did not realize that the algae came in with the well water he used. He thought the green matter was some inorganic residue that developed in the water. It was Ingen-Housz who recognized the

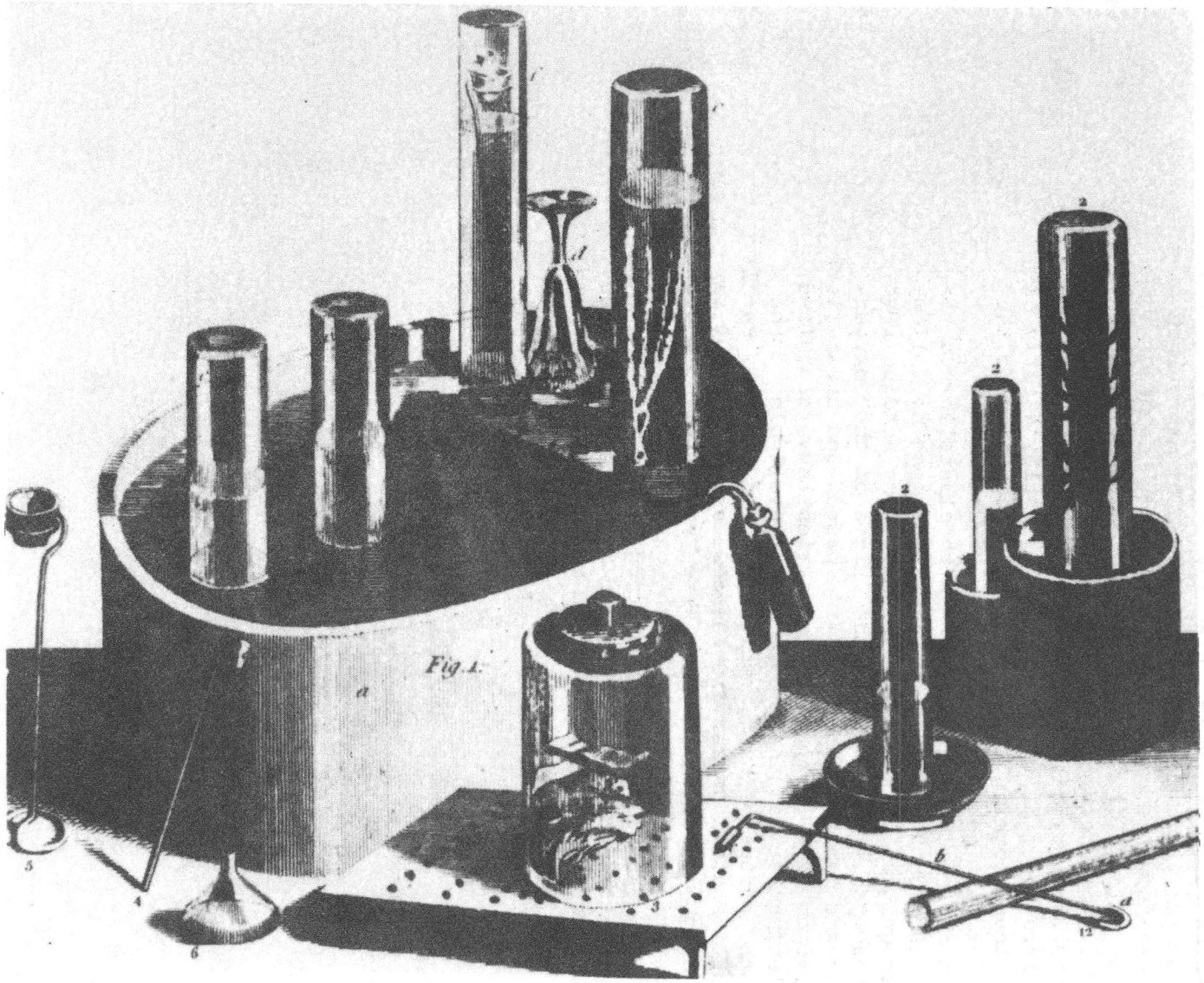

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Figure 1. Priestley experiment involving candle and mouse.

Joseph Priestly showed that air which became depleted upon burning a candle in it could be restored by photosynthesis of green plants — by a sprig of mint, by groundsel or, most rapidly, by spinach. Air that was sufficiently restored would sustain a living mouse (under inverted beer glass *d*).

Joseph Priestly, *Experiment and Observations on Different Kinds of Air.* Vol. I (1774).

green matter for what it really was, namely, microalgae (Figure 2). It was the failure of Priestley to perceive that he was dealing with plant material in the form of microalgae that deflected him from the realization deduction. His major concern was the balance of nature between plants and animals. This failure, together with the failure to appreciate the importance of light in the revitilization of vitiated air led to a certain irreproducibility in many of Priestley's early experiments, thus blocking him from first recognition of the importance of what he had seen.

Even in the early period of work with plants toward the end of the 18th century a design had already begun, following the recognition of the nature of oxygen by Antoine Lavoisier, a Franchman, in 1770 as to the origin of that oxygen. Ingen-Housz and Jean Senebier (a Swiss pastor and naturalist) who worked from 1782-1792, as well as Priestley, recognized the correlation between the amount of air available to the plant and the amount of oxygen which it could produce. But it was not until another Swiss, Nicolas deSaussure, in 1804 saw that both carbon dioxide and water were required by the plant that the question of the origin of the oxygen became an issue.

Early on, because of the correlation betwen the volume of fixed air, the carbon dioxide absorbed by the plant and the volume of oxygen generated, it seemed that the oxygen should

FIGURE 2. Algae (photograph by G. E. Calvin)

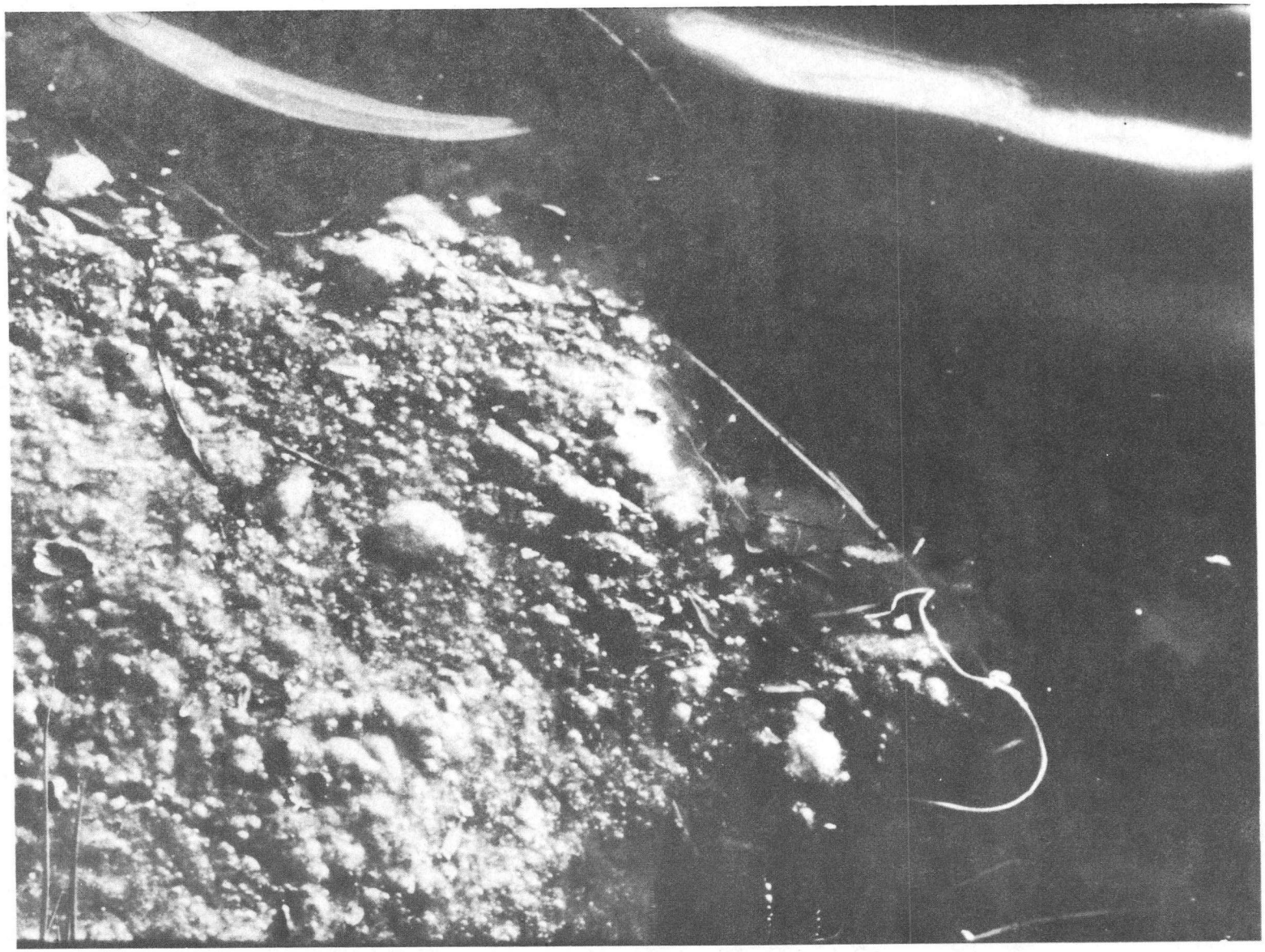

Figure 2. CBB 807-7973

be derived from the carbon dioxide. But deSaussere, by material balance experiments, was able to show that water must also be involved and thus the question of the origin of the oxygen was opened again. The general scheme, which deSaussere produced in 1804, involving both CO_2 and water and at least trace elements of the soil, to produce the plant material and gaseous oxygen, is essentially the same as the one existing when we began our work one hundred and fifty years later.

While van Helmont, Priestley and Ingen-Housz began with whole plants and were gradually able to show that only the green parts were necessary for the revitilization of the air, Priestley inadvertently had encountered green algae in his experiments but failed to recognize them. It was not until well into the 19th century that it was possible to demonstrate these effects entirely in the absence of living plants, using only the green parts extracted from them--Senebier, deSaussure

Only one other important contribution occurred in the 19th century and that came from the German physician, Mayer, in 1845, who saw that the plants were essentially converting light energy into chemical energy. It is of interest to note that another name not usually associated with plants was also at work at the beginning of the 19th century. Sir Humphrey Davy, the Englishman most of us know as the inventor of the miner's lamp for preventing explosions in coal mines, took up the work of Priestley and Ingen-Housz in 1800 and essentially repeated and advanced the gas exchange work with plants on a more quantitative basis. He was aware of the chemical nature of both oxygen, water, hydrogen and carbon dioxide, as well as the role of light, so that his measurements were early precursors of the type that dominated plant physiology well into the 20th century. Unfortunately, Davy did not publish his work until the appearance of his "Elements of Agricultural Chemistry, A Course of Lectures for the Board of Agriculture" in 1815. (2)

Two other chemists also made efforts in the 19th century to surmise the route by which carbon dioxide was converted into the plant substances: Liebig, an early agricultural chemist in 1843 felt that the plant acids were the intermediates whereas Beayer, another German, in 1870 thought it would be much simpler to go via carbon monoxide and formaldehyde to carbohydrate. Thus, the outline for the present-day photosynthetic cycle had been established.

The question of the origin of the oxygen was again addressed by C. V. van Niel in 1931 (3) on the basis of an analogy between the behavior of photosynthetic bacteria using hydrogen sulfide as a substrate instead of water. He formulated the whole process of the green plant as one involving the removal of hydrogen from water and its addition to CO_2,

leaving the oxygen behind. The confirmation of this idea was firmly established by Ruben, Hassid and Kamen in 1939 using water enriched with oxygen-18 to demonstrate that this was indeed a source of the oxygen evolved by the plants.

Ruben and Kamen performed the first experiment on tracing the route of CO_2 using a short-lived isotope of carbon, carbon-11, and were able to show an intial formation of certain acids, but the lifetime of carbon-11 (22 minutes) was too short to allow a detailed tracing of what has turned out to be a rather complex pathway. (4) Thus the matter stood until 1945 when we began our work.

III. ELUCIDATION OF THE PATH OF CARBON IN PHOTOSYNTHESIS

My own interest in the elucidation of the photosynthetic process stems from an entirely different source. During my postdoctoral years with Professor Michal Polanyi at the University of Manchester in England (1935-1937) I was introduced to the notion of electron transport, particularly in respiration, and the Thunberg idea of hydrogen transport to oxygen as the mechanism of metabolic conversion of reduced nutrients. The important role of heme complexes in these activities was impressed upon me, and the relationship between hemes and chlorophyll, the green pigment clearly responsible for the absorption of light by the green plant, was evident. This followed, of course, the clear description of the structure of chlorophyll by R. Wilstätter and A. Stoll in 1918. Both heme and chlorophyll belong to the class of compounds known as porphyrins or cyclic tetrapyrroles.

By the time I came to the University of California in Berkeley in 1937 I had already developed a great interest in the mechanism by which porphyrins played such an important role in biological oxidation-reduction reactions, both in the plant and in animals. Following the termination of World War II, after long-lived (3000 years) carbon-14 became available, I was able to take up that study seriously in 1945. Thus, one hundred years had elapsed from the recognition by Mayer that plants were essentially machines for the conversion of light energy into chemical energy until the time we began our study of how the plant achieved this result, using tracer carbon.

In contrast to the early workers who started with whole plants and gradually came down to either microalgae (inadvertently as Priestley did or deliberately as Sannebier did), we started our efforts from the very beginning using a micro green algae for the experiments. A few experiments were done

with barley shoots and other wild plants that we could harvest around the laboratory, but the major activity became cent ered around the microalgae, *Chlorella*, *Scenedesmus* and a few others (Figure 2).

The work was begun with Andrew A. Benson in 1948 and was finally completed by J. A. Bassham in 1954, showing the possibility that a preparation of chloroplasts not only produced oxygen, as shown by Robin Hill in 1937, but also could perform the entire task of capturing sunlight and producing carbohydrate (Figure 3). (5-7) Many students, postdoctoral visitors (from all over the world as well as the United States), staff and faculty were involved in this search from 1948 to 1954, which culminated in the publication of twenty-two separate articles carrying the title "The Path of Carbon in Photosynthesis". During this period we were able to trace in detail the steps the carbon atom atom passed through on its way from gaseous carbon dioxide until its primary deposit as a hexose molecule. Many secondary metabolic observations were made during that period, and are still continuing today, on how plants are able to convert the primary formed sugar into all its other components, including hydrocarbons, as well as proteins. We are still involved in this search.

FIGURE 3. The photosynthetic carbon reduction cycle

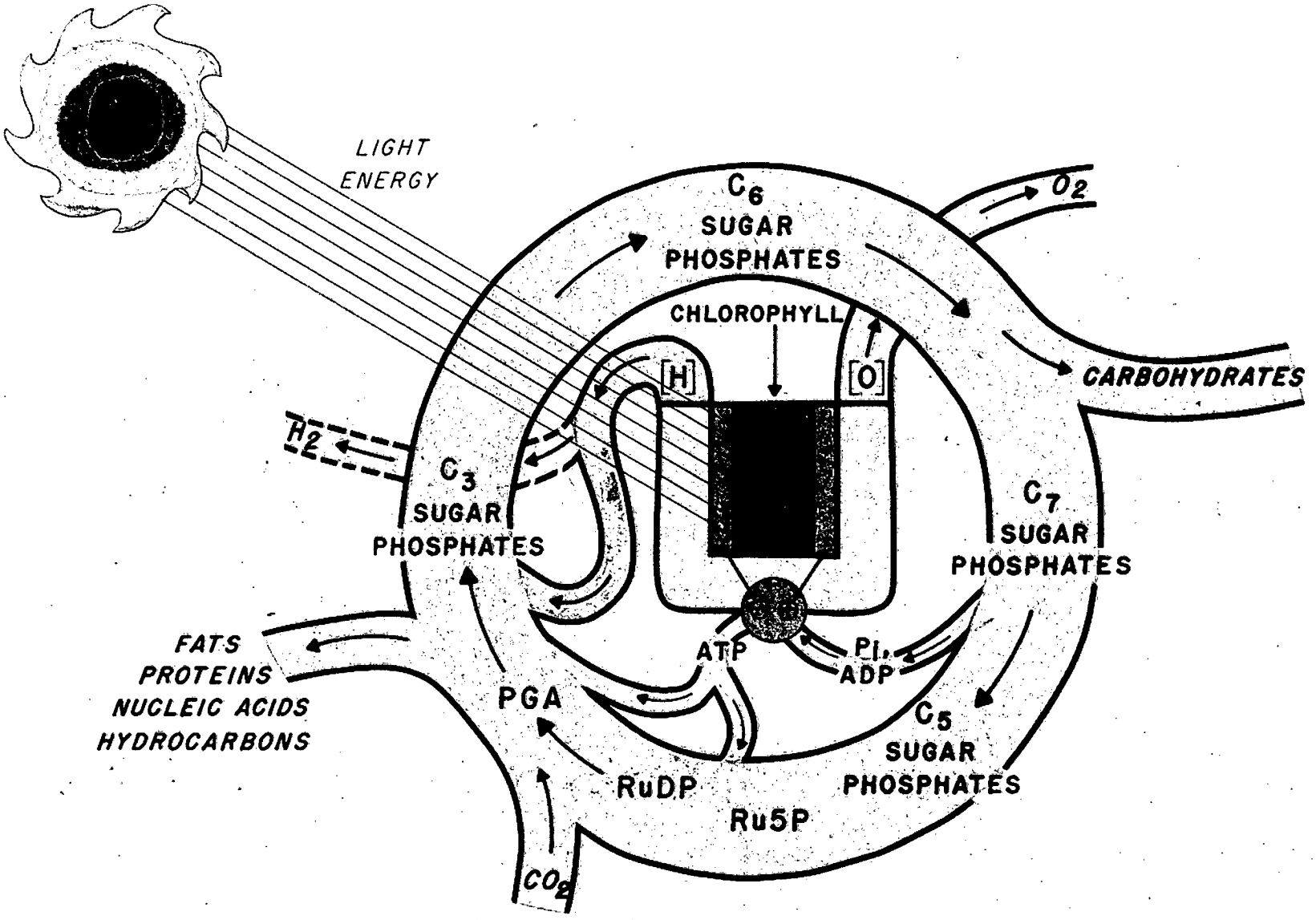

Figure 3.

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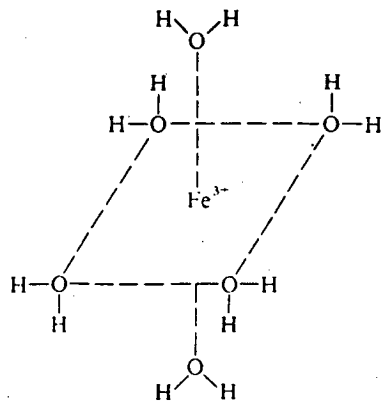
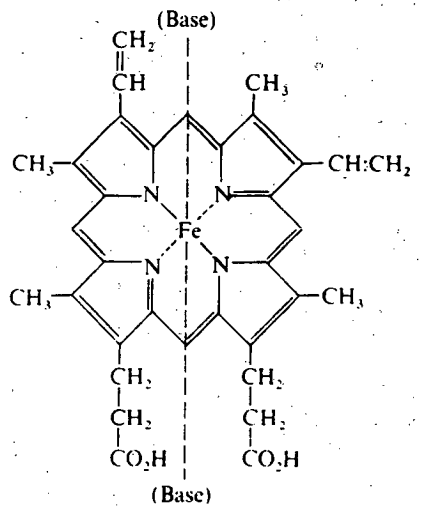
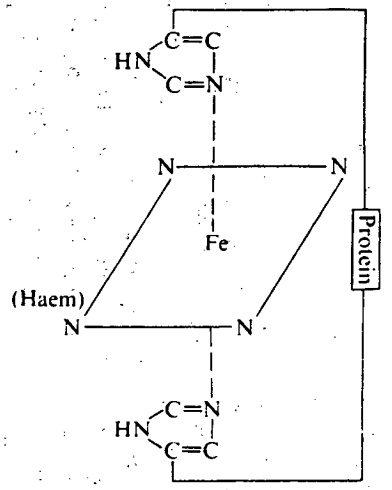
IV. CHEMICAL EVOLUTION

Another point of contact between this modern work on the unraveling of the path of carbon in photosynthesis and its historic predecessors was a common interest which it engendered in several of its early practitioners, as it did in me, concerning the nature of the origin of life itself. One of the chemical facts which we were able to use in our attempt to describe the transition from naturally occurring inorganic catalysts to biologically generated highly specific catalysts was the relationship between the catalytic function of iron and iron oxide on oxygen evolution from peroxidase compared to that of the iron heme and finally the catalyst itself (Figure 4). This kind of relationship and the widespread distribution of porphyrin-type molecules led to our first experiments, following Haldane and Oparin, in which we sought to demonstrate the possibility of CO_2 reduction in nonliving things by a suitable energy source; this was done in 1951 using ionizing radiation produced by the 60-inch cyclotron at Berkeley on a solution containing CO_2 and hydrogen and some iron salts. The result was the reduction of the CO_2 to a whole series of reduced compounds. (8) In view of the fact that CO_2 now appears to be the principal source of carbon-containing materials on the primitive earth (9,10), our original experiment, using ionization radiation, is more relevant than it has been in the intervening years during the time it was believed that the primitive earth's atmosphere was reduced. (11) The experimental confirmation of the theories of chemical evolution has raised this intellec-

FIGURE 4. Evolution of a catalyst







	Aqueous ferric ion	Haem	Catalase
Catalytic activity $ml^{-1} s^{-1}$ at 0 C	10^{-5}	10^{-2}	10^{5}
	(a)	(b)	(c)

XBL 831-7561

Figure 4.

tual exercise from a solely theoretical activity to one including experimental fact in the years since Haldane and Oparin performed their pioneering experiments. (12)

In addition, the molecular record in ancient rocks (2 to 3 billion years old) established the presence of biogenic hydrocarbons during that period, (13) corroborating the later morphological evidence in the form of primitive algal mats resembling the blue-green algae of today (Figure 2). Some of these same "chemical fossils" (14) appeared in analyses of returned lunar samples, (15) giving further experimental proof to early theories of the origin of organic matter.

V. PLANTS AS A SOURCE OF FUEL AND MATERIALS

Having mapped most of the biosynthetic routes used by modern plants on the way from carbon dioxide through carbohydrate and the group of molecules designated as hydrocarbons, that is to sterols and esters from fatty acids and isoprenoids, we are now ready to begin an attempt to use that information to fulfill some of our modern fuel and material needs. In fact, our present activities constitute two quite distinct approaches to the solution of these problems, both stemming from our studies of the photosynthetic process insofar as it is now understood. These activities are:

(1) To use the plants themselves as the generating factories for energy storage and organic materials that we need. This means not only the proper selection of plants to produce the materials (fuels) which our society requires, but also the genetic modification of the plants to (a) improve quantum efficiency, (b) to increase the yields of desired chemicals and (c) to modify the quality of the chemicals.

(2) To use the information which we have so far obtained about the physics and physical-chemistry of the quantum conversion process to design and construct totally synthetic devices capable of capturing the quanta of sunlight and storing it in some useful chemical form.

Most of the work in this latter area of activity has been pointed toward the production of hydrogen and oxygen from water, which the plant can do. More recently it has been realized that the generation of oxygen, at least, is in a sense a loss of values of intermediates on the way to oxygen, and it would be better to use the oxidizing intermediates to produce high value oxidation products that cannot otherwise be

made easily. Finally, we are just approaching the possibility of using the intermediate reducing agents on the way to hydrogen to capture and reduce CO_2, probably by methods not at all resembling those used by the modern plant. In all likelihood these will be one-carbon reduction products such as formic acid, carbon monoxide, formaldehyde, ethanol and methane.

A. Petroleum Plantations

We are now faced with the problem of selecting plants best suited for the storage of energy and the production of liquid fuels and materials. Since the first product of all green plant reduction is carbohydrate, the first step toward the end of energy sufficiency via plant production has been taken by making use of the most efficient carbohydrate-producing plant we have, namely, sugar cane, and converting that sugar, by a second fermentation step, to liquid fuel (ethanol). Brazil, which is the largest sugar cane growing country in the world, started such an energy efficient alcohol fuel program in 1975 and today their productivity of fermentation alcohol from sugar cane is over 10 billion liters per year. The Brazilians have redesigned or modified automobile engines so they can use either 95% alcohol, gasoline, or some combination thereof. Brazil is even considering the possibility of building an entire petrochemical industry based on alcohol through ethylene as well as a sucrochemical industry based on the primary sugar production productivity as well.

However, it is quite clear that it would be much more valuable to us to find plants that carry the reduction of CO_2 all the way to the fully reduced hydrocarbon rather than stopping at the carbohydrate which, as you have already seen, requires a second step to produce a useful liquid intermediate. Such plants exist today and, in fact, one of them has been a commercial crop for over one hundred years. This is the Hevea rubber tree which had its origin in the Amazon forests in Brazil but which is now cultivated on large plantation scale in Southeast Asia and Africa. The Hevea belongs to the family *Euphorbiaceae*, and a closer examination of this plant family reveals another genus, Euphorbia, much larger than the genus Hevea; all species of Euphorbia are latex producers. This latex, however, is an emulsion of a much lower molecular weight hydrocarbon (approximately 500) in water than that of Hevea (1 to 2 million). The number of species of the genus Euphorbia is very large, about 2000, and they have all types of growth habits, from small, fast growing plants to large slow growing cactus like plants, most of which are capable of growth in semiarid regions.

It is possible to select a plant species for its physical and agronomic properties as well as its chemical properties. We have done this to some extent and have performed a number of experiments on one such type, *Euphorbia lathyris*, commonly called "gopher plant", which have been encouraging. (16) This particular species can grow in one year on as little as 12 inches of water, but, of course, would be more productive with 15-18 inches of water. The *E. lathyris* grows normally to a height of 2-4 feet, but must be planted each year. A very closely related species, *E. esula*, however, is a perennial, in that it dies down in the winter and regrows from the root in the spring. *E. esula*, however, does not seem to have the hydrocarbon content per unit dry weight that *E. lathyris* does, and as we shall see later, a hybrid of these two species might be a very desirable plant, if it could be produced.

Euphorbia lathyris has been grown in many parts of the world on an experimental basis. The first of these plantations was developed in California (Figure 5) showing the plant at the age of about 5 months. The same species (*E. lathyris)* is under cultivation near Madrid, Spain (Figure 6) where it has gone to seed in preparation for a much larger plantation.

FIGURE 5. *Euphorbia lathyris* plantation, Northern California.

Figure 5 CBB 800-13496

FIGURE 6. *Euphorbia lathyris*, Madrid, Spain

Another plant family, most members of which are latex producers, is the *Asclepiadaceae*, commonly known as milkweeds. A number of varieties of this group have been explored as fuel crops. One of the most attractive in terms of productivity for semiarid regions for its fuel and materials capabilities is *Calotropis procera*, and the plantation is shown in Australia (Figure 7). (17)

B. Analytical Methods for Hydrocarbon Extraction in Plants

The process for extracting the oil and other material from the Euphorbias is relatively simple. It involves cutting the whole plant, drying it in the field as a first stage (and possibly in a kiln as a second stage) and then extracting the dried material in a process very similar (and slightly simpler) to the one commercially used for seed oil extractions, particularly for soybeans and sunflower oils. The processing scheme, including weight and energy at each stage for a 1000 dry tons per day pilot plant for *Euphorbia lathyris* is shown in Figure 8. Here we see that the oil production of that 1000 dry tons

Figure 6. CBB 821-533

FIGURE 7. *Calotropis procera* plantation, Australia

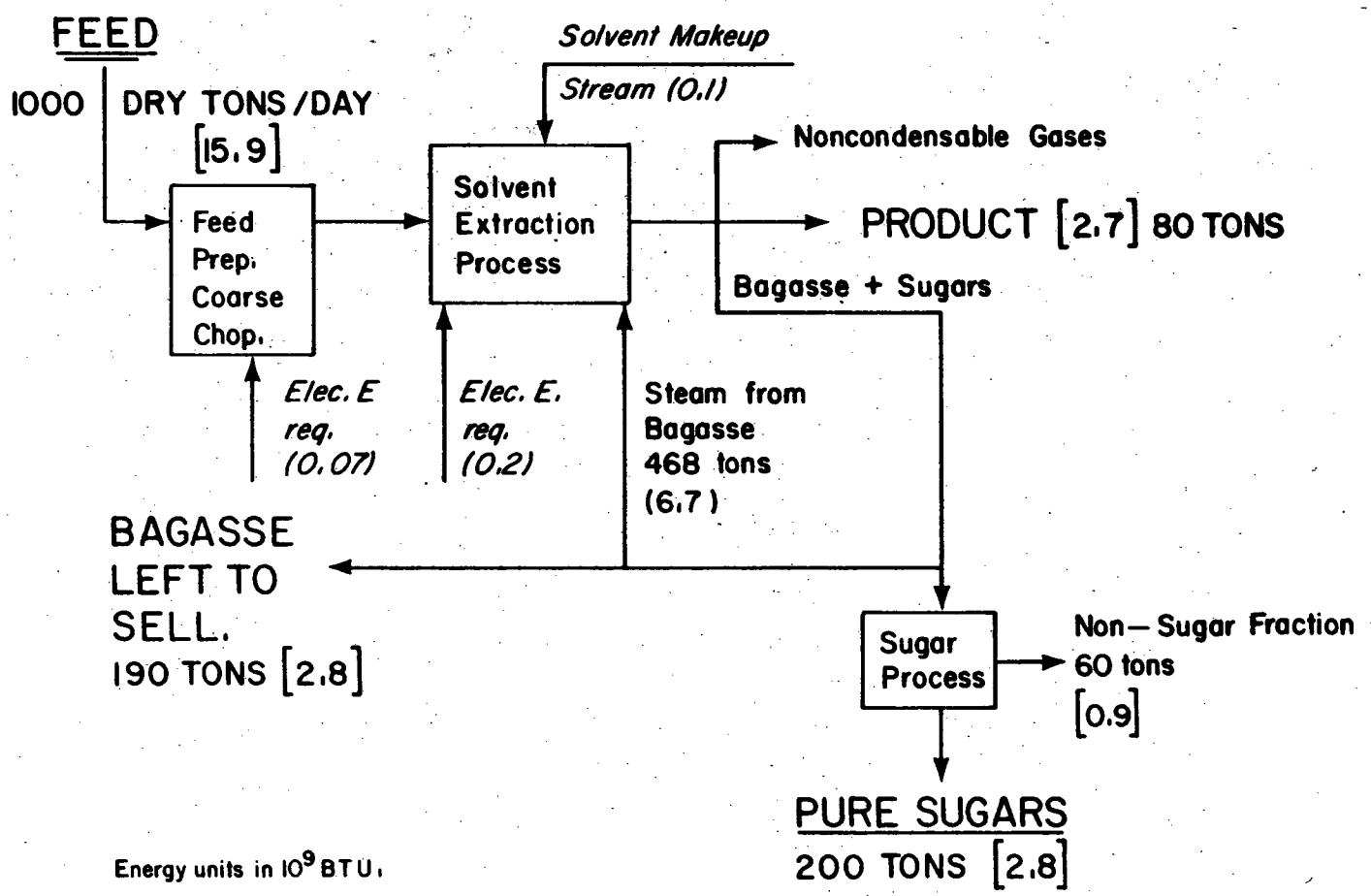
per day plant is 80 tons of oil and, in addition, another 200 tons of fermentable sugar are obtained following the oil extraction. This 200 tons of fermentable sugars would yield about 100 tons of alcohol when carried through the fermentation process. Of the 700 tons of bagasse remaining, about 500 tons would be used to generate the steam required for all the processing work, leaving behind 200 tons of bagasse in excess which could be used to generate steam for power or fertilizer.

A sample of the crude oil from *E. lathyris* resulting from such an extraction was submitted to a zeolite cracking step by the Mobil Corporation, using a shape selective catalyst, with the results shown in Figure 9. Since the zeolite was designed to produce high octane gasoline, it does indeed do so with the crude feedstock from the *E. lathyris*. However, since most of the molecules in the crude feedstock are C_{30} triterpenes, some of them sterols and sterol esters, it behooves us to examine them for possible high value products before the cracking operation is performed, to increase the total value of the whole process and make it more nearly approaching economic viability. (18)

Figure 7. CBB 829-7854

Figure 8

Conceptual Processing Sequence to Recover Terpenoids and Sugars from Euphorbia lathyris



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Products of conversion over Mobil zeolite catalyst of Euphorbia lathyris terpenoids (heptane soluble) (adapted from Nemethy et al 1980).

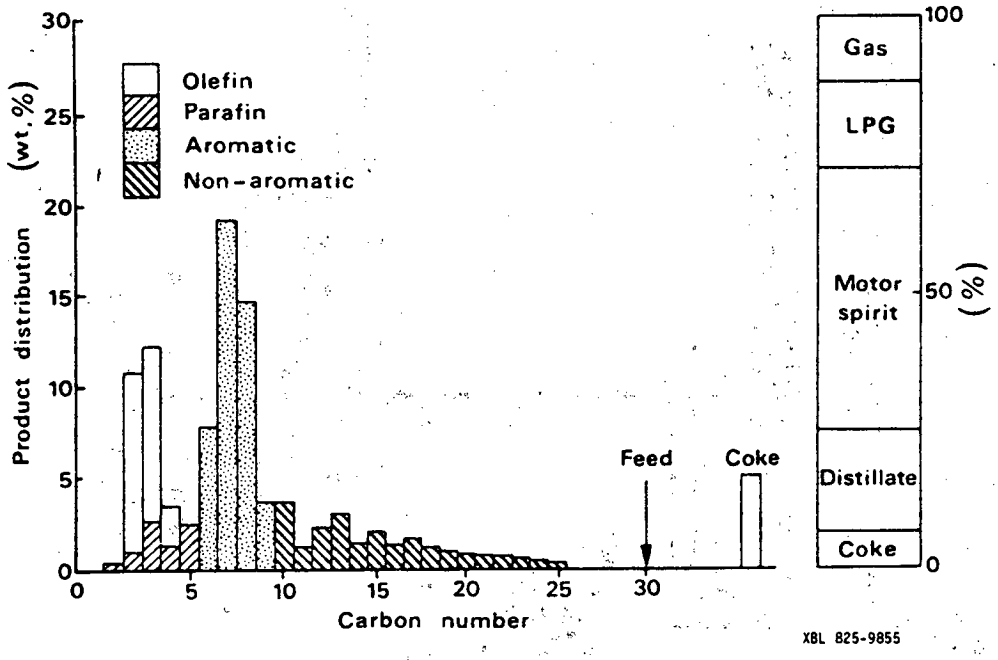


FIGURE 9. Products of conversion of *Euphorbia lathyris* heptane-soluble terpenoids over Mobil zeolite catalyst (18)

C. Biosynthetic Pathways in Plants

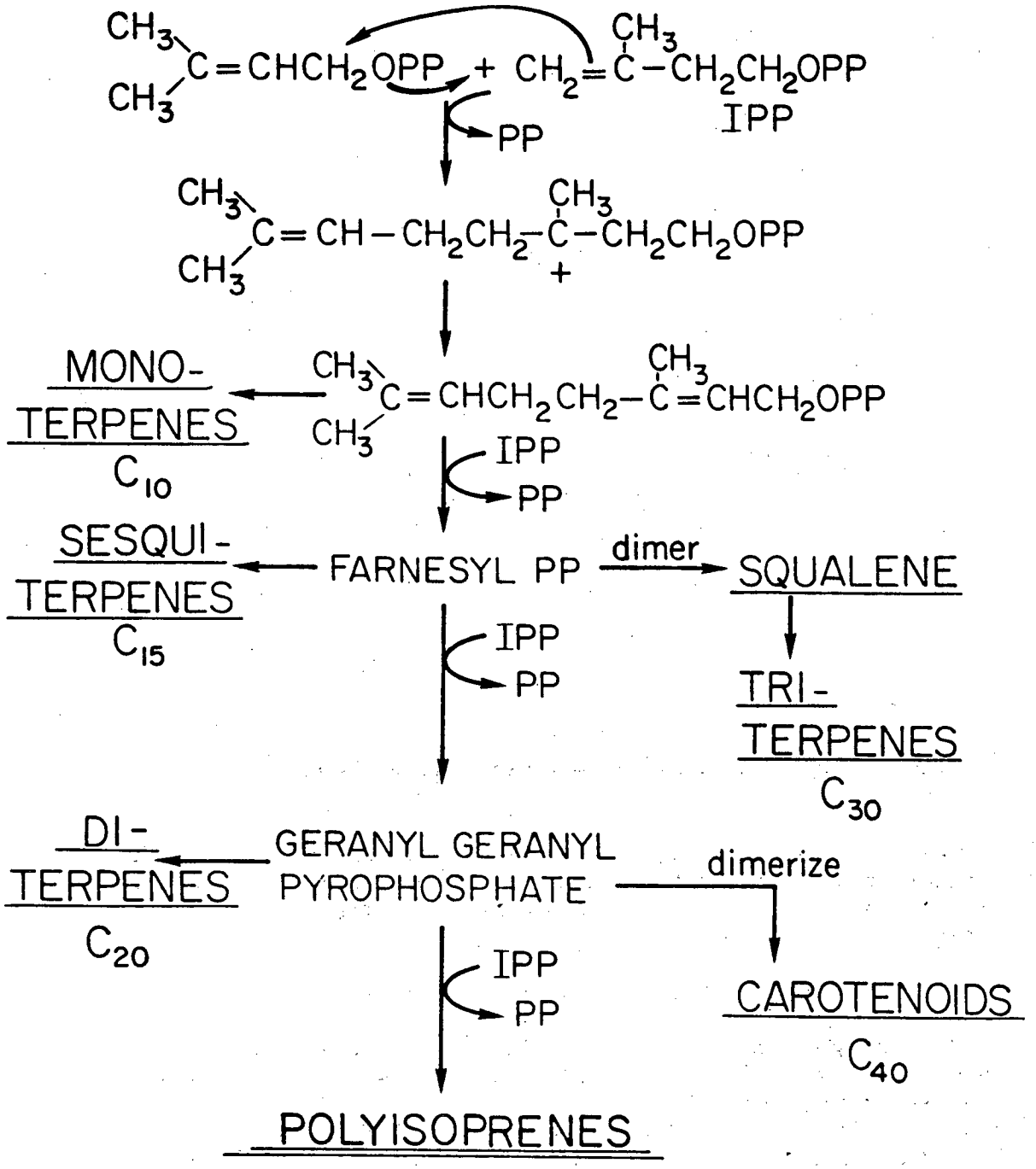
Since most of these reduced compounds from *E. lathyris* are isoprenoids it is worthwhile examining the biosynthetic scheme for producing them (Figure 10). While a detailed map of the biosyntheses in *E. lathyris* has not been constructed, by using the general information available on other isoprenoid biosyntheses, the scheme postulated from carbohydrate to pyruvate through acetyl CoA to mevalonate and thence to isopentenyl pyrophosphate (shown at the top of Figure 10) seems likely. From isopentenyl pyrophosphate (IPP) the cycle goes into the primary polymerization sequence which leads first to monoterpene pyrophosphate which can be cyclized to monoterpenes, or on to farnesyl pyrophosphate which can be cyclized to sesquiterpenes or dimerized to C_{30} linear isoprenoids of the squalene type. It is this latter route which we believe the *E. lathyris* takes, and the end products are cyclic triterpenes of the C_{30} group which have been esterified at various points by relatively long fatty acids of various sizes, also made

FIGURE 10. Pathways of triterpenoid biosynthesis in plants

from acetyl CoA. We will return to a description of this particular biosynthetic pathway later when we introduce the possibility of modifying it to produce more desirable end products.

D. Oils from Plants and Trees

We have examined a number of oils from various plant types. (19) These are shown in Figure 11 from left to right: The C_{30} triterpenoid oils obtained from *E. lathyris*, oil from Marmeleiro (a Croton), diesel-like fuel from *Copaifera langsdorfii*, extract of the fruits of *Pittosporum undulatum*, Jojoba oil and Andiroba oil (*Carapa guariensis*). The material from the *Euphorbia lathyris* is similar to the oil from *Calotropis procera*, and the oil from the Marmeleiro, found in Brazil, consists almost entirely of C_{10} terpenes. (The sample was kindly supplied by Prof. Afranio Aragao Craveiro of the Universidade Federal do Ceara, Fortaleza and Prof. Jose Carioca, also of Universidade Federal do Ceara.) The oil from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C30
DI-
TERPENES
C20
GERANYL GERANYL
PYROPHOSPHATE
dimerize
CAROTENOIDS
C40
POLYISOPRENES

XBL-798-4985

FIGURE 11. Oils from plants and trees

the Brazilian tree *Copaifera langsdorfii* is just as it is drained from the tree itself and consists entirely of cyclic sesquiterpenes, some twenty-five of them; this material has undergone no processing whatsoever. The oil is drained from the tree, in a tapping operation similar to that for tapping a sugar maple, and the product can be used as a diesel fuel with no further processing. The fruits of *Pittosporum undulatum* were extracted to give the oil. Other species of this tree grow wild in the Philippines, such as *Pittosporum resiniferum*, where the fruits are used for illumination and are called "petroleum nuts". The oil from the *P. undulatum* (found in California) consists almost entirely of C_{10} materials, 80% being myrcene and the rest α-pinene. The Jojoba oil has been obtained from the seeds of the desert plant *Simmondsia chinensis* and the oil is a monoester of a long chain fatty acid with a long chain alcohol group. The productivity of this plant per unit area of land per unit of time seems to be lower than for the other oils shown in Figure 11, but the value of the oil is certainly high. It has properties as a lubricant which are dif-

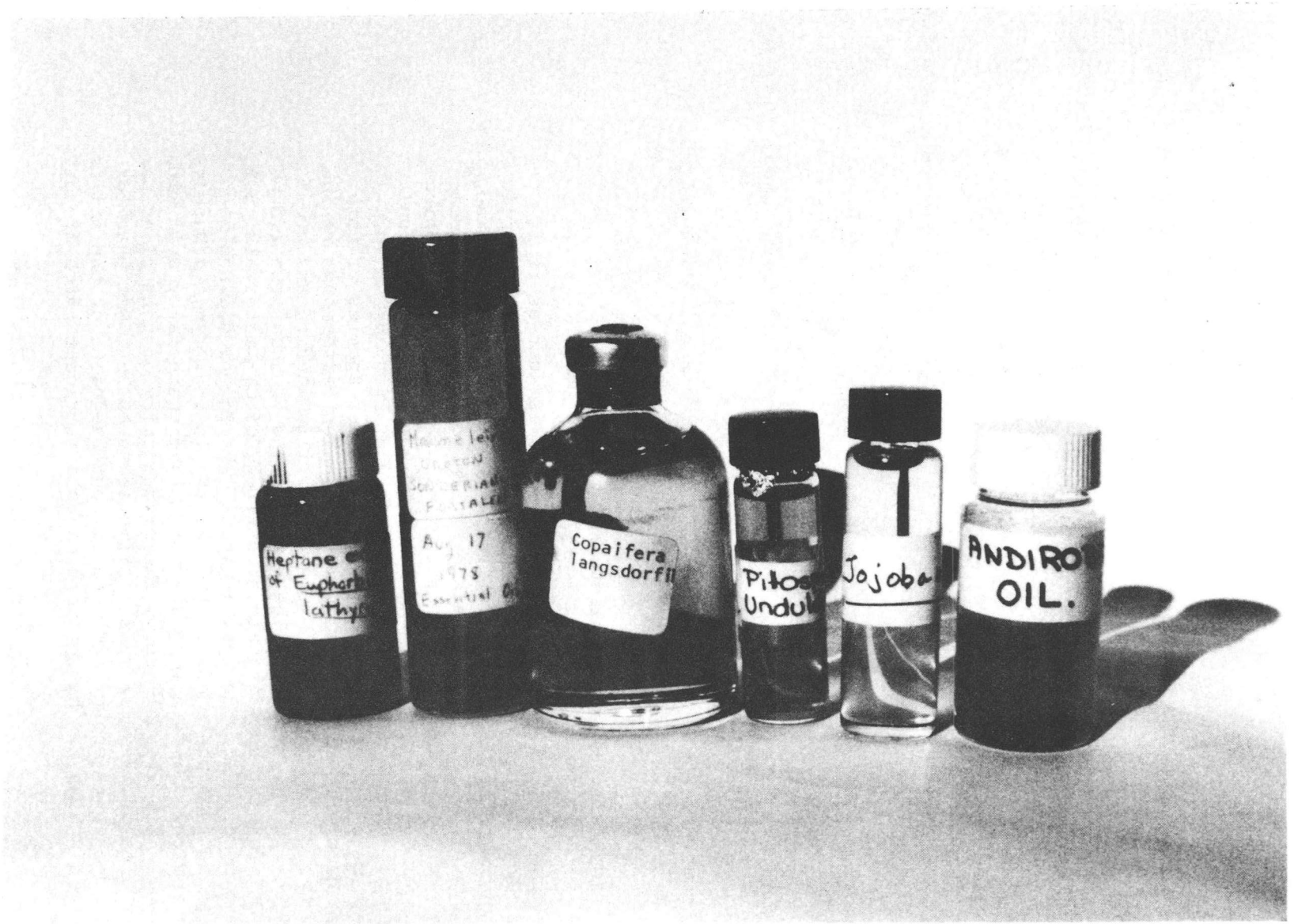

Figure 11.

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ficult to find in any other plant and Jojoba has become a commercial crop in the American southwest and possibly will spread to other parts of the world as well. The final vial contains Andiroba oil, a seed oil from a Brazilian plant called *Carapa guariensis*, and it is a triglyceride, as are other seed oils such as palm nut oil. The Andiroba oil is being explored as a possible source of fuel in Brazil, as are other glyceride groups from plants such as sunflower and soybeans in the United States, both of which are food products. At least in the United States, until the economics for government subside for set-aside land are modified, plant seed oils seem not to be economic for use as a raw material for fuels.

E. Genetic Engineering for Hydrocarbons in Plants

One possible scientific activity which seems appropriate now is to make use of what we know about the biosynthetic route to the various terpenes in different plants and try to modify both the yield and the quality of the terpenes. (20,21) We know that *E. lathyris* and *C. procera*, for example, can produce one such group, the C_{30} triterpenoids. It might be possible to change the direction the farnesyl pyrophosphate takes in *E. lathyris* which you have seen is a dimerization to C_{30} followed by cyclization, to a direct cyclization of the C_{15} pyrophosphate. This would produce sesquiterpenes such as those found in *Copaifera langsdorfii* or *Copaifera multijuga*, members of the family *Leguminosae*.

In order to begin such an effort, the first step would be to culture the protoplasts of *E. lathyris* and develop the steps required to regenerate fertile plants from protoplast culture. Some of these steps have long since been taken. We have used this process for *E. lathyris*.(19,22) We have not yet tried another species of Euphorbia, *E. esula*, but since *E. lathyris* and *E. esula* are so closely related it seems possible that this process will be a success and a useful hybrid could be created. One objective would be to perform somatic hybridization of the protoplasts of these two Euphorbia species and eventually try to generate a hybrid having the productivity of the *E. lathyris* and the hardiness of *E. esula*.

We, however, have succeeded in culturing protoplasts of *E. lathyris* , and as a demonstration of our ability to separate protoplasts of different kinds we have performed an exploratory experiment with *E. lathyris* protoplasts. (22) A sample was divided into two parts, one stained with fluorescein isothiocyanate which fluoresces in the yellow to green and the other sample stained with rhodamine isothiocyanate which fluoresces red. These two different stained cell samples were mixed and induced to fuse. The experiment was performed in an effort to

separate the fused from the unfused cells. The fused cells would present only one of the fluorescing colors. Figure 12 shows the fused and unfused cells used in this experiment. The flow cytometry apparatus used to count the separated cells is shown in Figure 13. (22)

Our ability to generate a whole plant from protoplasts has been demonstrated. It is possible to see shoots arising from a callus generated from *Euphorbia lathyris* protoplasts. Since this photograph was taken (June 1982) both roots and shoots have been produced from the same callus(Figure 14).

FIGURE 12. Fused *Euphorbia lathyris* protoplasts

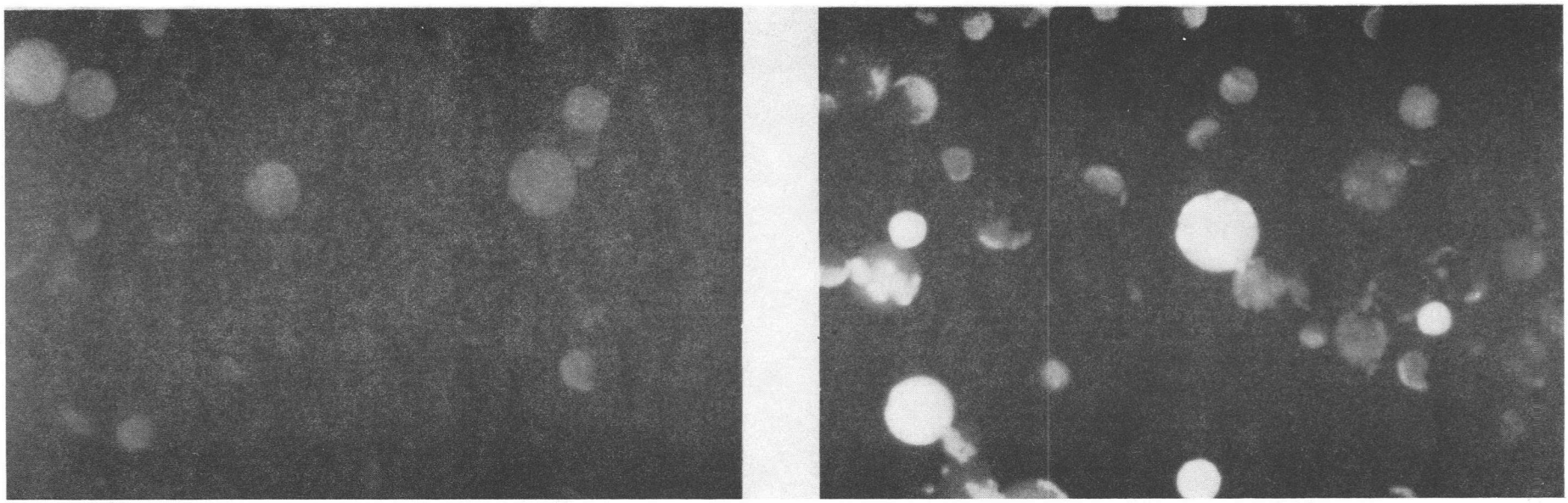

Figure 12.

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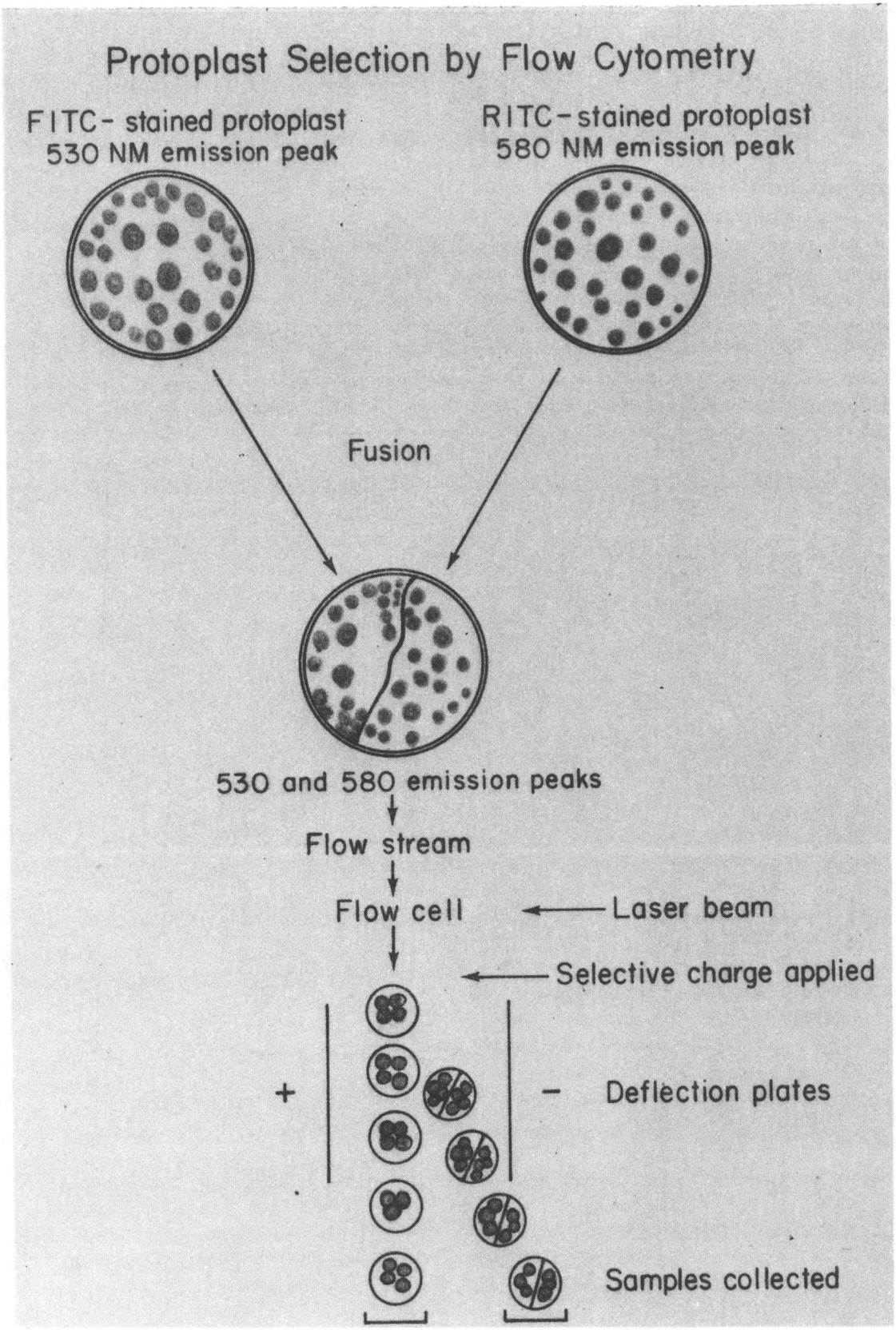

Figure 13

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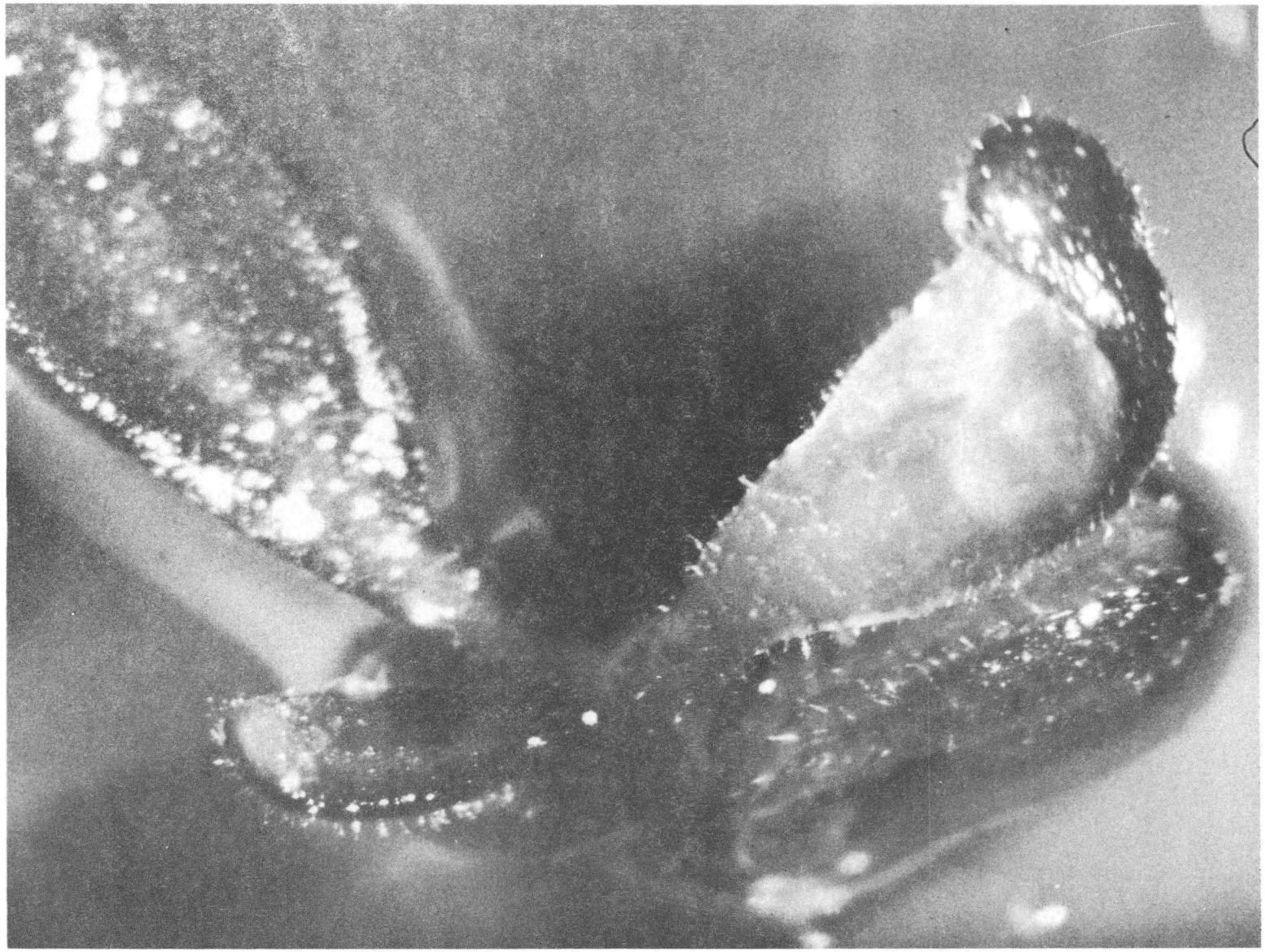

Figure 14. E. lathyris shoot from protoplast callus (Redenbaugh & Kawaguchi, Plant Genetics, Inc.)

CBB 829-8432

A more difficult and longer term approach would be to transplant the gene for farnesyl pyrophosphate cyclase from a plant which contains it (such as *C. multijuga* or *C. langsdorfii)* to a plant which does not, *E. lathyris*, using the principles of genetic engineering. This operation would require first the characterization of the farnesyl pyrophosphate cyclase and then the isolation either of its messenger RNA or the DNA gene fragment itself by a suitable process and then the re-insertion of this copy gene DNA into the protoplast of *E. lathyris*. Models for all of these steps, save the last one, exist, so it seems possible to me that we should be able to achieve the last step using either a liposome packaged gene or a gene attached to a suitable vector such as the T_1 plasmid of agribacteria.

This kind of process is exactly analogous to carcinogenesis produced by activation, transposition or integration of a pre-existing information produced by the DNA damage done by chemical carcinogens and the repair systems that they induce.(23)

VI. ARTIFICIAL PHOTOSYNTHESIS

Ultimately all the land resources which are capable of growing any kind of plants will undoubtedly be required for food production. We must therefore begin now to design and construct totally synthetic devices for the capture of the quanta of the sun and the storage of these quanta in some stable, useful chemical form. This problem has been approached from several different directions, which are manifestations of what I believe to be the essential principle used by the green plants to achieve and stabilize the primary quantum conversion step, and that is "photoelectron transfer across a phase boundary".

The first example of this principle which was used was the surface of a solid semiconductor in contact with a liquid phase and dependent upon the surface potential of the semiconductor to achieve the charge separation after electron transfer to the conduction band. We first did this experiment with zinc oxide covered with the sensitizing layer of chlorophyll. (24) We were able to show that visible light absorbed by the chlorophyll led to photoelectron transfer to the conduction band and that chlorophyll cation radical left on the surface of the zinc oxide was then neutralized by electrons from a donor in the solution. This use of a semiconductor surface has been carried much further by other investigators.

The first was Honda in Japan (25) who used titanium oxide and ultatviolet light to generate oxygen from water. This has been elaborated by, among others, Grätzel (26) in Switzerland who has used titanium oxide suitably coated with both hydrogen and oxygen generating catalysts so that the same titanium oxide could be used to generate both the products of the photolysis of water.

We took another step in this direction by coupling both an n-type and a p-type semiconductor to make use of all of the wavelengths of the solar spectrum to generate a potential capable of electrolyzing water. This was accomplished by devising a cell made of iron oxide and gallium phosphide. (27) This approach has been further strengthened by Somorjai (28) who replaced the gallium phosphide with another iron oxide doped in the opposite direction.

Since our early experiments (29) we have focussed almost all our attention on another way of achieving phase boundary electron transfer which resembles more closely the biological systems in the green plant. (24,30-32) An electron micrograph of chloroplast lamellae is shown in Figure 15. In this photograph you can see the membrane structure of the chloroplast upon which the components, both sensitizers and electron transfer agents, are situated. These components are presumed to achieve a two-quantum process shown diagramatically in Figure 16 in which electrons are moved in two

FIGURE 15. Electron micrograph of chloroplast lamellae

FIGURE 15. Electron micrograph of chloroplast lamellae

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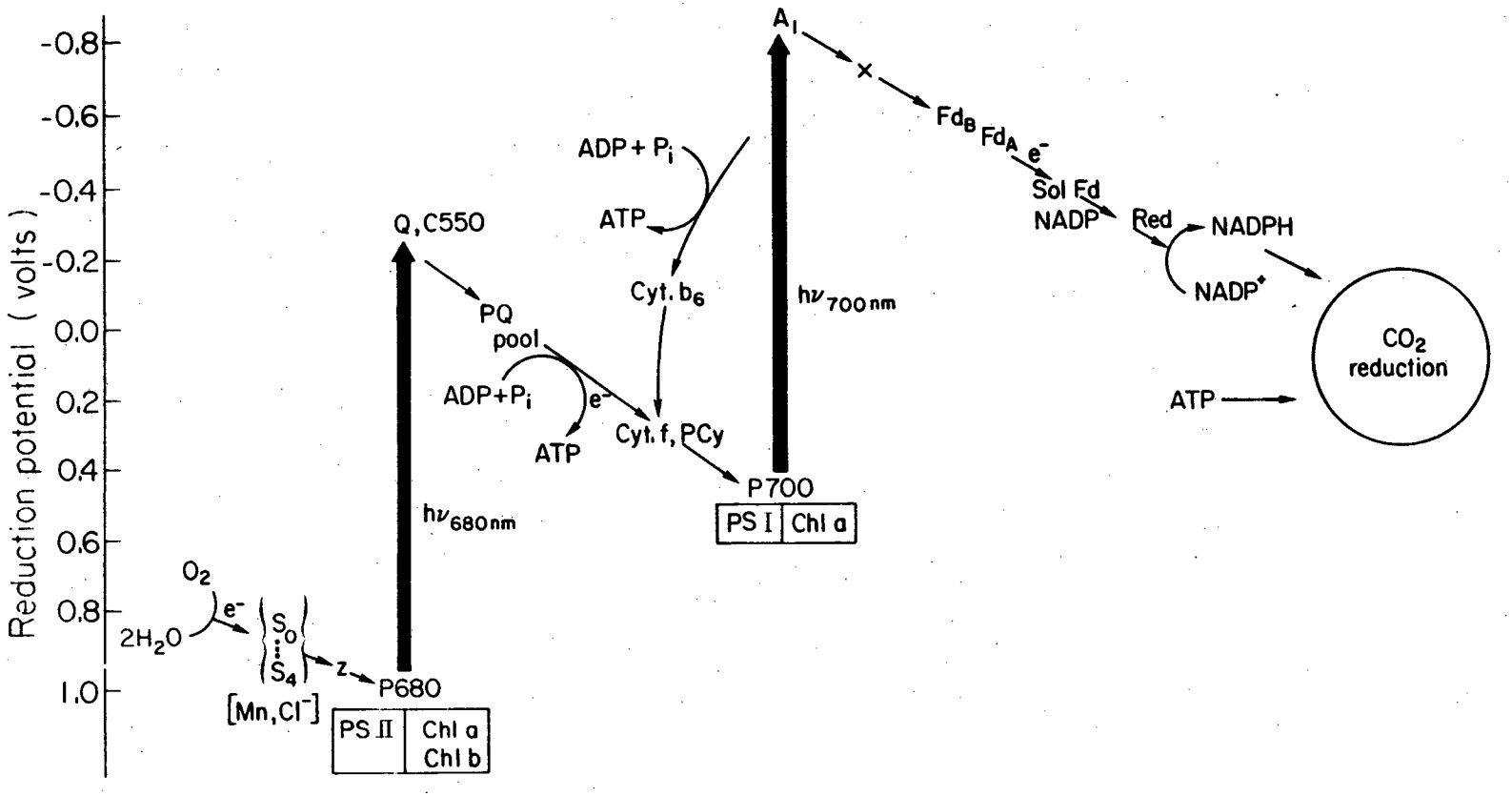

FIGURE 16. Photoelectron transfer scheme (Z-scheme)

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stages from the redox potential of oxygen on the water side to something well above the redox level of the molecular hydrogen on the reduction side, with concomitant generation of ATP between the two photoprocesses. Since we are not required to generate ATP between the two, it seems likely that we should be able to devise a membranous process, or process involving a surface, which would transfer the electron from the redox potential of oxygen to that slightly above hydrogen, using a single quantum, something which could be achieved with a single quanta of 700 nm or less.

A. Photosensitized Electron Transfer across Bilayer Membranes

Our problem, therefore, is to devise a system in which we transfer an electron across a membrane with a single quantum. The product of that single quantum transfer can then be stored for long enough times so that several of them (two for hydrogen production and four for oxygen production) could be combined at some later time. To demonstrate this concept we have chosen to use vesicles made of both natural and synthetic lipids, a diagrammatic representation of which is shown at the top of Figure 17. It is possible to construct an unsym-

FIGURE 17. Photoelectron transfer scheme

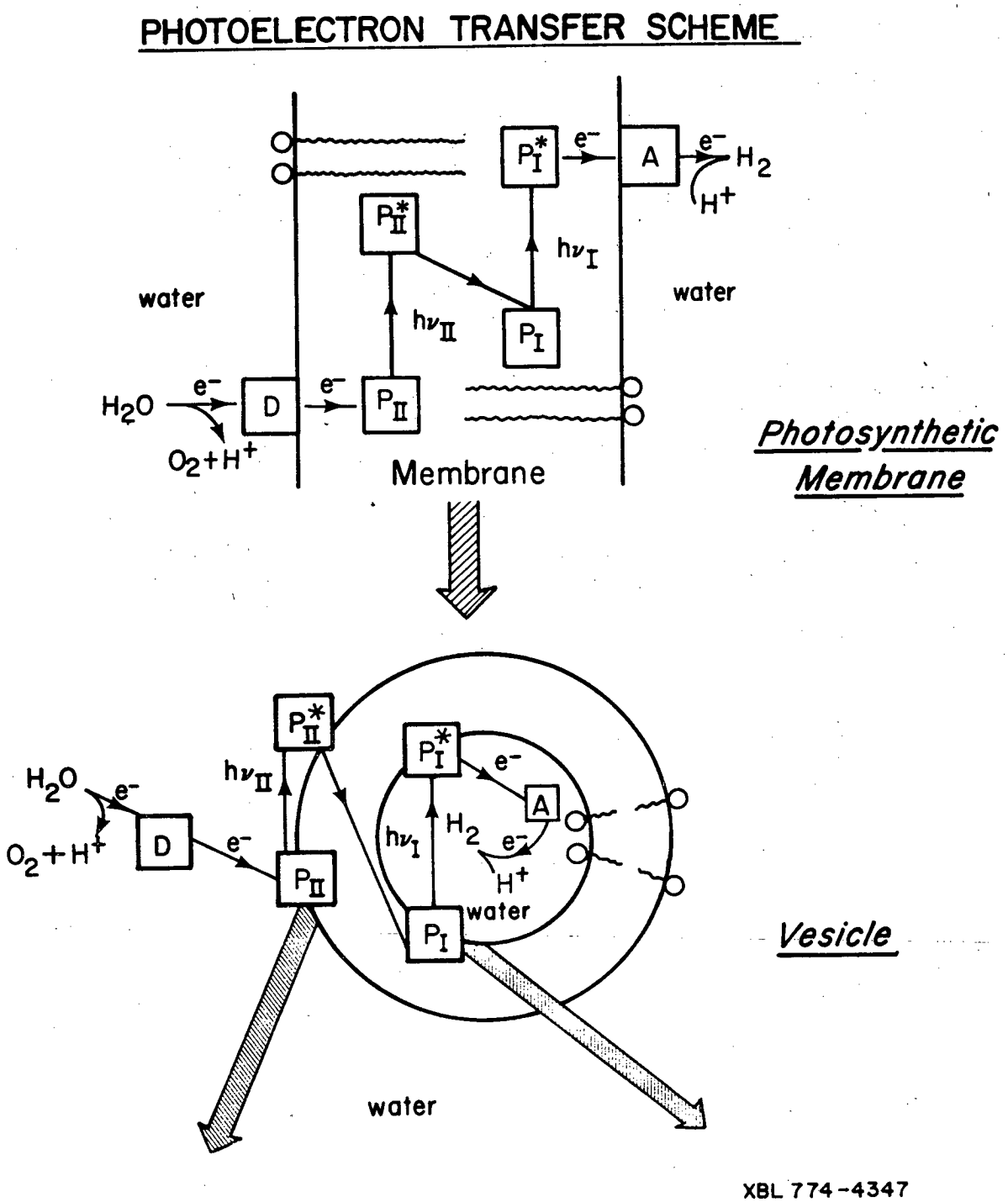

FIGURE 17. Photoelectron transfer scheme.

metrical membrane which has the same surfactant sensitizer on both sides but different catalysts on the inside and outside. One such detailed system is described in Figure 18. All the components are designated.In this case, the acceptor and the hydrogen generating catalyst are on one side of the vesicle, the acceptor being the heptylviologen and the catalyst being a colloidal platinum. The sensitizer which is present on both sides of the membrane is, in this case, a surfactant ruthenium trisbypyridyl compound. On the inside there are two different donor systems shown (Figure 18), one of them a sacrificial donor (EDTA), that is, a donor which is decomposed in the course of the irreversible reaction, the other being a water soluble ruthenium trisbypyridyl complex with a heterogeneous surface upon which the four "holes" so generated (the ruthenium in the +3 state) can accumulate to produce molecular oxygen from water.

Our fisrt task then is to demonstrate the photoelectron transfer across the membrane such that the oxidation and reduction products are generated on opposite sides and cannot back react. We have succeeded in doing this, using heptylviologen without the platinum as a measure of electron transfer to the outside and the sacrificial donor (EDTA) as the electron source on the inside of the vesicle. (33) We have been able to show that the ruthenium +3 compound being produced, either chemically or photochemically, will indeed generate oxygen from water in the presence of ruthenium oxide catalyst or cobalt oxide catalyst. (34) It is therefore possible to perform a photochemical electron transfer from the inside to the outside of the membrane, producing a reduction product on one side of the membrane and an oxidation product on the other, which cannot back-react because the membrane prevents it. This is the basic, fundamental structure of photosynthetic membranes in green plant chloroplasts.

Another step would be to replace the sacrificial donor (EDTA) with some compound that could act as donor in the membrane and could be regenerated in some useful. way. We have done this in the membrane system using surfactant Zn-porphyrin as the sensitizer and surfactant Mn^{III}-porphyrin as the donor, arranged as shown in Figure 19. (35) Upon illumination in the absorption band of the Zn-porphyrin it is clear that not only is the PVS reduced (rise at ∿610 nm)but the manganese is oxidized to Mn^{IV} (rise at ∿420 nm). This Mn^{IV} appears to be capable of oxidizing a useful substrate such as an olefin or to oxidize water in the presence of a suitable catalyst to regenerate reduced manganese (Figure 20).

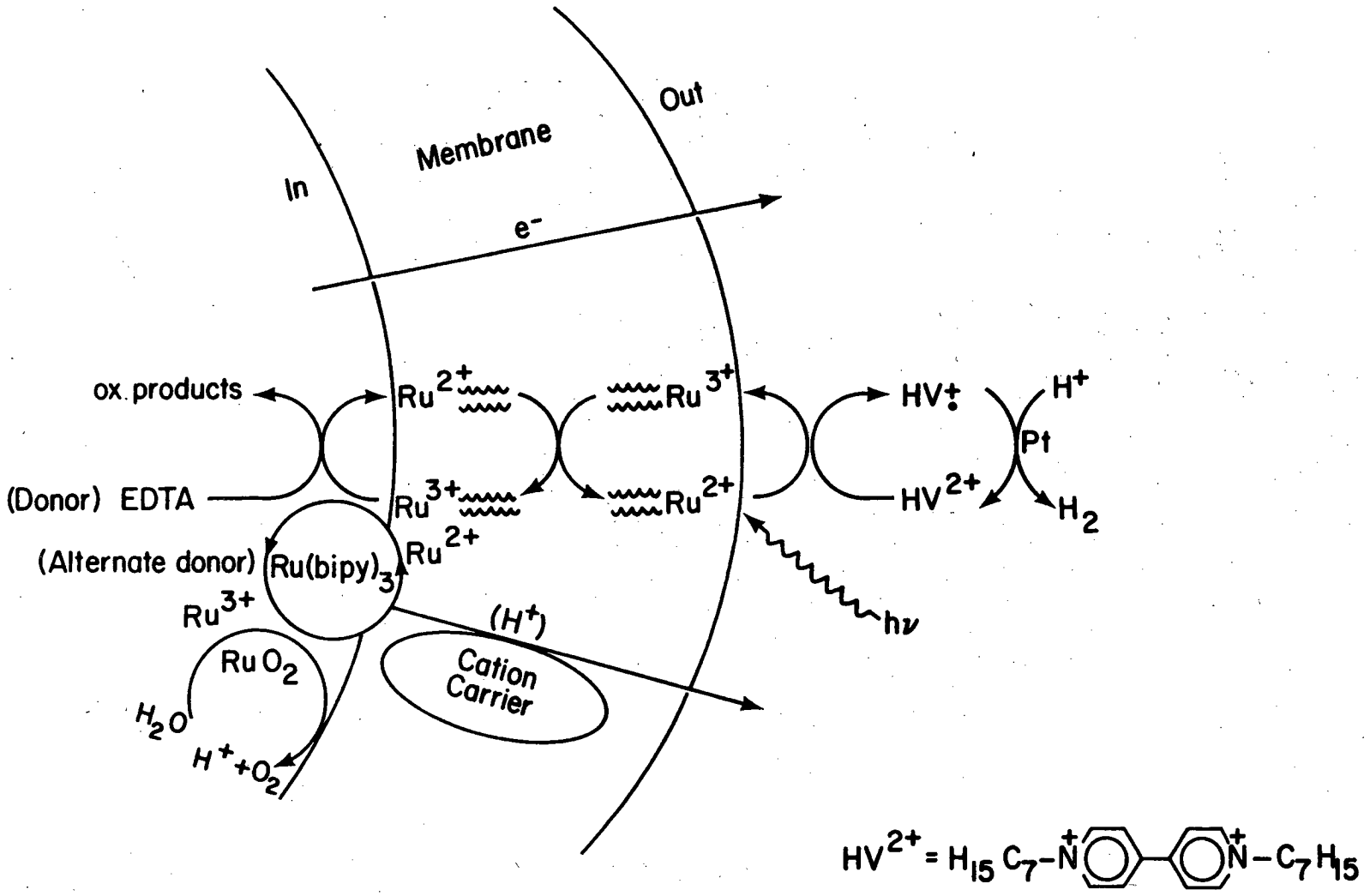

FIGURE 18. Scheme for photosensitized electron transfer across a lipid vesicle wall.

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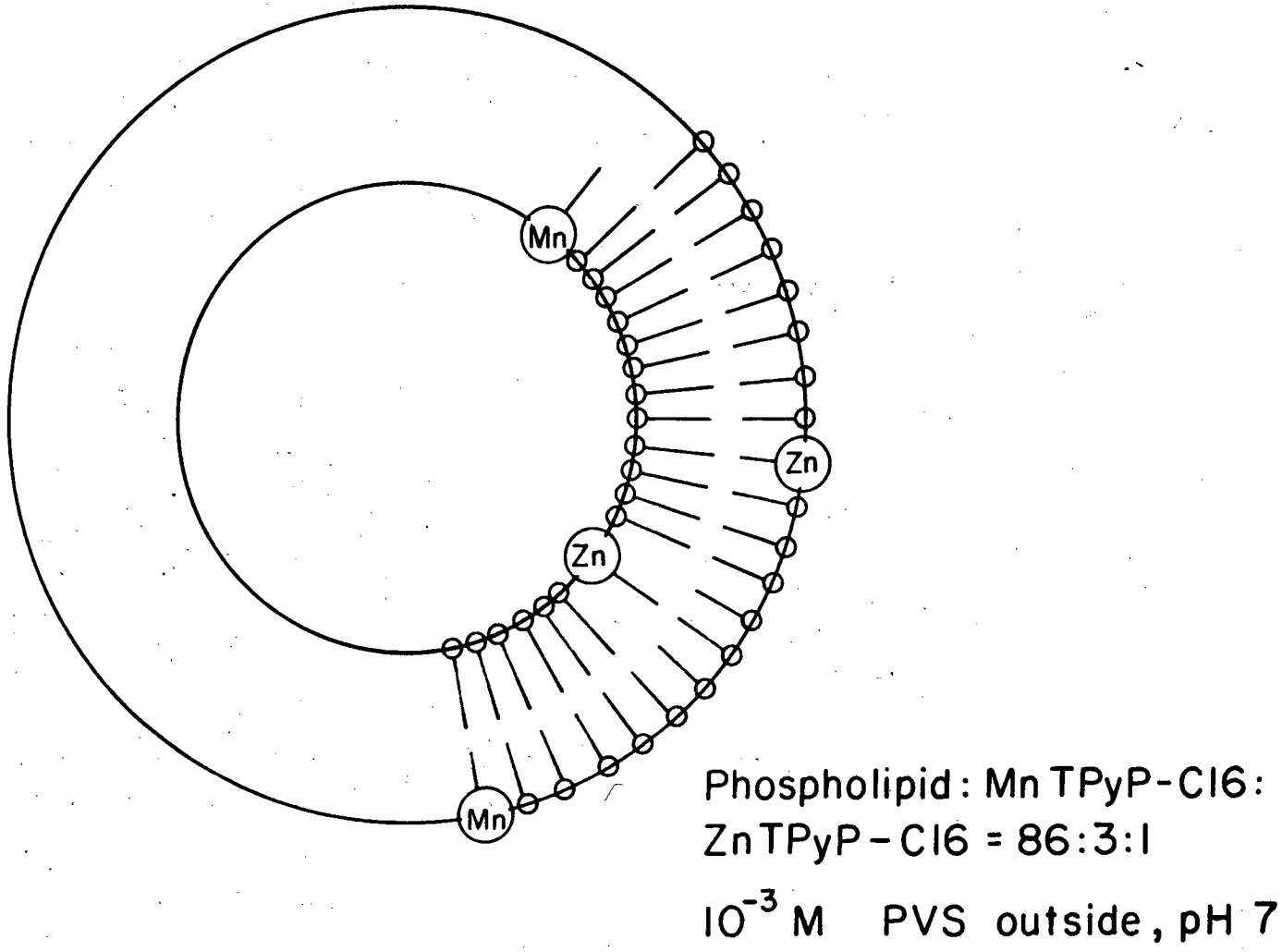

FIGURE 19. Single unilamellar vesicle prepared by sonication. Ratio between phospholipid and photosensitizer of 86:3:1 with PVS added outside of vesicle.

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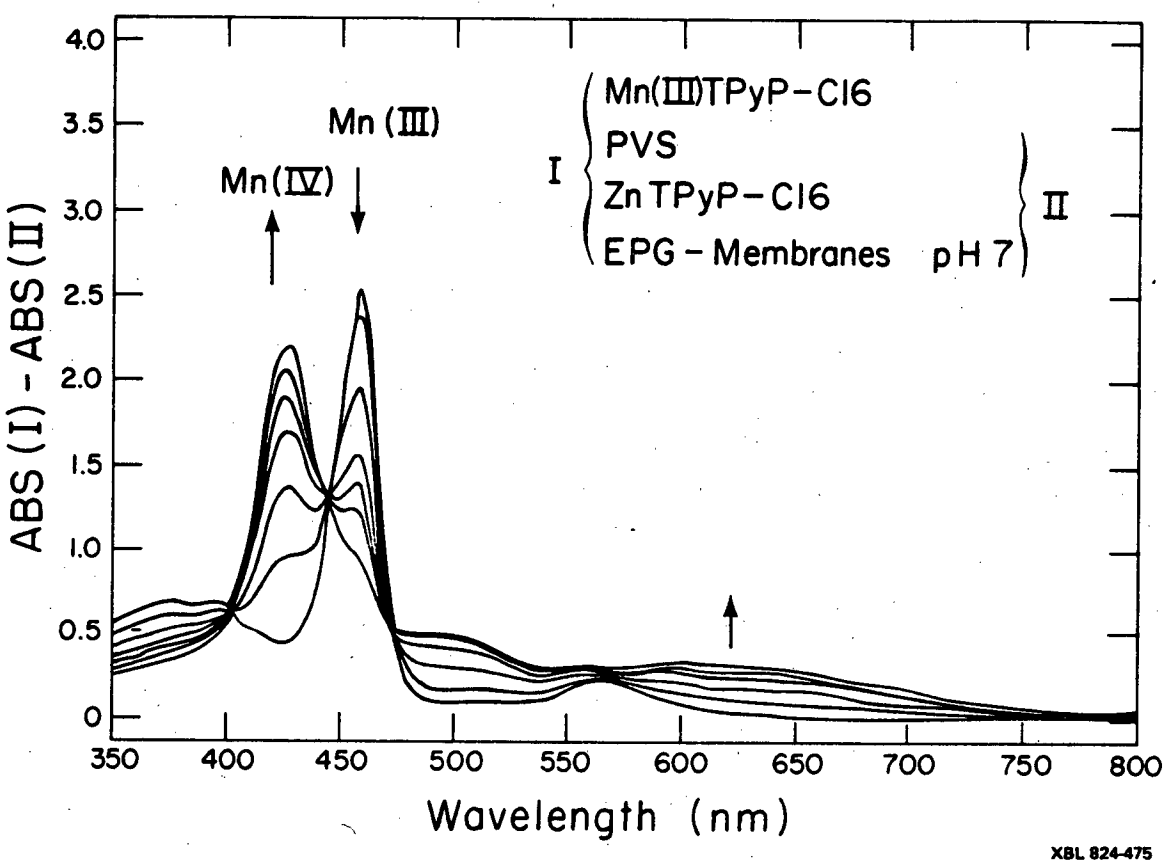

FIGURE 20. Difference spectra of system I vs system II.

B. Photosensitized Electron Transfer Reactions in SiO_2 Colloids

It occurred to us that it might be possible to use a surface potential to achieve separation without having the charge go across the membrane boundary. It is possible to arrange the system in such a manner that the surface potential of the interface will be the controlling factor in the efficiency of electron transfer if the right particles are used with the correct charge distribution and proper structure of donors/acceptors/sensitizers. (36) We chose silica particles which are highly negatively charged to test this hypothesis, performing the experiments at pH 9 to be sure that the positive sensitizer is closely attached electrostatically to the negatively charged surface. The neutral acceptor, propylviologen sulfonate, can approach either a positive or negative surface. When the sensitizer is illuminated and excited it can pass its electron to the neutral acceptor, creating a negative radical which changes the charge on the ruthenium sensitizer from +2 to +3. The Ru^{3+} is held more tightly to the negatively charged surface and the negatively charged electron acceptor is ejected from the field around the negative particles. Thus, the back-reaction of the two materials which should energetically go back downhill is prevented (Figure 21). That this is indeed the case is shown by flash photolysis experiments whose result is shown in Figure 22. (37) With the analyzing beam set at 602 nm to monitor the propylviologen sulfonate radical, we see, in A, that the back-reaction for the decay of that radical is rapid in homogeneous solution. In the presence of 0.2% SiO_2 in B, however, the back-reaction is indeed very much slower. Intermediate potentials may be established in the SiO_2 particles by the addition of salt (C), or by lowering the pH (D), in which case some back reaction is again observed.

The next step in this artificial photosynthetic sequence will be to find an electron acceptor to replace the hydrogen generating system which will not only accept the photoelectrons but also bind carbon dioxide, and thus allow their combination, leading to a reduced product perhaps as far as methane.

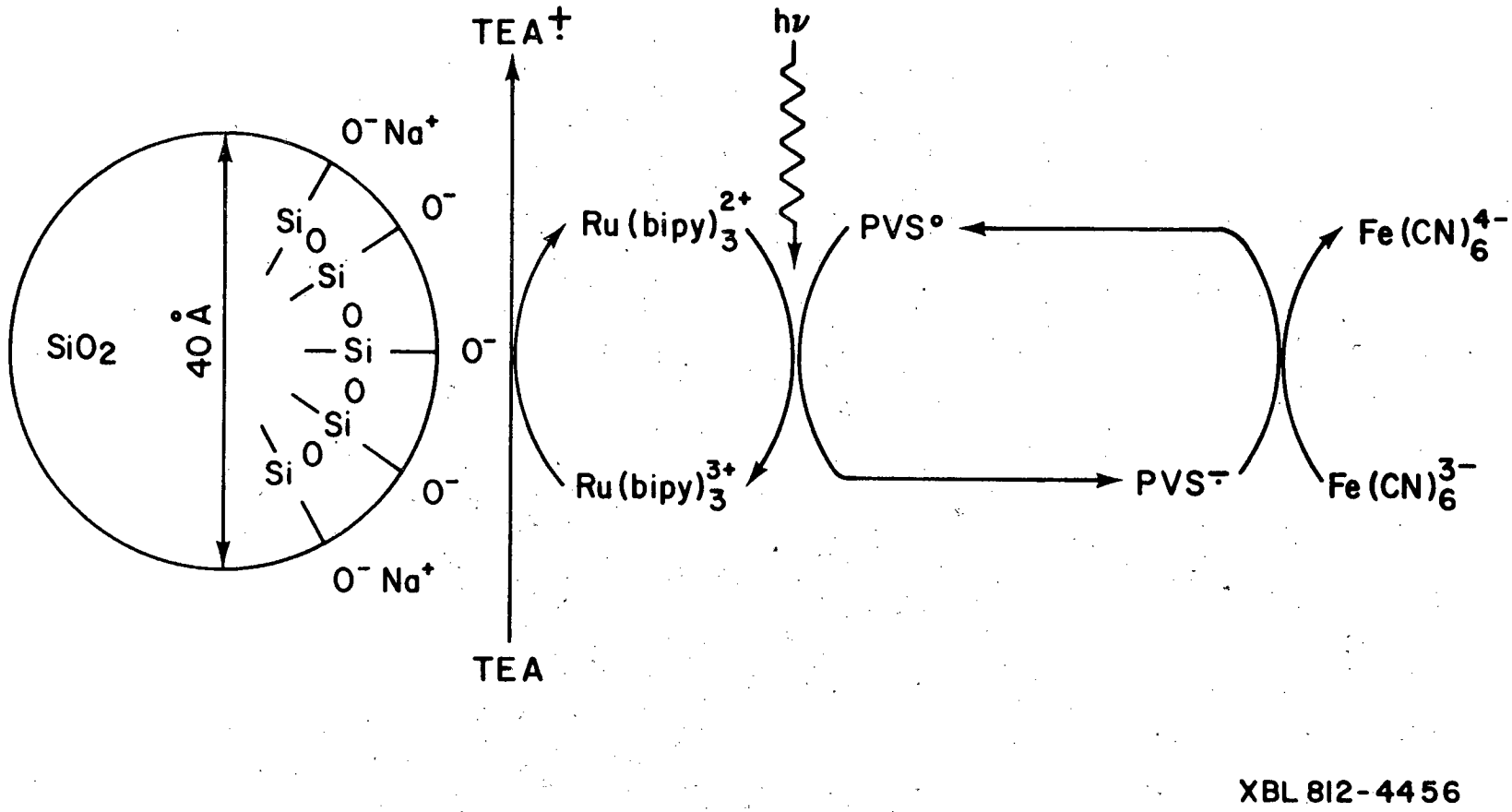

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FIGURE 21. Schematic function of SiO_2 particles in separating multicharged photoproducts.

FIGURE 22. Results of flash photolysis experiment using SiO_2 particles.

C. Device for Photodecomposition of Water

It now remains for us to put one of these entire systems into some useful form. One way to do this would be to use thin hollow fiber membranes which have been constructed for water desalinization and artificial kidneys. A collection of such hollow fibers with Zn-tetraphenylporphyrin as sensitizer (Figure 23) is capable of sensitizing the oxidation of Mn-porphyrin to the Mn^{IV} oxidation state. With the use of suitable catalysts such as ruthenium oxide, cobalt oxide or perhaps even manganese oxide we would obtain Mn^{II}. Furthermore, it seems that Mn^{IV} porphyrin is capable of oxidizing an olefin or water and returning to the Mn^{II} state by this route. (35,38) Whether this will be *the* economic factor which makes the entire process viable or not is yet to be determined.

At this point it is probably worth remembering a quotation from Priestley (1774) which referred to his correspondence with Lavoisier: (39)

"It is pleasant when we can be equally amused with our own mistakes, and those of others. I have voluntarily

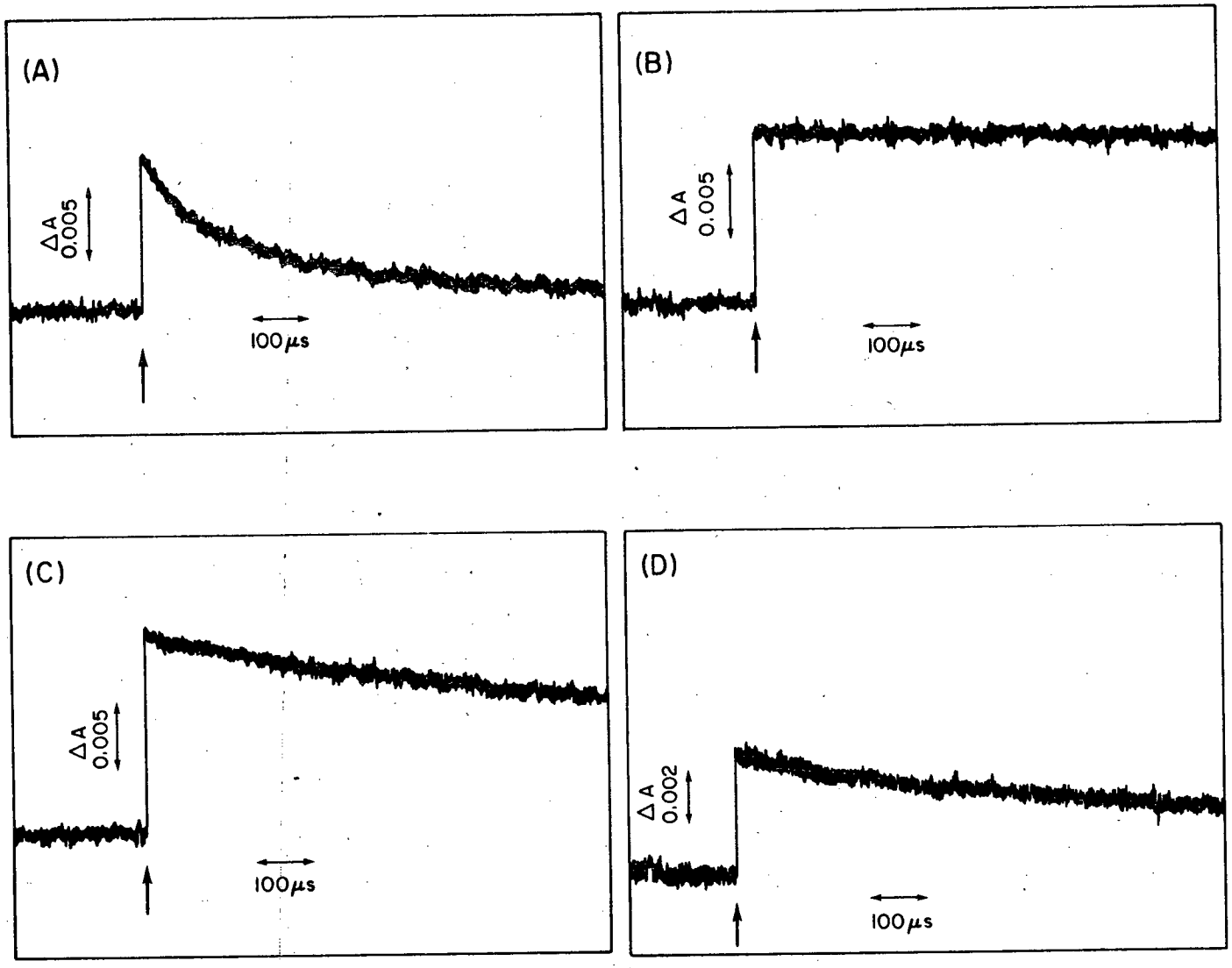

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FIGURE 22. Results of flash photolysis experiment using SiO_2 particles.

FIGURE 23. Hollow fiber apparatus for photodecomposition of water.

> given others many opportunities of amusing themselves with mine, when it was entirely in my power to have concealed them. But I was determined to shew how little *mystery* there really is in the business of experimental philosophy, and with how little *sagacity*, or even *design*, discoveries (which some persons are pleased to consider as great and wonderful things) have been made.

VII. CONCLUSION

We have traced a route from the primeval carbon dioxide through the modern green plant to a totally synthetic system which will use visible light from the sun to generate both fuel and chemicals. We are using two different methods to achieve the purpose of lessening dependence on the products of ancient photosynthesis (oil and gas), the first being the development of hydrocarbon-producing plants for fuels and materials and the second being the creation of artificial photosynthetic systems to mimic the green plant to achieve

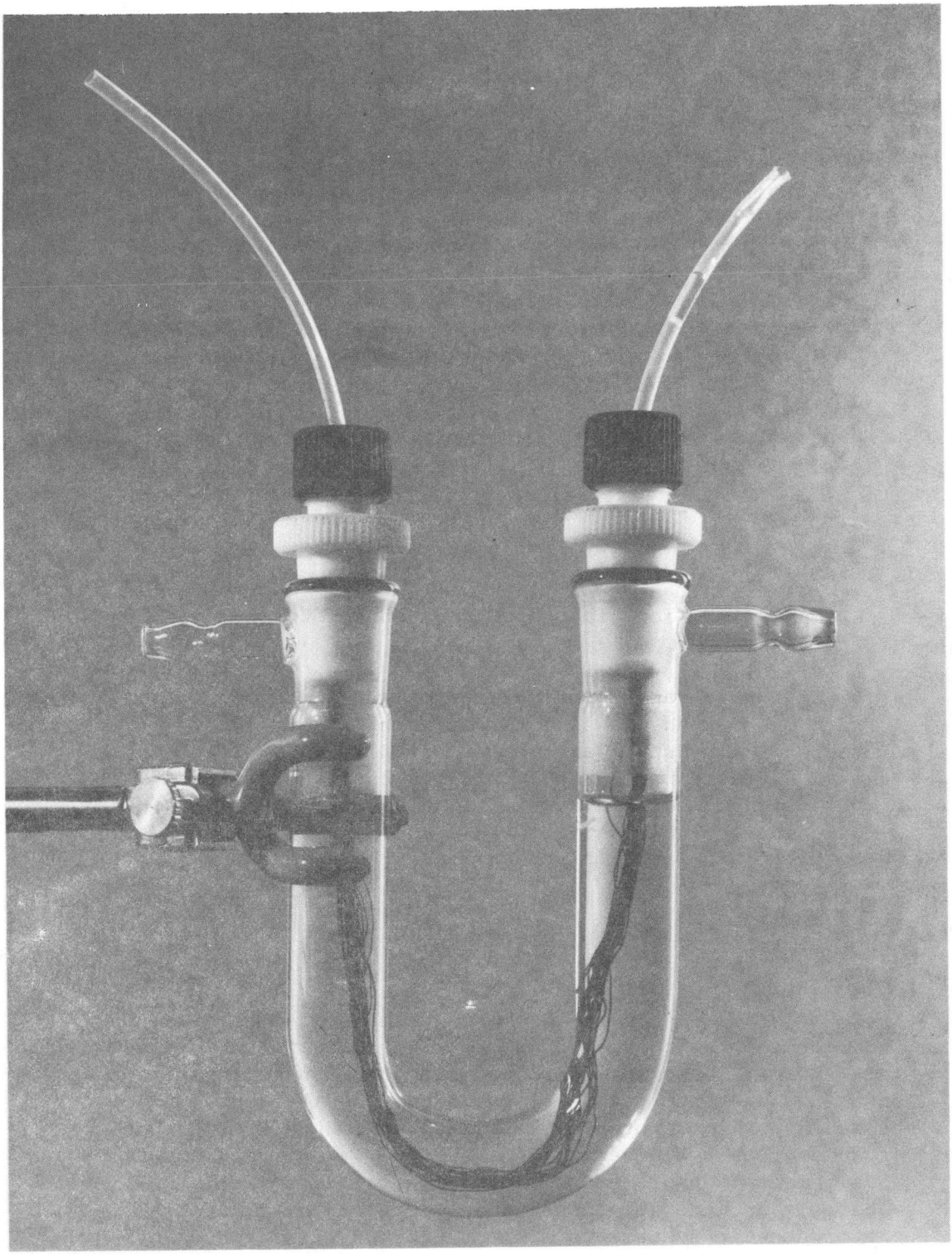

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FIGURE 23. Hollow fiber apparatus for photodecomposition of water.

the photolysis of water, with the concomitant production of hydrogen as a fuel and molecular oxygen, or intermediate oxidation and reduction prdoucts, for further chemical use.

Leonard Nash, in his study of the historical basis for the relationship between plants and the atmosphere in 1952 used the phrase "artificial photosynthesis" to describe "photosynthesis in the absence of plant life". (40) He said then that "the achievement of truly artificial photosynthesis appears to lie unforeseeably far in the future". The experiments on the use of totally synthetic systems which are underway throughout the world will achieve the goal of understanding and effective control of natural photosynthetic processes much more rapidly than Nash anticipated thirty years ago.

A representation of modern evolutionary pathways from the primitive steps described earlier to plants, animals and human life is depicted by "Metamorphose" by the Dutch artist, M. C. Escher in 1939-1940 (Figure 24).

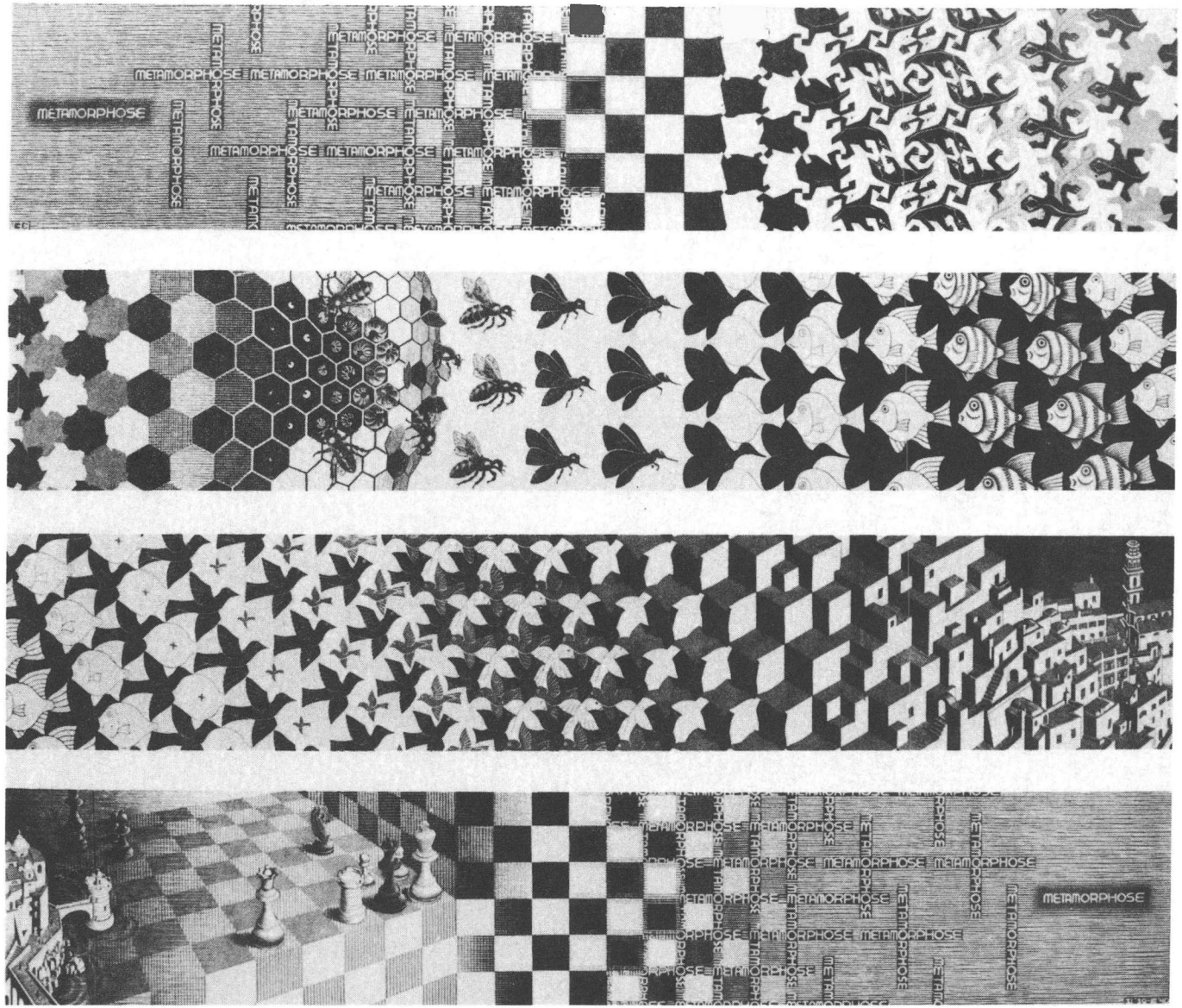

FIGURE 24. "Metamorphose", M.C. Escher (1939-1940). CBB 732-727

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