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Ernest O. Lawrence Radiation Laboratory

BIO—ORGANIC CHEMISTRY

QUARTERLY REPORT

June, July, August 1959

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BIO-ORGANIC CHEMISTRY QUARTERLY REPORT

June, July, August 1959

M. Calvin, Director

Lawrence Radiation Laboratory and Department of Chemistry
University of California, Berkeley, California

September 24, 1959

PRELIMINARY EXPERIMENTS ON THE ACTION SPECTRA AND KINETICS OF THE PHOTOINDUCED ESR SIGNAL IN SPINACH CHLOROPLASTS

Power B. Sogo* and Lou Ann Carter

Experiments have been started on the physical behavior of the photoinduced ESR (electron spin resonance) signal in photosynthetic material. Previous studies[1, 2] in this laboratory may be summarized as crude measurements of rise and decay times as a function both of the organism containing the photosynthetic material and of the temperature of the sample. No particular attention was paid to the wave length or intensity of the irradiating light. The influence of these variables is of primary concern in this paper.

Action Spectra

A typical action spectrum of the steady-state concentration of photoinduced unpaired electrons in spinach chloroplasts at room temperature and at a light intensity of 2×10^{15} quanta/sec is shown in Fig. 1. The spectral resolution of the exciting light was obtained from a combination of band-pass interference filters and colored glass filters. This resulted in a fairly broad-band illumination with a half-maximum band width of about 400 A .

The most interesting feature of this spectrum is the wave-length position of the peaks. These are shifted about 700 A toward longer wave lengths from the positions of maximum absorption of chlorophyll. This result would tend to rule out practically all the chlorophyll contained in the sample as the primary light acceptor for the process of spin production. A possible explanation of the shift is in the sample geometry in our experiments, which is very bad for the measurement of action spectra. The sample consists of a viscous, aqueous paste of spinach chloroplasts which has been

*Present address: Physics Department, San Diego State College, San Diego, California.

[1] Sogo, Pon, and Calvin, Proc. Natl. Acad. Sci. 43, 387 (1957). University of California Radiation Laboratory Report No. UCRL-3713, March 1957.

[2] Sogo, Jost, and Calvin, Radiation Research, Supplement 1, 511 (1959). University of California Radiation Laboratory Report No. UCRL-8358, July 1958.

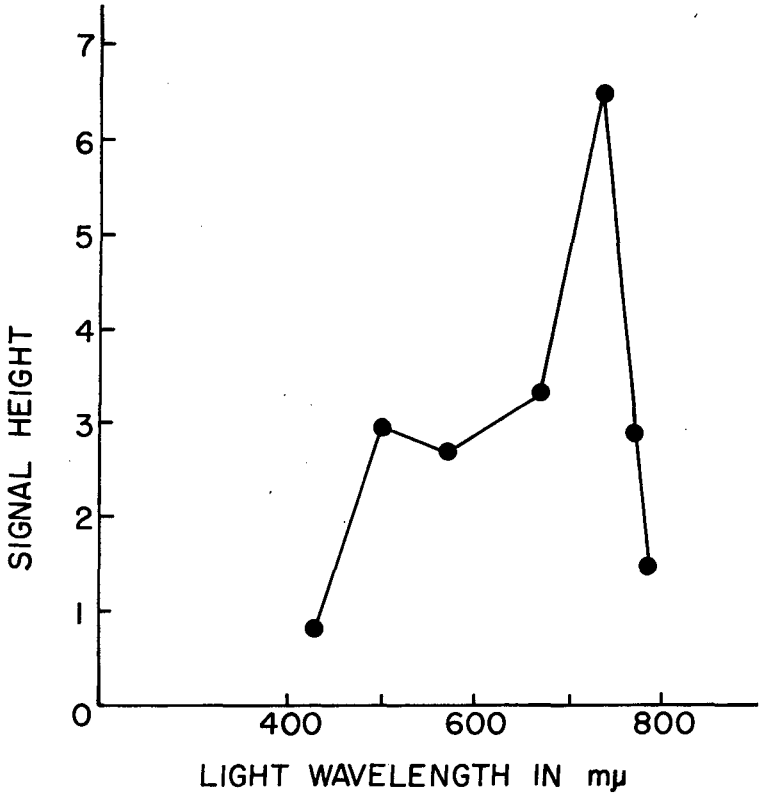

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Fig. 1. Action spectrum of photoinduced unpaired electrons in spinach chloroplasts (light intensity: 2×10^{15} quanta/sec).

painted on a silver rod. The thickness of the sample is about 0.5 mm. The packed chloroplast sample is very dense optically, so that strongly absorbed light penetrates only a short distance while weakly absorbed light penetrates a longer effective distance. When one considers this effect together with light-saturation behavior, then one expects large signals from weakly absorbed light and small saturated signals from strongly absorbed light.

The best sample for the measurement of a true action spectrum is one that is thin enough so that one can assume a uniform light intensity throughout the volume of the sample. The present spectrometer does not possess the required sensitivity for measurements on such small samples.

Kinetics

Preliminary experimental data indicate that the dynamic behavior of the photoinduced ESR signal in spinach chloroplasts at room temperature, and with an exciting-light wave length of 7400 Å, may be described by an equation in the form

$$\frac{dn}{dt} = QI_0 \left(1 - \frac{n}{N_0}\right) - \frac{n}{T_1} - \frac{n^2}{T_2} ,$$

where n is the number of unpaired spins per unit volume,

Q is the maximum quantum efficiency for the production of unpaired spins,

I_0 is the incident light intensity,

N_0 is the maximum number of unpaired spins per unit volume that can be produced by light,

T_1 is a unimolecular decay time,

T_2 is a bimolecular decay time.

The implications of this equation agree with reasonable physical intuition, namely, (a) the probability of producing an additional unpaired spin depends on the number of unpaired spins that exist, and (b) at very high concentrations of unpaired spins, recombination becomes probable and a bimolecular decay law results.

Inadequate spectrometer sensitivity has precluded an adequate test for the validity of the above equation. However, it appears that below a light intensity of about 10^{14} quanta/sec one observes the following phenomena, which, if rigorously confirmed, will establish the above equation for the range $n \ll N_0$:

(a) The steady-state signal is directly proportional to the light intensity.
(b) The rise time is equal to the decay time.
(c) The rise and decay follow an exponential law.

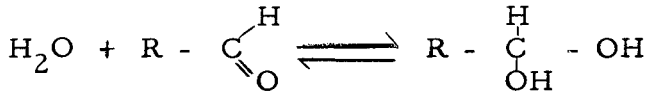
At higher light intensities, the steady-state signal height begins to saturate with respect to the light intensity. The rise time no longer equals the decay time and the initial portion of the decay curve approximates a bimolecular type of curve.

The range $n << N_0$ the signals are so small compared with spectrometer noise that there is some doubt of the validity of Conclusions (a) through (c) above. A substantial increase in the basic sensitivity of the ESR seems most important for further insight into the nature of this signal.

NMR STUDY OF ACETALDEHYDE-WATER MIXTURES

E. Lombardi* and Power B. Sogo†

Acetaldehyde and trimethylamine are known to be the principal products of the radiation decomposition of crystalline choline chloride.[1] In order to investigate the migration of protons from the ethanol group of the choline molecule during radiation decomposition it is necessary to know whether acetaldehyde can exchange hydrogen atoms with water. This must be known because water is used to separate the radiation products. Therefore we undertook the study of mixtures of acetaldehyde and water. Using the technique of high-resolution NMR, we have established that there is no exchange of protons in acetaldehyde, either for protons in water or for deuterons in D_2O. We have also calculated a value for the equilibrium constant between the two forms (free and hydrated). The general expression of this equilibrium is



We have used samples of acetaldehyde, propionaldehyde, and isobutyraldehyde that were purified by distillation. The purity of H_2O and D_2O was ascertained by NMR. All the spectra have been taken with a Varian Model V-4300 B spectrometer working at 40 Mc. The chemical shifts are expressed in ppm of the external magnetic field by the relation

$$\delta = (H - H_{ref}) \times 10^6 / H_{ref} ,$$

where H is the resonance field for the sample and H_{ref} is the resonance field of protons in water.

The spectrum of pure acetaldehyde consists of a quadruplet at $\delta = -4.3$ and a doublet at $\delta = +4.1$. The quadruplet represents absorption by the aldehydic proton, which has been split by spin-spin interaction with the three protons of the methyl group, while the doublet represents absorption by the methyl group, split by the aldehydic proton.

When D_2O is added to pure acetaldehyde (Fig. 2) we observe the same acetaldehyde spectrum, consisting of a quadruplet at $\delta = -4.3$ and a doublet at $\delta = +4.1$. In addition, a quadruplet at $\delta = +0.7$ and a doublet at $\delta = +5$ appear. We have assigned these additional lines to the hydrated form of the acetaldehyde, with the new quadruplet representing absorption by the proton of the acetal group, and the new doublet representing absorption by the methyl group.

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†Present address: Physics Department, San Diego State College, San Diego, California.

[1] Lemmon, Parsons, and Chin, J. Am. Chem. Soc. 77, 4139 (1955).

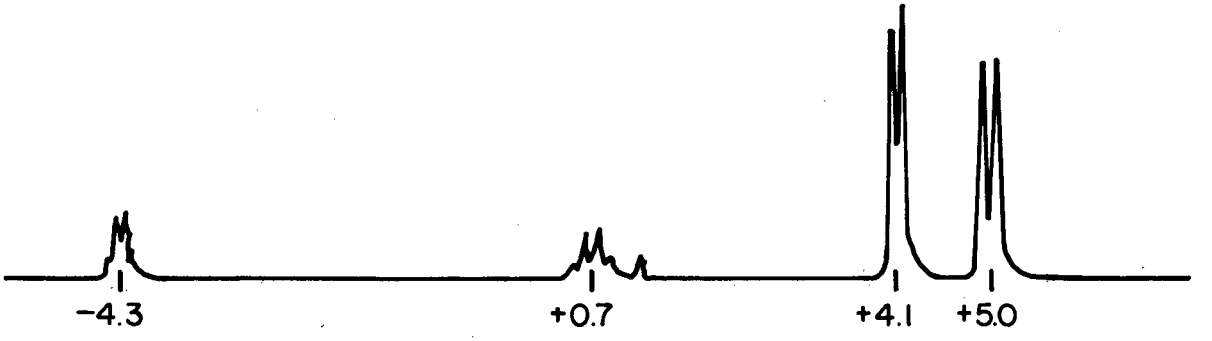

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Fig. 2. NMR spectra of mixture containing 1 part acetaldehyde to 1 part D_2O.

The intensities, but not the positions, of the peaks assigned to the hydrated form depend on the relative proportions of acetaldehyde and water. The assignment is further supported by the fact that the hydrated form is less polarized than the aldehydic form.

The same effect has been observed in mixtures of propionaldehyde and water or D_2O, and of isobutyraldehyde and water or D_2O.[2] The data are reported in Table I.

Table I

Chemical shift of nonequivalent protons in aldehydes and their hydrated forms

(The values are expressed in ppm of the external magnetic field and are estimated to be accurate to ± 0.2 ppm)

	Acetaldehyde	Acetaldehyde + H_2O	Acetaldehyde + D_2O
-CHO	-4.3	0.6	0.7
$-CH_3$	4.1	5.0	5.0
	Propionaldehyde	**Propionaldehyde + H_2O**	**Propionaldehyde + D_2O**
-CHO	-4.3	0.3	0.4
$-CH_2$	2.7	3.6	3.6
$-CH_3$	4.1	4.1	4.1
	Isobutyraldehyde	**Isobutyraldehyde + H_2O**	**Isobutyraldehyde + D_2O**
-CHO	-4.1	0.6	0.6
CH	3.0	3.7	3.7
$-CH_3$	4.3	4.3	4.3

It is possible to calculate the value of the equilibrium constant by using the relation

$$K_{eq} = x / (a-x)(b-x) ,$$

where x = equilibrium concentration in moles/liter of the hydrated form,
a = initial concentration of aldehyde in moles/liter,
b = initial concentration of H_2O or D_2O in moles/liter.

From the areas under the absorption peaks of the pure and the hydrated form we obtain the ratio $x/(a-x)$, from which we calculate x. The results of many experiments, using different amounts of acetaldehyde and water or D_2O, gave for acetaldehyde at 25°C

$$K_{eq} = \frac{(2.7 \pm 0.1)(10^{-2})}{\text{moles/liter}} .$$

[2] However, in mixtures of acetone and water no formation of the hydrated form has been observed.

INTERACTIONS OF RECOILING TRITONS WITH METHYL HALIDES

I. M. Whittemore, Michael Szwarc,* and Richard M. Lemmon

Tritium may be produced by the reaction $He^3(n,p)H^3$. The thermal neutron cross section for this reaction is very high--5500 barns.[1] When this irradiation is carried out in the presence of organic compounds, high percentages of the total activity formed are found in the starting compounds and in other compounds not originally present.[2]

Work has been started on the general problem of the fate of the recoiling tritons (recoil energy 0.19 Mev) in the presence of the compounds of the methyl halide series. Attempts will be made to distinguish "hot" reactions from "thermal" reactions and to assess the degree of localization that takes place upon capture of the tritons.

Irradiations are being carried out in the pool-type reactor (LPTR) at the Livermore site of the Lawrence Radiation Laboratory. Various facilities for irradiation are available, including in-pile columns, tubes adjacent at the core, and thermal-column irradiation facilities.

A vacuum line has been constructed for carrying out the various manipulations required. This line is illustrated in Figs. 3, 4, and 5. Figure 3 shows the main vacuum line used for storing gases, filling bulbs for irradiation, and preliminary separation of the reaction products. Figure 4 shows the combustion train used to separate H_2 and CH_4, two of the expected reaction products. Figure 5 shows a vacuum manifold used in the separation of the methyl halide from the less volatile products.

The various gases needed in the experiments are introduced through trap A (Fig. 3) and into bulbs B, C, D, and D'. He^4, H_2, CH_4, and methyl halide are stored in the bulbs. He^3, from which all residual tritium activity has been removed, is introduced through stopcock 11 into bulb N. Small quantities can be removed from N by trapping it between stopcocks 12 and 13, and this quantity can be measured in a calibrated portion of the vacuum line bounded by stopcocks 12, 14, 16, 18, and the McLeod gage M.

Methyl halide may be introduced into the evacuated quartz irradiation bulb L in known quantity by means of the calibrated volume C'. With the line evacuated and stopcock 2 closed, methyl halide is slowly let out of its large bulb into the vacuum line, and when the desired pressure is indicated on the manometer F, stopcock 5' is closed. The rest of the halide is removed from the line and the contents of C' vacuum distilled into L, which

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[1] D. J. Hughes and R. B. Schwartz, Neutron Cross Sections, BNL-325, July 1958, p. 3.

[2] F. S. Rowland and Richard Wolfgang, in International Conference on Radioisotopes in Scientific Research, Vol. II (Pergamon Press Inc., 1958), p. 105.

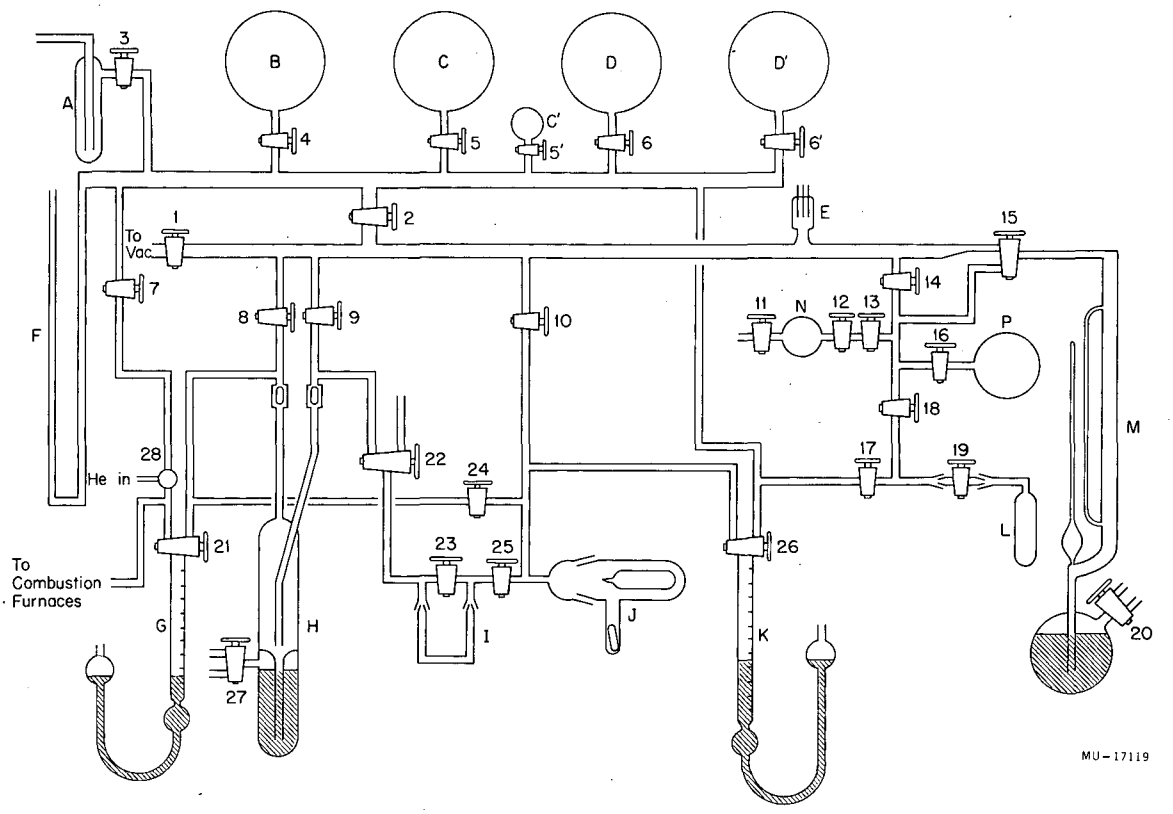

Fig. 3. Vacuum-line apparatus for work on methyl halide and recoiling tritium.

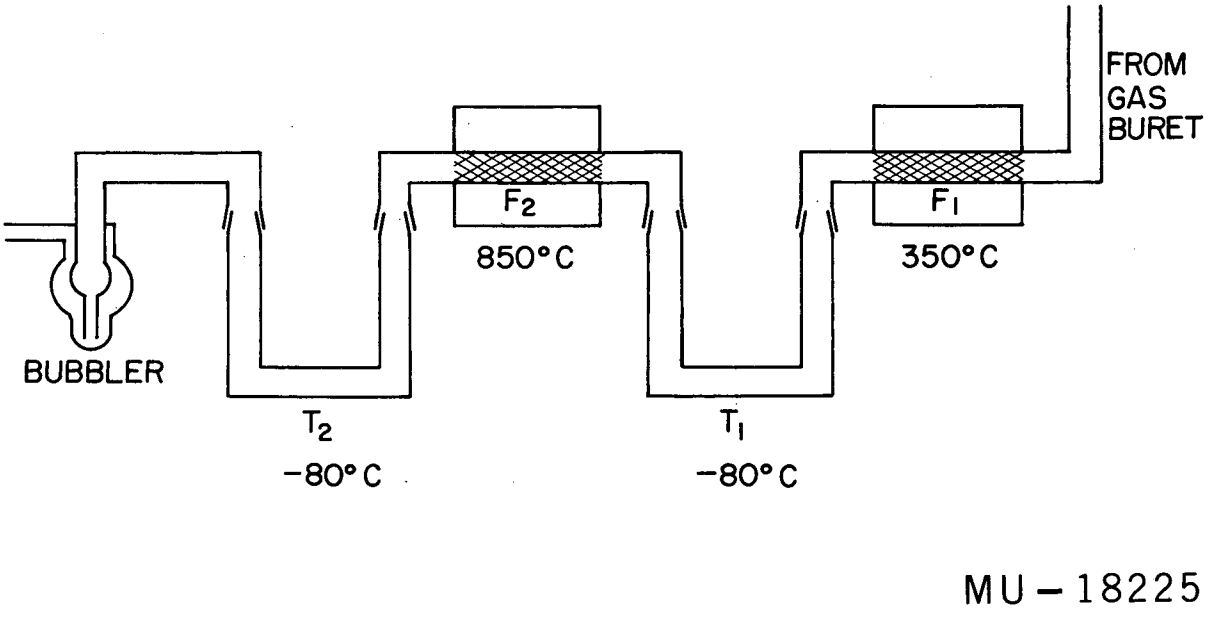

Fig. 4. Combustion train for separate combustions of methane and hydrogen.

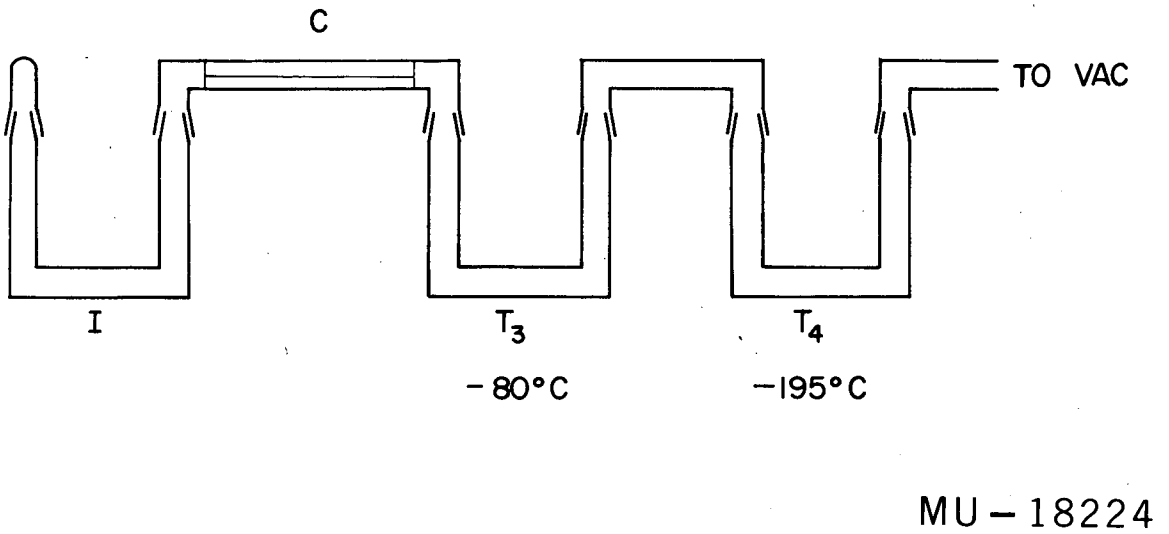

Fig. 5. Vacuum-line apparatus for separation of water and methyl halide.

is cooled with liquid nitrogen. Stopcock 17 is closed and 18 opened. He^3 is allowed to equilibrate with L and then L is sealed off. The amount of He^3 remaining in the line is measured and the quantity of this gas sealed in the bulb is calculated by difference.

When the introduction of He^4 as a moderator in L is desired, a modified procedure is used. A mixture of He^3 and He^4 can be placed in P and then the same procedure as for introduction of He^3 may be followed.

After irradiation, a bulb is introduced into the breaking chamber J along with a small quantity of water. J is equipped with a side arm containing an iron bar that can be used for breaking off the neck of the quartz bulb. The water is frozen and the breaking chamber, the trap I, the Toepler pump H, and the gas burets G and K are evacuated. A measured quantity of H_2 and CH_4 are put in K and introduced into J, and the quartz vessel is broken (stopcocks 24, 25, and 10 closed). After a short period to allow the gases to mix and for the HX to exchange with the water, the Toepler pump is started and the gas mixture is allowed to circulate through stopcock 25 (trap I frozen in liquid N_2, stopcock 23 closed), stopcock 22, and H, and return to J through 24. In this way, all materials with no appreciable vapor pressure at liquid nitrogen temperature are trapped in I. This includes H_2O and all expected products except H_2 and CH_4. The He^4, H_2, and CH_4 can be collected (in large fraction) by closing stopcock 24 and collecting in the gas buret G. The fraction of these gases collected is determined, and the gases are passed through the combustion train (Fig. 4).

F_1 and F_2 are tube furnaces containing CuO wire. F_1 is kept at 350° and under the conditions of gas flow used, 99 + % of the H_2 is oxidized to water while less than 1% of the CH_4 is converted to water. T_1 is a dry-ice-cooled trap to collect the H_2O formed in the first furnace. F_2 is kept at 850° and quantitatively converts the methane to water and carbon dioxide. The H_2O is trapped in T_2 at dry-ice temperature. After all the gas has been introduced from the gas buret, the combustion train is slowly swept with He^4 introduced through stopcock 28 (Fig. 3) until all the water has been frozen in the traps. The H_2O is quantitatively washed from the traps with a liquid scintillation solution into a counting vial, and the tritium activity counted in a liquid scintillation counter.

The methyl halide and the H_2O in trap I in Fig. 3 are removed from the vacuum line and placed on a vacuum manifold (Fig. 5) while still cold. One side of trap I is capped, the other side attached to the front of the manifold. T_3 is cooled to -80° and T_4 is cooled to -196°. I is allowed to warm slowly to room temperature. If the partial pressure of methyl halide is kept below its vapor pressure at -80°, none of the halide condenses in T_3 and it passes into T_4 (any other products that meet this criterion will also pass into T_4). The less volatile materials, especially H_2O in this case, condense in T_3. Too rapid transfer of the material in I into the rest of the manifold is prevented by the fine capillary C, 1/2 mm in diameter. Tests with radioactive H_2O and with CH_3I (separately) have shown the separation of these two products to be quantitative. The H_2O in T_3 can be counted directly in a liquid scintillation counter, as can also the products in T_1 and T_2 in Fig. 4. If the methyl halide is CH_3I, it too may be directly counted. If the trap contains one of the more volatile halides, the substance may be transferred while still frozen to the main vacuum line, then to the gas buret G, and burned in the combustion train in the same manner as the CH_4 and H_2.

Work done thus far with the apparatus has been of a preliminary nature aimed at achieving reproducibility of results and improving the mechanical techniques and manipulations.

PROPOSAL FOR THE DIRECT VIEWING OF A RADIOACTIVE PAPER CHROMATOGRAM

Paul M. Hayes

Paper chromatography of plant extracts containing C^{14}-labeled compounds is the major analytical tool of our photosynthesis research. Several thousand papers are run each year. It is virtually impossible to speed up the process of two-dimensional chromatography, but it is now possible to shorten the succeeding step, radioautography. It is normal for the finished paper chromatogram to be placed next to a sheet of x-ray film (in a darkroom) for a period of time (several days to weeks), allowing the film to be exposed to the C^{14} beta particles. This waiting period is lost time if the data shown on the film indicated poor chromatographic separation, or some other previously unknown error or mishap to an experiment. The actual dollar loss is difficult to measure but is not so important as the loss of time. Our group normally has several postdoctoral visitors working on different phases of the problem of photosynthesis. For them the loss of time is particularly serious, as they are generally here less than one year.

It appears that the components are now available to assemble a device for showing directly, and at full scale, the radioactive areas of a paper chromatogram. This device will be assembled with the following components:

a. the paper holder and scintillator,
b. the image intensifiers,
c. the image orthicon (or vidicon, if applicable) b and c may be combined),
d. the read-out TV monitor,
e. possibly a storage tube between c and d may be either necessary or desirable.

The paper holder and scintillator constitute a purely physical problem and should present few real difficulties; there may be some innovations involved with the actual scintillator support. The image intensifiers already exist in many forms. These light amplifiers have various kinds of gains: electrical and (or) electronic, and minification. It is now possible to "stack" these intensifiers and "see" a single photoelectron. The output screen of the intensifiers tends to be small, apparently less than 1.5 in. in diameter. To get the "information" from this small screen size to an area the size of the original chromatogram requires the use of a TV camera tube--either the vidicon, or the much more sensitive image orthicon. The information as seen by the orthicon may be of such low level that the addition of an integrator in the form of a storage tube will be necessary. The storage tube has the ability to store the image of the TV picture for periods from several minutes to hours. Finally, the image would be shown on the appropriate TV monitor, which we can use also to vary the contrast and possibly improve the picture of the radioactivity on the chromatogram.

Once the information can be displayed on the TV monitor, there will be several ways of storing or recording the data as shown. There can be the storage tube as just described, or a Polaroid camera arranged to give a proper record of the picture either for notebook or publication; or, lastly, roll film can be mounted to the side of the TV screen in such a way that when wanted, it can be drawn out over the face of the TV and exposed, and then used as the x-ray film is now used.

The image intensifier would be the heart of the proposed instrument. Such intensifiers have been built onto the face of image orthicon or TV camera tubes. In fact, there is now a three-stage intensifier orthicon with sufficient gain to see a single electron. The image intensifier is an outgrowth or improvement of the image converter that converted infrared energy to visible light. The first uses were primarily for battlefield surveillance and weapon sights, as the initial development can just prior to World War II. Later, other applications included use in the fields of infrared microscopy, entomology, dermatology, ophthalmology, and photomicrography. Recently, radiologists who wanted to reduce x-ray dosage, and nuclear physicists who wanted to track high-energy particles by crystal scintillations, have developed uses for the image intensifier. The armed services saw the need for improvement in the field of night vision and sponsored a great deal of basic intensifier research.

Basically, the intensifier contains only a few elements: the photoemissive front element, or photocathode; the lens stack, or appropriate grid and anode; and the phosphor screen.

The photoemissive surface is an evaporated, semitransparent coating of cesium, potassium, antimony, and sodium. This coating is several times as sensitive to visible light as the cesium-antimony photocathode, and has only about one-tenth the dark emission. The electrons emitted from this surface, having a distribution corresponding to the input image, are accelerated by electrostatic fields and focused on the fluorescent screen at the other end of the tube. In the cascading type of intensifier the fluorescent screen and the photoemissive surface of the succeeding stage are combined in a single element.

As already indicated, there is a newly developed intensifier orthicon tube available with as many as three image intensifiers in tandem ahead of the supersensitive orthicon. This is a single unit, with each stage dependent on the other.

No mention is made here regarding resolution or light-input levels. This information is dependent on each type of intensifier, and is generally available.

H_2 ASSIMILATION BY ADAPTED SCENEDESMUS UNDER HIGH LIGHT INTENSITIES

Richard Goldsby and Roderic B. Park

Twenty years ago Gaffron[1] showed that the algae Scenedesmus could be made to assimilate molecular hydrogen. This assimilation takes place under low light intensities in properly adapted Scenedesmus, and is augmented by the presence of CO_2. The adaptation consists of the anaerobic incubation of Scenedesmus in the dark under an atmosphere of molecular hydrogen for two or more hours. This light-dependent assimilation of hydrogen and CO_2 has been termed "photoreduction."[2] The presence of O_2 in sufficient concentration after adaptation causes de-adaptation, and also causes a reversion to normal photosynthesis.[2] The saturating light intensities commonly used in photosynthesis experiments are far in excess of the de-adaptation intensity.

Presently, some attempts are being made in this laboratory to use hydrogen-adapted Scenedesmus and tritium gas as a system for the study of the path of hydrogen in photosynthesis. However, under the conditions of classical photoreduction, the uptake of hydrogen is limited by the necessarily low light intensities imposed by the phenomena of de-adaption. It is generally thought that the increased amount of molecular oxygen generated at the higher light intensities somehow destroys a part of the photoreductive apparatus that was formed during the anaerobic adaptation. In hopes of avoiding the apparently deleterious effects of molecular oxygen, we added CMU (3-chlorophenyl-1, 1-dimethyl urea) — a compound known to inhibit photosynthetic oxygen evolution— to Scendesmus prior to adaptation and subsequent exposure to light.

Experimental Procedure

A suspension of Scenedesmus obliquus grown under constant-density conditions[3] was centrifuged at 900 g and resuspended in a 1:1 mixture of the supernatant Myers media[4] and distilled water. A portion of the resultant algal suspension was centrifuged at 1300 g for 10 minutes to obtain a measurement of the cell volume, while aliquot portions of the remainder were pipetted into Warburg flasks. At this point, various amounts of a saturated solution (3×10^{-4} M) of CMU in distilled water was added to some of the Warburg flasks.

The flasks were attached to the manometers and placed in the Warburg apparatus and agitated while a stream of either 96% H_2: 4% CO_2 or 96% air: 4% CO_2 was passed through the vessels. After this pretreatment each flask-manometer combination was used to study the net rate of uptake of the 96% H_2: 4% CO_2 mixture or the net rate of oxygen evolution at various light intensities. The temperature was maintained at 24°C during the pretreatment and measurement. The light intensities incident on the surface of the Warburg flask were measured with a photocell.

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[1] H. Gaffron, Nature 143, 204 (1939).

[2] H. Gaffron, Am. J. Botany 27, 273 (1940).

[3] J. A. Bassham and M. Calvin, The Path of Carbon in Photosynthesis (Prentice-Hall, Englewood Cliffs, New Jersey 1957,) pp. 32-33.

[4] J. Myers, Plant Physiol. 22, 509-597 (1947).

Results and Discussion

The curves in Fig. 6 illustrate the behavior of Scenedesmus that has been exposed to a variety of anerobic conditions. Curve 1 shows the expected pattern for Scenedesmus adapted by pretreatment under an atmosphere of 96% H_2: 4% CO_2 in the dark. When the algae are exposed to light intensities below 230 foot candles (ft-c), a net gas uptake is observed. This gas evolution shows a reversion to normal photosynthesis, indicating that the algae have been de-adapted by too intense light.

The points of Curve 2 show that when Scenedesmus is pretreated under 96% H_2: 4% CO_2 in the presence of light, oxygen evolution takes place at both low and high light intensities. There is no net assimilation of the 96% H_2: 4% CO_2 mixture. The algae do not adapt when incubated under anaerobic conditions in the light.

Curve 3 demonstrates that Scenedesmus pretreated in the presence of 1.4×10^{-5} M CMU and under an atmosphere of 96% H_2:4% CO_2 in the presence of light do indeed show a net assimilation of the gas mixture even at light intensities far in excess of 230 ft-c. At high light intensities the algae show a marked resistance to de-adaptation.

In Curve 4, it is seen that a pretreatment in the dark, under an atmosphere of 96% H_2:4% CO_2 and in the presence of 1.4×10^{-5} M CMU, yields an adapted algae which shows the greatest resistance of de-adaptation by high light intensities.

The effect of CMU on oxygen evolution at 460 ft-c in Scenedesmus that have been incubated in the dark under an atmosphere of 96% H_2:4% CO_2 has been investigated. It was found that at 460 ft-c O_2 is evolved at the rate of 4.9 $\mu l/mm^3$ wet packed cells/10 min when no CMU is present. When 1×10^{-4} M CMU is present, oxygen evolution, although reduced to 39% of its value in the uninhibited system, is still appreciable.

CONCLUSIONS

In the presence of CMU, adapted Scenedesmus shows a marked resistance to de-adaptation under conditions of high light intensity. Also, if CMU is added during the anaerobic incubation it is possible to adapt algae in the light; this is not possible without CMU under the conditions of this experiments.

The parameter measured in these experiments was always either net gas evolution or net gas uptake. The very great possibility of concomitant hydrogen assimilation and oxygen evolution in adapted Scenedesmus could not be assessed under the conditions of these experiments. If such an effect were present to an appreciable degree, the apparent hydrogen uptake measured in these and earlier[2] experiments would be less than the actual hydrogen uptake. An effort will be made to examine in a quantitative manner the extent of any concomitant hydrogen uptake and oxygen evolution.

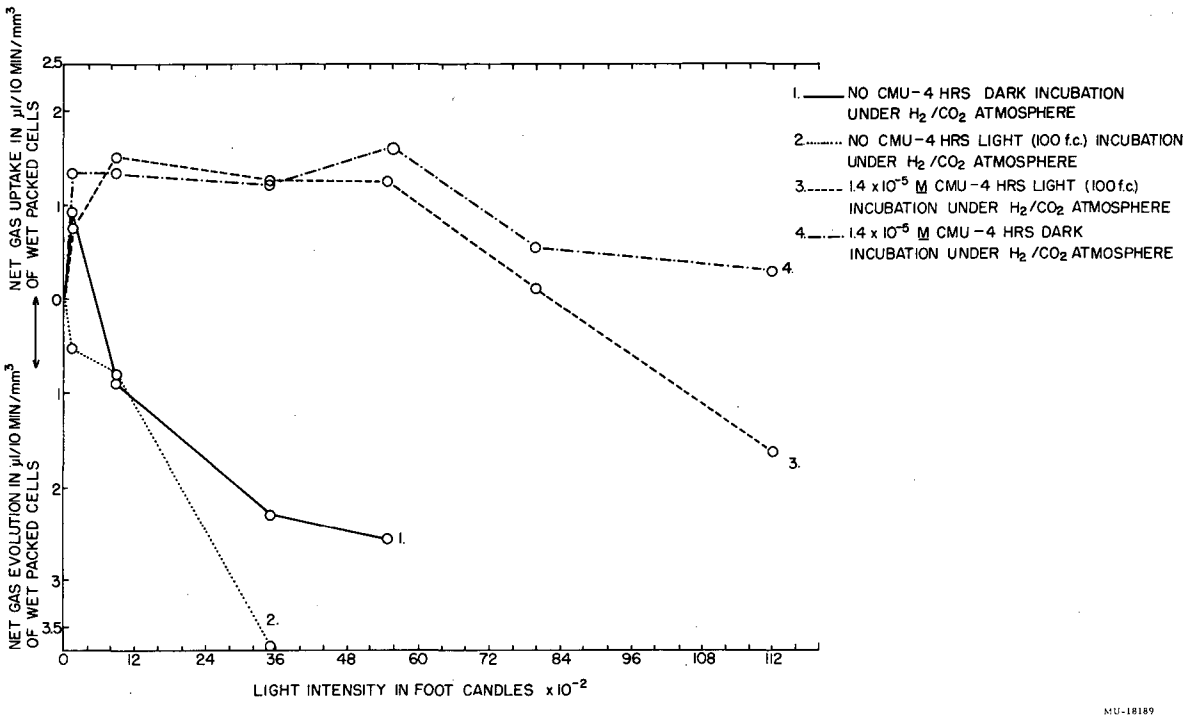

Fig. 6. Behavior of Scenedesmus exposed to a variety of anerobic conditions.

THE KINETICS OF FORMATION OF 2-CARBOXY-4-KETOPENTITOL FROM $C^{14}O_2$ IN CHLORELLA PYRENOIDOSA

Karel P. Louwrier

V. Moses and M. Calvin have described the identification of 2-carboxy-ketopentitol phosphates as products of photosynthesis.[1] The γ-keto acid is structurally closely related to the β-keto acid and, since the latter is suggested as an intermediate in the formation of phosphoglyceric acid from CO_2 and ribulose disphosphate, an experiment was performed to find whether the γ-keto acid is formed by a carboxylation reaction or is in equilibrium with a compound formed by such a reaction.

If the γ-keto acid is formed by a carboxylation reaction, then it can be expected that the concentration of the compound will decrease when the CO_2 concentration decreases. This will also be the case when the keto acid is in equilibrium with a compound formed by a carboxylation reaction. If the keto acid is formed in an irreversible way from such a precursor, than a slow decrease of the concentration can be expected after the CO_2 pressure has been decreased.

Chlorella pyrenoidosa was grown under steady-state conditions in an apparatus described earlier.[2] The medium was 10^{-3} M sodium phosphate buffer at pH 7.2. In order to adapt the algae to this environment, the suspension was flushed with air containing 2% CO_2 for 30 minutes in the light. After this period, $C^{14}O_2$ was injected and samples were taken after different times. After 7 min photosynthesis in $C^{14}O_2$-containing atmosphere, the CO_2 pressure was diminished by circulating the gas phase through a 10% KOH solution. The algae were killed in ethanol so that the final ethanol concentration was 80%. The extraction and chromatography were carried out, with the results shown in Fig. 7. The keto acid diphosphates were extracted and phosphatased. The phosphatase products were mainly γ-keto acid, which usually appeared in two spots, and ribulose (Fig. 8). The radioactivities found in ribulose and keto acids after various times are given in Table II. These data indicate that the γ-keto acid is not formed by a direct carboxylation reaction unless it is the stable end product of an irreversible carboxylation reaction. The growth of C^{14} activity in ribulose resulting from the phosphatasing compounds in the keto acid phosphate area after decrease of the CO_2 pressure suggests that such ribulose does not result from decomposition of an unstable CO_2 fixation products.

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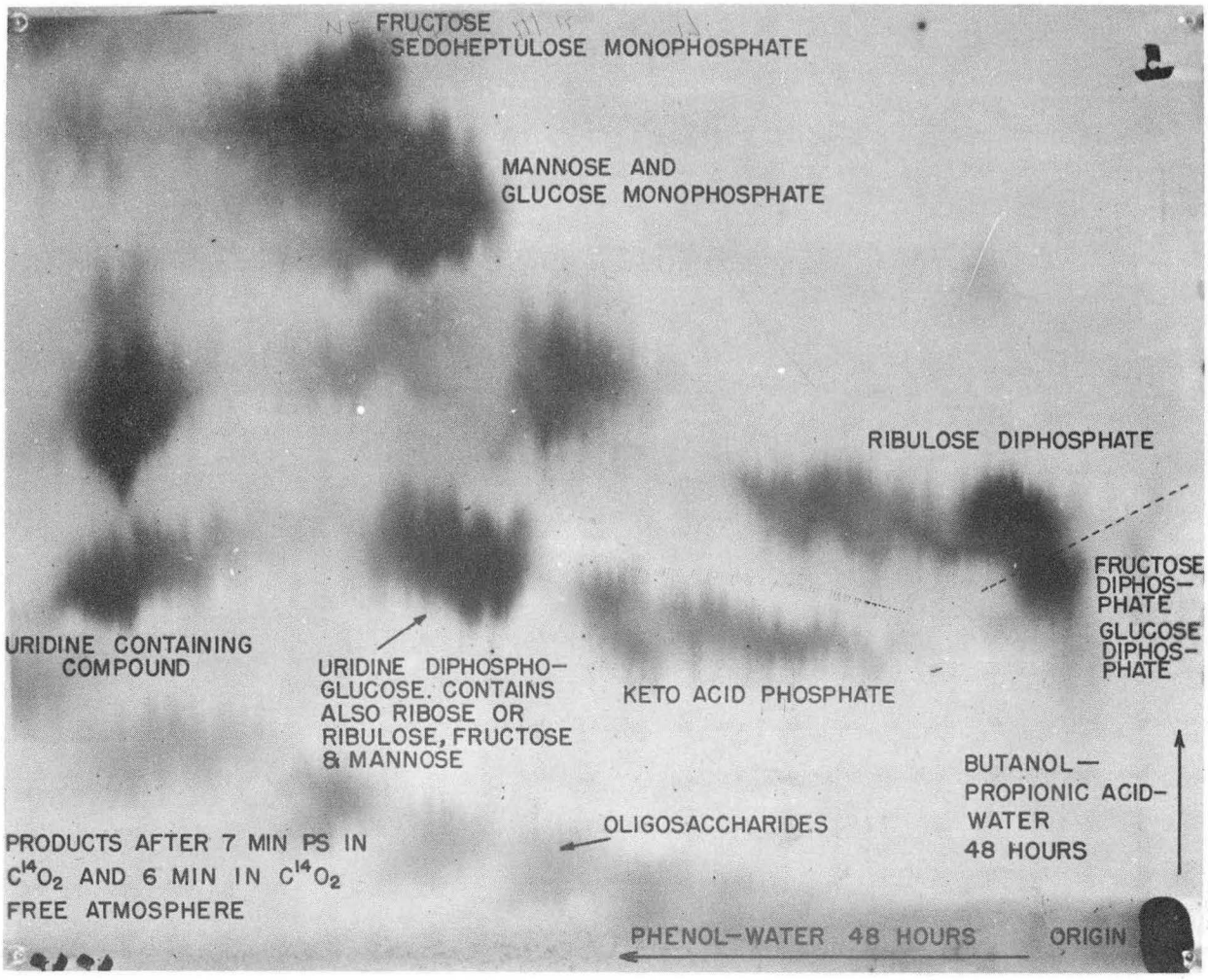
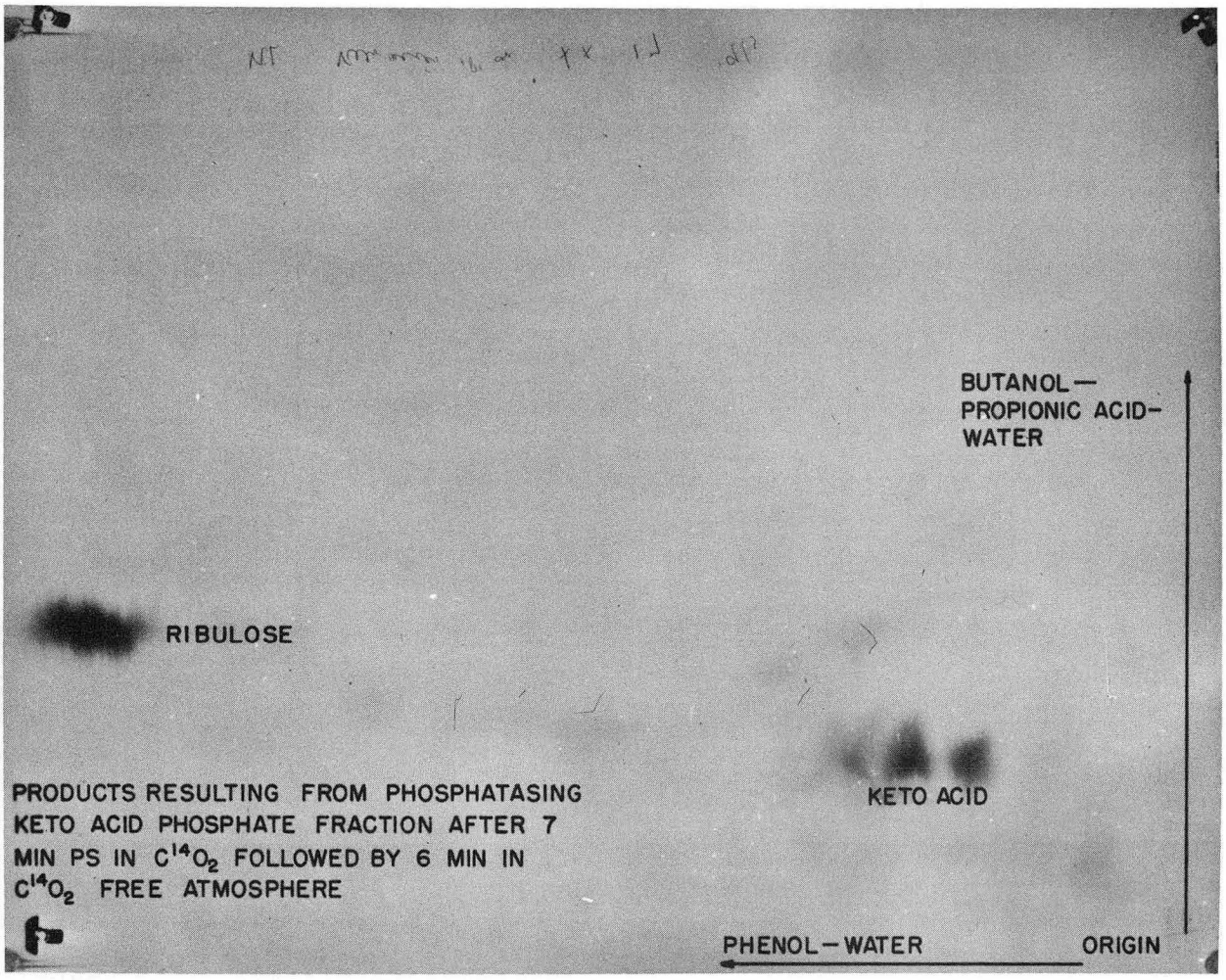
[1] V. Moses and M. Calvin, Proc. Natl. Acad. Sci. 44, No. 3, 260 (1958).

[2] J. A. Bassham, "Photosynthesis," in Encyclopedia of Plant Physiology, Vol. V (Springer-Verlag, Germany in preparation); an earlier form of the apparatus is described by Osmund Holm-Hansen, Paul Hayes, and Patricia Smith, Current Methods of Algal Culture, in Chemistry Division Quarterly Report, UCRL-3595, Oct. 1956, p. 56.



ZN-2258

Fig. 7. Products after 7 min PS in $C^{14}O_2$ and 6 min in $C^{14}O_2$-free atmosphere.



ZN-2257

Fig. 8. Products resulting from phosphatasing keto acid phosphate fraction after 7 min PS in $C^{14}O_2$ followed by 6 min in $C^{14}O_2$-free atmosphere.

Table II

Appearances of radioactivity in compounds of the diphosphate area as a function of time and CO_2 pressure

Time of PS with $C^{14}O_2$	Keto acid (cpm)	Ribulose obtained by phosphatasing keto acid diphosphate area (cpm)	Ribulose diphosphate (cpm)
45 sec	122	-	1746
1 min	224	-	2758
1 min 30 sec	450	64	3621
2 min	775	71	3135
3 min	872	161	3149
7 min	1239	517	2032
CO_2 diminished			
7 min 10 sec	1172	482	4748
7 min 25 sec	1245	539	5417
7 min 35 sec	988	546	616(?)
8 min	1246	586	6425
8 min 30 sec	1170	607	10782
9 min 35 sec	1272	762	7439
12 min 35 sec	634	1095	11698

FIXATION OF $C^{14}O_2$ BY FRAGMENTS PREPARED FROM SONICALLY RUPTURED CHLOROPLASTS

Roderic B. Park, Karel P. Louwrier, and Ning G. Pon

It was recently shown that sonically ruptured chloroplasts fix $C^{14}O_2$ as rapidly as, and very often more rapidly than, intact chloroplasts.[1] The enhancement of CO_2 fixation rate observed in sonically ruptured chloroplasts was interpreted as a permeability effect with respect to certain phosphorylated cofactors added externally to the enzyme system.

In further experiments on sonically ruptured chloroplasts, it was of interest to know how the functions of the photosynthetic process are distributed among the various particles obtained on sonic rupture. The sonically ruptured chloroplasts were separated centrifugally into their various components, and these components were studied with respect to their enzymatic, chemical, and physical properties. A flow sheet for the preparation of chloroplast fragments is given in Fig. 9.

If spinach chloroplasts are observed under oil inversion in the light microscope, very prominent dark areas, about 0.4 μ in diameter, are observed throughout the chloroplasts. These dark areas are thought to be due to high chlorophyll concentrations, and are called "grana." It might be expected that such portions of the chloroplasts would contain relatively large chlorophyll-to-nitrogen ratios. It was of interest to find whether we had prepared any fractions of unusually high chlorophyll-to-nitrogen ratio that might correspond to grana. Nitrogen content of the sample was determined by the Kjeldahl technique and the chlorophyll content was determined spectrophotometrically. The chlorophyll-to-nitrogen weight ratios of the various chloroplast fractions are given in Table III.

It is noted that whole chloroplasts have a chlorophyll-to-nitrogen ratio about one-half that of the precipitates, with the exception of the 145 K ppt (30 min). Apparently a large portion of the protein associated with whole chloroplasts is released as soluble protein when the chloroplast is osmotically or sonically ruptured. The released protein then appears in the 145 K supernatant, accounting for the low chlorophyll-to-nitrogen ratio in this fraction. The relatively low ratio of the 145 K precipitate has been shown by analytical ultracentrifuge data to be due to the precipitation of some soluble proteins at 145 K along with the small green fragments. The truly interesting feature of these numbers is that the chlorophyll-to-nitrogen ratios of different fractions are very similar. This result would not be expected if "grana" were being purified and isolated. Apparently, the chloroplast fragments we use are randomly broken pieces of the chloroplast lamellar structure. Another observation consistent with the notion that we are isolating randomly broken chloroplast fragments is that the concentration ratios of the visible pigments, as assigned spectrophotometrically, are similar for the various fractions.

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[1] R. B. Park, K. P. Louwrier, and N. G. Pon, in Bio-Organic Chemistry Quarterly, UCRL-8848, June 1959, p. 28.

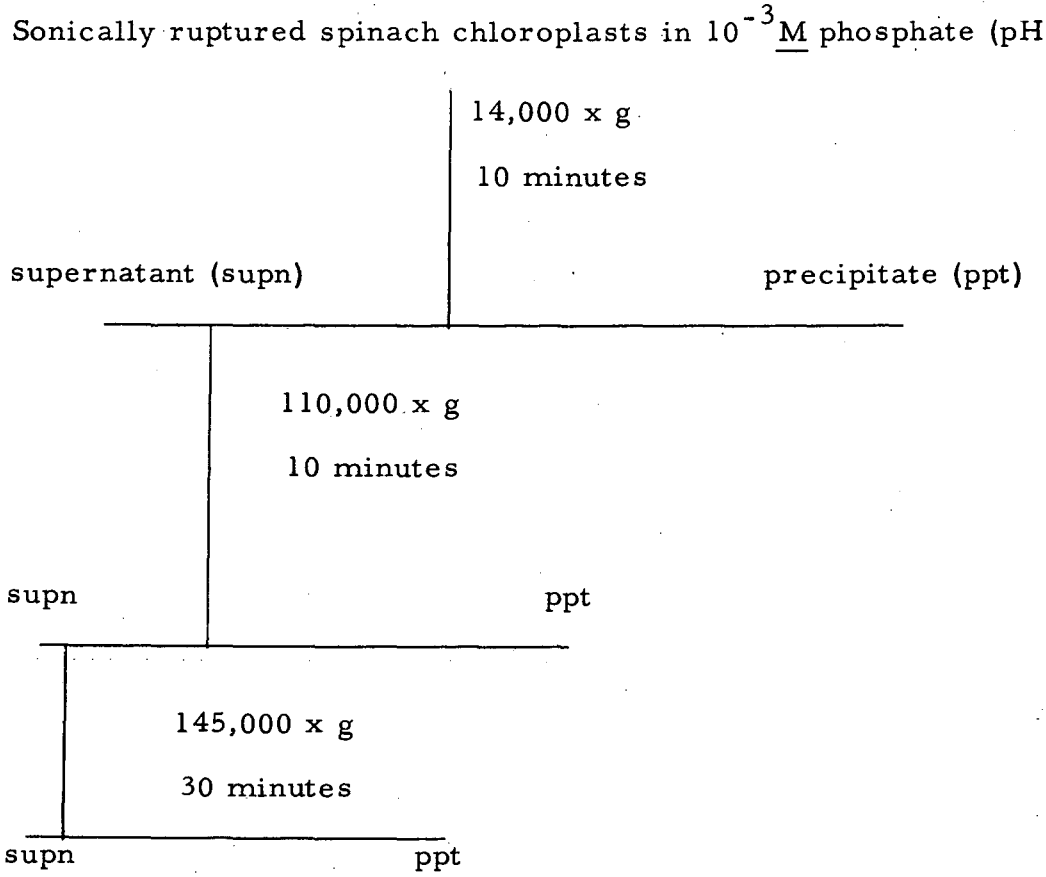

Fig. 9. Typical flow sheet for preparation of chloroplast fragments. All operations were performed at or near $0^{o}C$.

Table III

Chlorophyll-to-nitrogen weight ratio

Fraction	Chlorophyll (mg) / Nitrogen (mg)	Particle size (μ)
Leaves	0.32	
Whole chloroplasts	1.06	4 — 5
600 — 5 K ppt[a]	1.91	
5 K — 10 K ppt	1.80	
10 K — 20 K ppt	2.07	1 — 2
20 K — 40 K ppt	1.99	
40 K — 145 K ppt	1.82	
145 K ppt (30 min)	1.37	0.1
145 K supn	0.52	

[a]600 — 5 K ppt means the precipitate obtained between 600 g and 5000 g. The 145 K supn is the supernatant obtained after 30 minutes' centrifugation at 45,000 g.

Electron microscopy of metal-shadowed particles from the various fractions has further clarified the nature of the system with which we are working. In general, the particles precipitated between 600 and 145 K x g are sheets about 100 A in thickness, and of various diameters. The largest diameters observed are about 40,000 A, about the diameter of an intact chloroplast. The average diameters of the lamellar particles associated with various centrifugal fractions are given in Table III. The smallest particles obtained at 145 K are about 800 A in diameter. If one assumes that the particle dimensions observed in the electron micrograph are correct and that the partial specific volume of the protein is about 0.7, a knowledge of the chlorophyll-to-nitrogen ratio of the particle makes it possible to calculate roughly the number of chlorophyll molecules per particle. This number is about 50,000, and it is considerably larger than the photosynthetic unit of Emerson and Arnold ($\sim$2000 chlorophyll molecules per reducing center).[2,3] Electron microscopy of the supernatant from the 145 K precipitate shows predominantly spherical particles, the largest of which are 80 to 90 A in diameter. These proteins are also associated with the lamellar structures obtained at low g values.

$C^{14}O_2$ fixation by various fractions was studied in two ways. In one case, the fragments were resuspended in 1 ml 10^{-3} M phosphate (pH 7.3) and in the second case the fragments were resuspended in 1 ml of supernatant from the 145 K x g ppt that also contained 10^{-3} M phosphate at pH 7.3. The cofactors and $HC^{14}O_3^-$ were added to the system, which was then incubated for 30 min in the light or dark at 24°C.[1] The results from this experiment are given in Table IV. It is apparent that supernatant must be added to the chloroplast fragments to obtain high $C^{14}O_2$ fixation rates. The relatively high light-fixation rate of the 110 K – 145 K fraction in 10^{-3} M phosphate is apparently due to the precipitation of some of the supernatant protein along with the green particles at this high g value. Chromatograms of ethanol extracts from the 14 K – 110 K fraction and 145 K supernatant after incubation with $C^{14}O_2$ are given in Fig. 10. Mixture of the two fractions yields a much higher fixation rate. A chromatogram of the 14 K – 110 K particles plus 145 K supernatant after incubation with $C^{14}O_2$ is shown in Fig. 11. Though a small amount of reduction is carried out by the green fragments alone, it is evident that this effect is enormously enhanced by addition of supernatant to the reaction mixture.

It will be interesting to prepare still smaller chloroplast fragments in order to establish a minimum size for carrying out photosynthesis. We hope that study of the physical and chemical properties of such minimal-sized particles will further clarify our understanding of the architecture of the photosynthetic unit.

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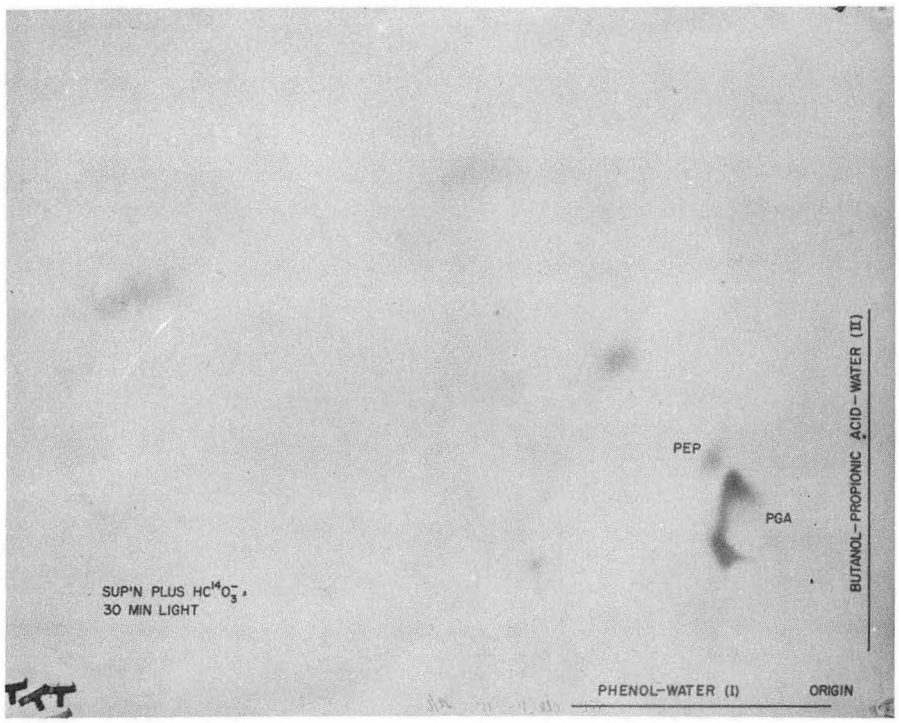
[2] R. Emerson and W. Arnold, J. Gen. Physiol. 15, 391 (1932).

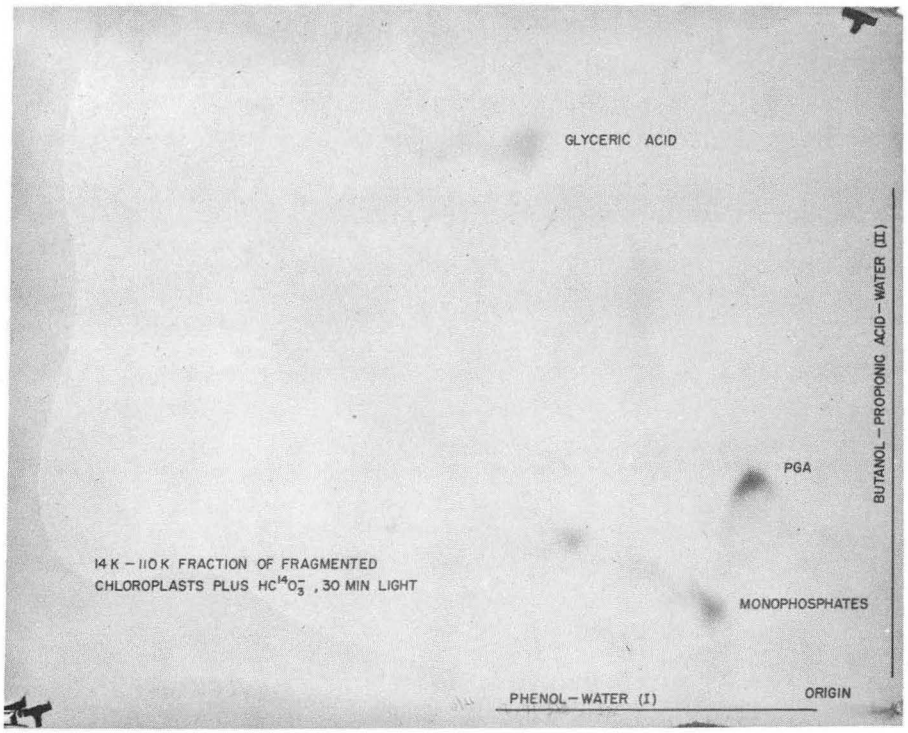
[3] R. Emerson and W. Arnold, ibid. 16, 191 (1932).

Table IV

$C^{14}O_2$ fixation by various fractions from sonically fragmented chloroplasts

Fraction	cpm/mg chlorophyll	
	Light	Dark
Total sonicate	2,100,000	40,000
0 — 14 K	76,000	9,100
0 — 14 K + supn	3,300,000	28,000
14 K — 110 K	18,000	4,200
14 K — 110 K + supn	3,000,000	22,000
110 K — 145 K	210,000	....
110 K — 145 K + supn	2,500,000	22,000
1 ml supn	30,000	53,000





ZN-2259

Fig. 10. Chromatograms of ethanol extracts of 14 K—110 K particles and 145 K supernatant after incubation with $HC^{14}O_3^-$ in light.

ZN-2256

Fig. 11. Chromatogram of an ethanol extract of 14 K—110 K particles plus 145 K supernatant after incubation with $HC^{14}O_3^-$ in light.

INHIBITION OF CO_2 FIXATION IN CHLORELLA PYRENOIDOSA BY 3-(p-CHLOROPHENYL)-1, 1-DIMETHYLUREA (CMU)

Karel P. Louwrier

CMU is known to inhibit both photosynthesis and the Hill reaction. The mechanism of this inhibition is not established, though the Hill-reaction experiments suggest that photosynthesis inhibition may be due to inhibition of photosynthetic phosphorylation or of the formation of reducing power. It was of interest to find in what way small concentrations of CMU affect the $C^{14}O_2$ fixation pattern in Chlorella. It might be expected that if CMU inhibits some aspect of the light reactions of photosynthesis (photophosphorylation and TPN reduction), the labeling pattern of CMU-inhibited Chlorella in the light should be similar to the normal dark-fixation pattern. Air containing 1% CO_2 was bubbled through 1 ml Chlorella suspension containing 2% wet packed cells for 15 min in light. One hundred µl of CMU solution, containing approximately 15 µg of inhibitor, was added to the algae. After a preincubation time, the mixture was flushed for 1 min with N_2. Then 6 µC C^{14} was added as $NaHC^{14}O_3$ and the cells were left in contact with the radioactive material for 3 min before they were killed by adding 4.4 ml of 100% ethanol. The cells were extracted as described earlier.[1] The different radioactive compounds were located by radioautography and their identifications were confirmed by co-chromatography (see Fig. 12). The radioactivity of the individual compounds and the results are recorded in Table V.

The radiocarbon found in various compounds indicates that the CMU enters the cell rapidly and that only the normal dark-fixation products are labeled in the presence of CMU, even in the light.

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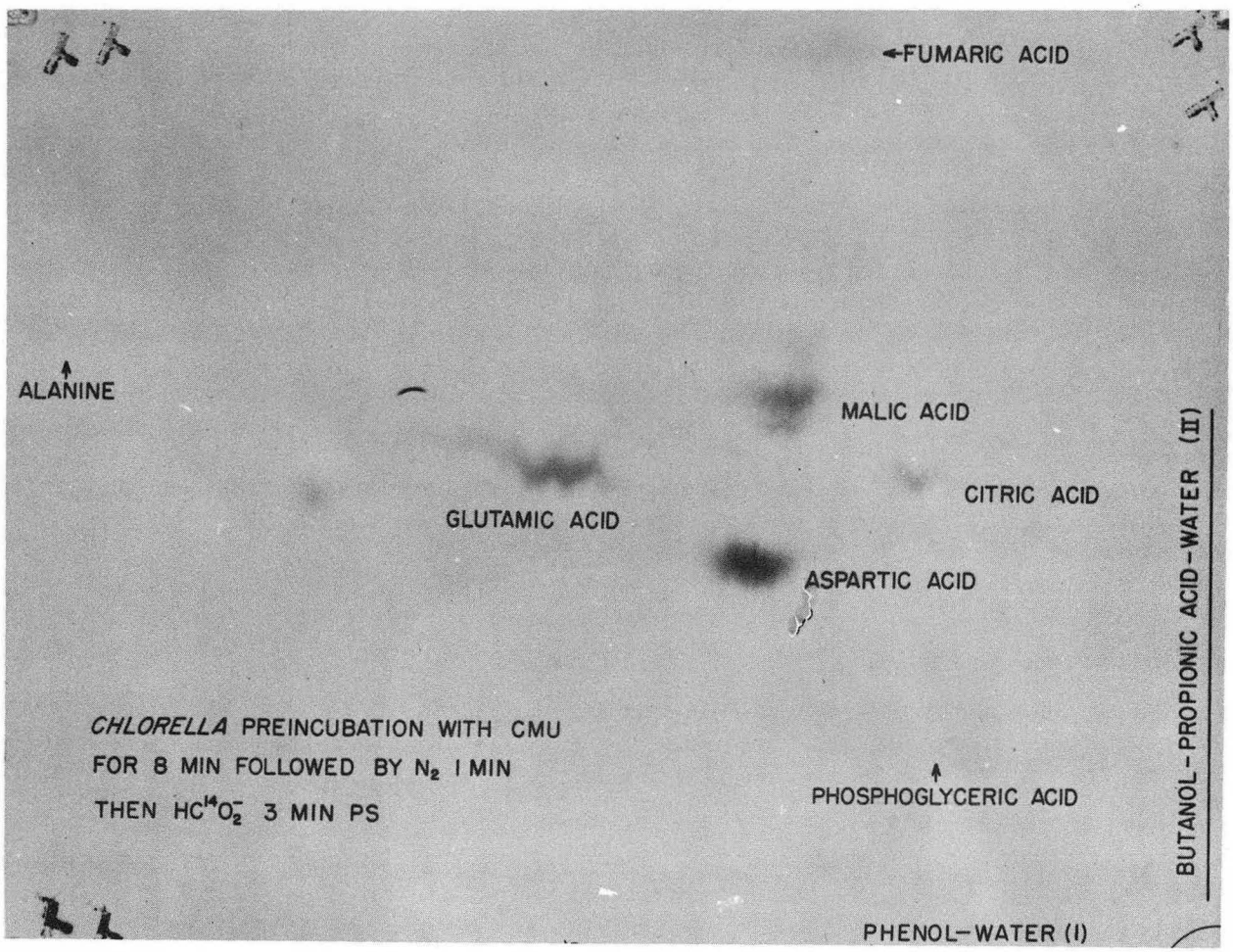
[1] Benson, Bassham, Calvin, Goodale, Haas, and Stepka, J. Am. Chem. Soc. 72, 1710 (1950).



ZN-2255

Fig. 12. Chlorella preincubation with CMU for 8 min followed by N_2 for 1 min, then $HC^{14}O_2^-$ 3 min PS.

Table V

Results: Radioactivity (cpm) in Various Compounds from CMU-inhibited Algae

Time of pre-incubation	PGA	Monophos-phates	Diphos-phates	Aspartic Acid	Citric Acid	Malic Acid	Fumaric Acid	Glutamic Acid	Alanine
64 min	-	-	-	858	40	288	40	280	-
32 min	40	-	-	1300	75	600	180	850	-
16 min	20	-	40	820	90	570	-	630	-
8 min	40	-	30	650	75	550	85	450	-
4 min	-	-	-	580	90	410	90	250	-
2 min	70	230	35	910	130	950	200	770	160
1 min	40	70	40	670	20	320	80	370	-
30 sec	120	120	110	600	40	590	80	250	60
15 sec	-	120	60	800	200	730	200	120	-
no CMU	8230	29700	37000	3640	270	8700	1900	1720	-

ATTEMPTED DEMONSTRATION OF FREE-RADICAL INTERMEDIATES IN REACTIONS CATALYZED BY PYRIDINOPROTEINS

H. R. Mahler[1] and L. Brand[1]

The over-all stoichiometry for the reversible oxidation-reductions catalyzed by pyridine-nucleotide-linked dehydrogenases may be represented as

$$SH_2 + PN^+ \xrightleftharpoons{\text{enzyme}} S + PNH + H^+ , \qquad (1)$$

where SH_2 is the reduced substrate, S is its oxidized form, PNH is the reduced pyridine nucleotide (DPNH or TPNH), and PN^+ is its oxidized form. This reaction involves the net transfer to two electrons plus a proton from a donor to an acceptor. A priori, then, the transfer step itself may be symbolized by one of the following three mechanisms:

$$\text{Donor} \longrightarrow \left[H^+ + 2\,e^-\right] \longrightarrow \text{acceptor}, \qquad (1A)$$

$$\text{Donor} \longrightarrow \left[H^\cdot + e^-\right] \longrightarrow \text{acceptor}, \qquad (1B)$$

$$\text{Donor} \longrightarrow \left[H:^-\right] \longrightarrow \text{acceptor}; \qquad (1C)$$

Equations (1A) and (1C) are essentially ionic mechanisms and entail the removal of a pair of electrons from the donor and its transfer to the acceptor simultaneously without a change in the multiplicity of either, while Eq. (1B) represents a free-radical path and would involve a change in multiplicity; a corollary would be the existence of half-reduced forms of the two compounds.

Presently available evidence would tend to rule out path (1A), at least as written (i. e., involving a free proton capable of exchange with those of the solvent) on the basis of the demonstration of direct hydrogen transfer between donor and acceptor.[2] Path (1C) is favored by the following lines of reasoning:[3]

(a) Chemical reduction of pyridine nucleotides, or of N-substituted nicotinamides in general, by reagents known to carry out this reaction by an ionic mechanism yield the 4-hydroderivatives either exclusively (dithionite) or in good yield (borohydride); reduction by free-radical reagents (x-irradiation of aqueous ethanol) or by free electrons (electrolytic reduction) gives rise to the 2- and (or) 6-hydroderivatives almost exclusively.

(b) The model experiment of Westheimer and his collaborators on the reduction of thioacetophenone by reduced N-benzyl-nicotinamide favor a hybrid ion mechanism.

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[1] Permanent address: Department of Chemistry, Indiana University, Bloomington, Indiana. We wish to express our appreciation to the personnel of the Bio-Organic Chemistry Group, especially to Dr. Power B. Sogo and Mrs. Lou Ann Carter for their courtesy and collaboration, and to Professor M. Calvin for making available to us the facilities of his laboratory.

[2] B. Vennesland and F. H. Westheimer in The Mechanism of Enzyme Action W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1954).

[3] For a brief review see H. R. Mahler, Ann. Rev. Biochem. 26, 17 (1957).

(c) The enzyme-catalyzed reaction resembles in many particulars the Meerwein-Ponndorf-Oppenauer equilibrium; the latter is believed to proceed by hydride ion transfer.

(d) No evidence for the existence of an unstable but kinetically distinct intermediate of any kind has been provided by careful studies in several different laboratories, especially of the reactions catalyzed by the two alcohol dehydrogenases from liver and yeast. For that, from liver, the only two intermediates detected are the complexes between enzyme and PN or PNH. For that from yeast, there is also evidence for the ternary complexes containing enzyme, PN, and SH_2; and enzyme, PNH, and S, respectively.

On the other hand, free-radical intermediates--i.e., path (1B)--have been postulated by Michaelis,[4] and preliminary evidence for their occurrence obtained by using electron spin resonance (ESR) techniques, has been provided by Commoner et al.,[5] at least for reactions catalyzed by yeast alcohol dehydrogenase and by glucose-6-phosphate dehydrogenase. We have studied in some detail the reaction mediated by two commercially available crystalline alcohol dehydrogenases (yeast and liver).

All reactions were carried out at room temperature in 0.1 M tris acetate buffer, at pH 8.0, and in both the forward and reverse directions. Among alcohol substrates tried were methanol, ethanol, n-propanol, ethylene glycol, and glycerol; among the aldehydes were acetaldehyde, propionaldehyde, and butyraldehyde. In general, 0.02 ml of substrate, 0.02 ml of pyridine nucleotide, and 0.02 ml of enzyme solution were tested singly and in combination in two ESR spectrometers for periods up to 1 hour after mixing. Concentrations employed varied from 0.01 M to M for the monohydric alcohols, up to 70% (by volume) for ethylene glycol and glycerol, from 10^{-3} M to 10^{-1} M for the coenzymes, and from 2 to 100 mg/ml for the enzymes. In none of these systems could an ESR signal be measured, even at the limit of sensitivity of the instruments. Control experiments indicated that 5×10^{11} unpaired electron spins would have been detected without ambiguity under the conditions employed.

A stable free radical was formed, however, in a chemical model reaction. When DPNH and riboflavin were mixed together in the solid state and a quantity of 70% ethanol, methanol, or glycol was added (just sufficient to hold the mixture in suspension) a green-colored material was formed in 5 min and the color was observed to intensify in the course of the next hour of standing at room temperature. Parallel experiments indicated a simultaneous increase in the ESR signal given by the mixture. In a larger-scale experiment approximately 100 mg of DPNH and 50 mg of riboflavin were dampened with 1 ml of 75% ethanol, allowed to stand for 1 hour at room temperature, and then dried *in vacuo* at the same temperature. The greenish-brown material so obtained gave a strong ESR signal which remained constant in intensity for 48 hours if the solid was kept at room temperature or up to 5 days at -20°. Comparison with DPPH[6] standards showed that 0.20 mg of

[4] L. Michaelis in The Enzymes, J. Sumner and H. Myrback, Eds., II-I (Academic Press, New York, 1951).

[5] Commoner, Lippincott, and Passonneau, Proc. Natl. Acad. Sci. U.S. 44, 1099 (1958).

[6] 1, 1-diphenyl-2-picrylhydrazyl

the solid corresponded to 10^{15} free radicals. The width of the absorption line (between points of maximum slope) was 2.6 gauss and the magnitude of the magnetic field at which resonance occurred was displaced 2.0 ± 0.5 gauss towards lower values than for DPPH. Suspension in a minimal volume of organic solvents (methanol, ethanol, chloroform, carbon tetrachloride, or acetone) or in water did not appreciably change these parameters, nor did cooling to liquid nitrogen temperatures lead to either an appreciable change in the line width or to the appearance of hyperfine structure. Treatment with hydroxylic solvents did, however, markedly influence the stability of the free radical; the half time for destruction was 30 min at room temperature in water, and 5 min in absolute methanol at 65° led to 99% destruction.

On the other hand, if the original preparation was carried out as before, but the last traces of solvent removed by brief exposure to a temperature of 60 to 65°, a reddish-brown material was obtained that exhibited an ESR signal of different characteristics. This showed a line width of 2.9 gauss and occurred at a field 13.0 ± 0.5 gauss lower than that of DPPH. The free radical responsible was more unstable; no detectable signal was obtained after 12 hours at 0° in the solid state.

EFFECTS OF D_2O ON DROSOPHILA

Ann M. Hughes

In a continuation of our studies of the effects of D_2O on Drosphila,[1] we have made experiments to determine the influence of D_2O on nondisjunction.

To determine the effect of D_2O on chromosome loss in the male, males carrying the gene for yellow on the X-chromosome and the wild-type allele for yellow attached to the Y-chromosome were grown on normal or 20% D_2O media. These males were mated with homozygous yellow females. The expected progeny would be yellow females and nonyellow males. Nondisjunction should result in approximately equal numbers of nonyellow females and yellow males. Loss of the Y-chromosome should result in increased numbers of yellow males only. In an examination of 12,782 progeny from treated males and 10,707 progeny from control males, there appeared to be no increase in the occurrence of nondisjunction. However, the percentage of flies showing loss of at least the sc^8 part of the Y-chromosome was increased from 0.04% in the controls to 0.17% in the treated, an increase that is statistically significant at less than the 1% level.

For a similar study in females, females carrying genes for y and f on one X-chromosome and genes for $yBvw^a$ on the other X-chromosome were grown on normal or 20% D_2O media. They were mated with males carrying genes for ycvvf on the X-chromosome and the wild-type allele for y attached to the Y-chromosome. In an examination of 11,378 progeny from control females and 12,743 progeny from treated females, the number of progeny resulting from nondisjunction increased from 0.3% in the controls to 0.6% in the experimentals, an increase that is statistically significant at the 1% level.

Throughout our experimental work we have consistently observed increased sterility in flies treated with D_2O, and decreased number of progeny from treated flies. Direct comparison of such effects in males and females of the same strain cannot be made in most cases, since different strains were treated in the various experiments. Therefore, one experiment was designed specifically to compare the effects of D_2O in the two sexes. Canton wild-type stock was grown on normal or 20% D_2O media. From these cultures, nontreated females were mated to nontreated males, treated females were mated to nontreated males, treated males were mated to nontreated females, and treated females were mated to treated males. Results of the sterility experiment are shown in Fig. 13. The decreased number of progeny is shown in Figs. 14 and 15. It appears from these results that in Canton flies, at least, the female is more sensitive to the effects of D_2O than the male, in regard both to sterility and to decreased number of progeny.

[1] Ann M. Hughes, in Bio-Organic Chemistry Quarterly Report, UCRL-8698, March 1959, p. 11.

STERILITY PRODUCED IN CANTON STOCK BY D_2O

	CONTROL	20% D_2O ♂♂	20% D_2O ♀♀	20% D_2O ♀♀ & ♂♂
Total Matings	51	49	56	46
% Sterile Matings	6	29	55	59

MU-18005

Fig. 13. Sterility produced in Canton stock by D_2O.

EFFECT OF D_2O ON NUMBER OF PROGENY

IN CANTON STOCK

	♀ & ♂ per Bottle	% Decrease from Normal	♀ per Bottle	% Decrease from Normal	♂ per Bottle	% Decrease from Normal
CONTROL	27	—	14	—	13	—
20% D_2O ♂ ♂	27	0	15	7 (increase)	12	8
20% D_2O ♀ ♀	7	74	3	79	4	69
20% D_2O ♀♀ & ♂♂	6	78	3	79	3	77

MU-18006

Fig. 14. Effect of D_2O on number of progeny in Canton stock.

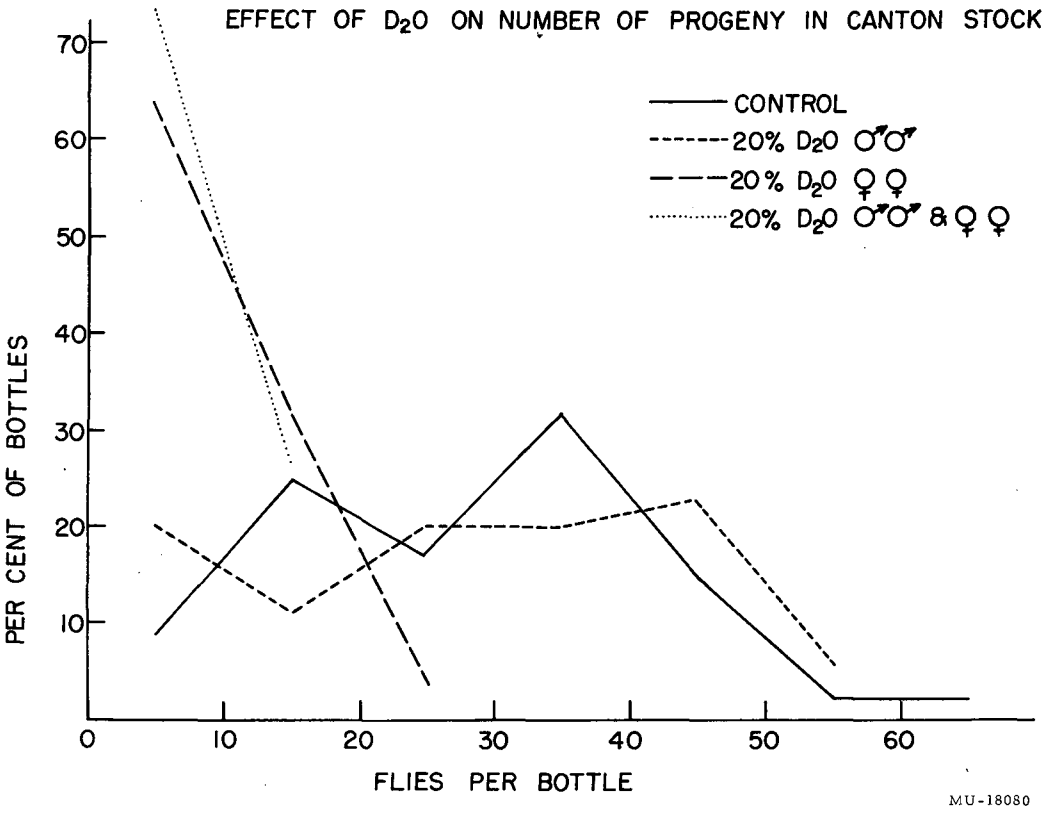

Fig. 15. Effect of D_2O on number of progeny in Canton stock.

THE EFFECT OF ORINASE ON RADIATION SICKNESS

Paul Weaver and Ann M. Hughes

It has recently been suggested[1] that Orinase (1-butyl-3-p-tolysulfonylurea) may be useful in the treatment of radiation sickness. Consequently, a study has been made of the effect of this compound on radiation sickness in NAMRU Swiss strain mice.

Eighty mice were irradiated in two groups of 40. The mice were placed in individual cages on a slowly rotating wheel so that each animal received 636 roentgens (10.6 r/min for 1 hr) of x-radiation. A 0.5-mm Cu filter and a 1.0-mm Al filter were used for the x-ray tube operating at 250 kv and 15 ma. Half of each irradiation group (i.e., 20 animals each, or a total of 40) was used as a control, the remainder being the experimental animals.

The Orinase solution was prepared by dissolving the crystals in a 1 N solution of NaOH and then restoring the pH to as close to 7 as possible without the Orinase's recrystallizing. The solution was then diluted with distilled water so that the concentration of Orinase was 2 mg/50 λ. Two mg of Orinase was administered daily by a subcutaneous injection. The controls were similarly injected with an equal amount of saline solution (0.9% NaCl by weight in H_2O). All mice were weighed approximately every other day.

The data, summarized in Figs. 16 and 17, suggest that Orinase does not counter the biological effects of radiation in this strain. Clearly, there is no significant difference between the average percent of weight change in the experimental and in the control groups. The higher mortality of the mice treated with Orinase may be attributable to the basic pH of the Orinase solution, which caused the experimental mice to suffer long-lasting skin ulcers.

In conclusion, it has been found that Orinase has no beneficial effect on radiation sickness in NAMRU Swiss strain mice.

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[1] Dr. James D. Hardy, and Dr. B. D. Polis (U.S. Naval Air Development Center, Johnsville, Pennsylvania), private communication.

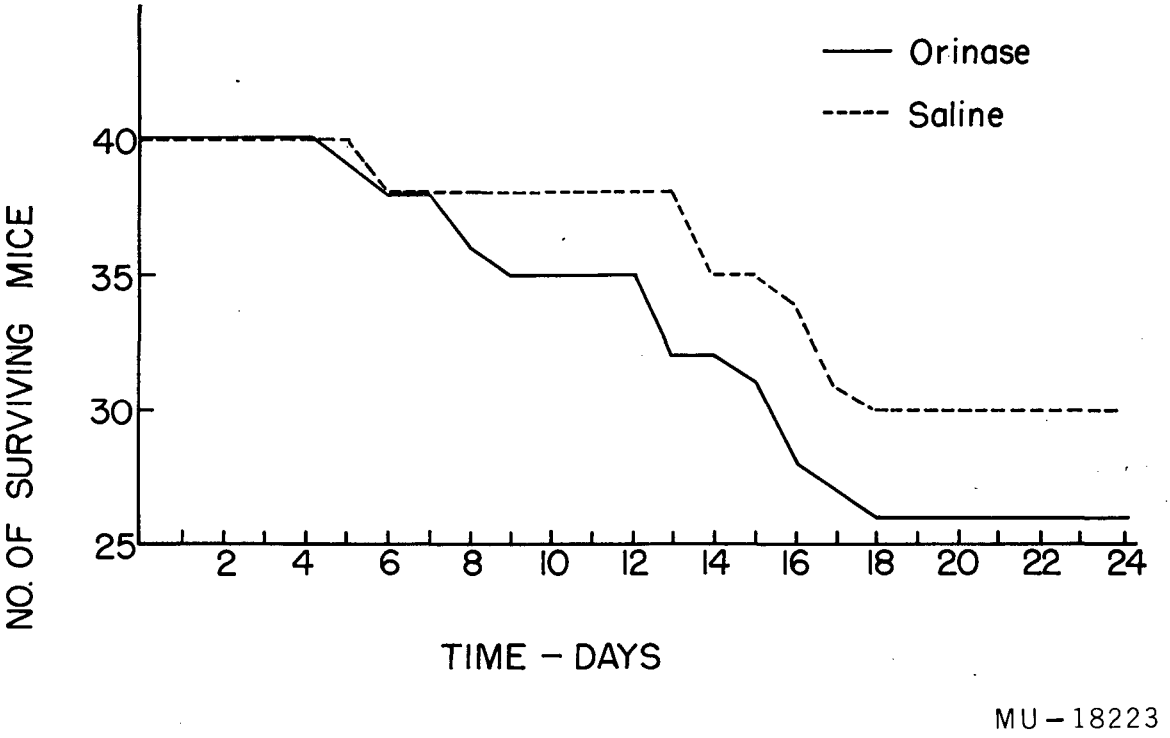


Fig. 16. Effect of Orinase on the mortality of irradiated mice.

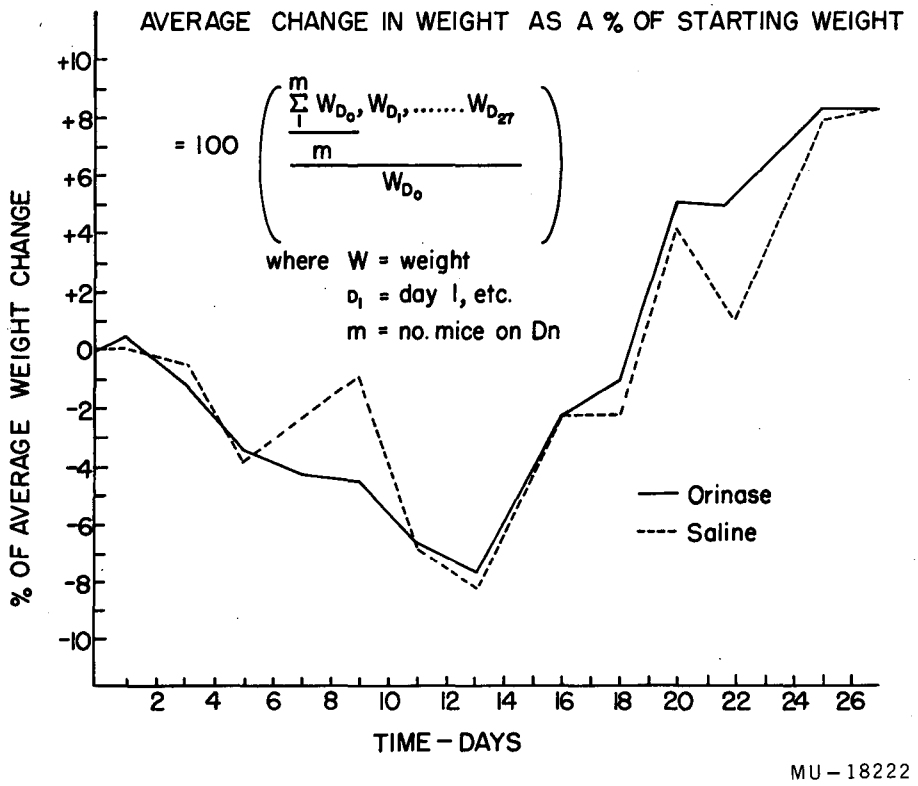


Fig. 17. Effect of Orinase on the weight of irradiated mice.

PRELIMINARY INVESTIGATION OF METEORITE "MURRAY"

Susan Vaughn

As a contribution to the problem of extraterrestrial life, an analysis of a carbonaceous meteorite was undertaken for the purpose of identifying organic matter within it. Although the surface of a meteorite becomes quite hot during its fall through the earth's atmosphere, it is well substantiated that the inside remains relatively cool. Thus, organic matter present would not necessarily be destroyed during the meteorite's earthbound flight. The meteorite under investigation was an observed fall on September 20, 1950 at 1:35 a.m. in Murray, Kentucky--hence its name. The entire meteorite weighed 7 kilograms, approximately 50 g of which was sent to this Laboratory from the Smithsonian Institution. It is classified as a carbonaceous chondrite, and is somewhat unusual because of its abnormally high percentage of carbon.

Although the main purpose of the analysis has been to determine the nature of the organic matter in the meteorite, some investigation of the inorganic material has also been undertaken. The results of the elemental analyses are presented in Table VI.

An x-ray diffraction pattern of the meteorite indicated much olivine (Fe, Mg-silicate) and serpentine ($3\ MgO \cdot 2\ SiO_2 \cdot 2\ H_2O$), with minor amounts of anorthite ($Ca\ [Al_2Si_2O_8]$) and perhaps a pyroxene close to hypersthene ($[Mg, Fe]\ SiO_3$). ESR data showed a band undergoing the Knight shift due to water of crystallization, probably that of the serpentine. The isotope ratio C^{13}/C^{12} was determined for samples of carbon dioxide from the meteorite and from terrestrial material. There seemed to be no significant difference in the ratios. The literature indicates that the isotope ratio of meteoritic carbon should lie intermediate between inorganic and organic terrestrial carbon.[1]

In order to separate any organic matter present from the inorganic, a series of solvent extractions was carried out. Chunks of the black meteorite were first powdered in a dry box with mortar and pestle. The extractions were made with freshly purified solvents. Great precaution was taken to keep the system free from organic contamination. A 9-g sample of the meteorite was successively extracted with CCl_4, $CHCl_3$, benzene, H_2O, ethanol, and benzene. Each extraction was made by refluxing the meteorite power with four 150-ml portions of a solvent for several hours. The meteorite sample was thoroughly dried before solvents were changed. The amount of material removed by each of the solvents was

[1] K. Rankama and Th. G. Sahama, Geochemistry (University of Chicago Press, Chicago, Ill., 1950), p. 548.

Table VI

Elemental analysis of meteorite "Murray"

Element or group	% of total meteorite	Element	% of total meteorite*
C	2.07	Al	1
H	0.90	B	0.3
N	0.08	Ca	0.5
S	0.375	Cr	0.5
P	0.02	Cu	0.3
Soluble $SO_4^{=}$	1.03	Mn	0.5
$SO_4^{=}$ from combustion residue	5.72	Ni	1
Combustion residue	92.7		
Fe	24.0		
Mg	10.1		
Silicate	15.8		

*These values are accurate only within a factor of two

Solvent	Wt. removed (mg)
CCl_4	20.1
$CHCl_3$	3.3
benzene	3.9
water	541.1
ethanol	9.3
benzene	0.0

These solvent extractions removed approximately 35% of the total carbon.

Wherever possible, the ultraviolet, visible, and infrared spectra of the extracts were taken. The spectra in the visible region showed no peaks. Infrared spectra were taken of the CCl_4, $CHCl_3$, and benzene extracts. The strongest bands were observed at 2900 wave numbers, indicating general aliphatic carbon-hydrogen bonds, and around 1400 wave numbers, indicating CH_2 and CH_3 groups. There was some indication of possible carbonyl, OH, and (or) NH groups around 1700 wave numbers. The ultraviolet spectra showed strong end absorption with suggestions of peaks (Figs. 18-22). The extinction coefficient of each at 260 mμ, based on g/ℓ concentration, is of the order of 1 to 10. The ninhydrin spray test for amino acids and the molybdate test for organic phosphate were attempted on small portions of the water extract. The results of both were, at best, doubtful.

The CCl_4, $CHCl_3$, benzene, and ethanol extracts were taken to the Shell Development Company for a mass-spectrometer analysis. The spectrum of the CCl_4 extract contained the greatest amount of material that could be volatized. Its major constituents were hydrocarbon fragments (aliphatic rather than aromatic), with a higher concentration of unsaturated groups than of alkyl groups. The molecular weights of the fragments were widespread and large ($>$ 200). The most intense peak of the spectrum occurred at $m/q=28$; this is accounted for by either N_2^+ or CO^+, probably the latter. This ion must have been formed by pyrolytic decomposition of certain molecules in the extract. The other three extracts exhibited even more unsaturation in the hydrocarbon fragments, and the molecular weights were considerably lower. The fragment NO appeared as a pyrolysis product from the ethanol extract.

Aromaticity having been discounted by the infrared and mass-spectra data, something else must be present to account for the high ultraviolet absorption. One supposition is that the material might be heterocyclic compounds such as purines or pyrimidines. To test this possibility the extracts were submitted to a systematic change of pH from neutral to acidic and basic to see if the peaks would shift their positions. HCl gas and ammonia were bubbled through the extract samples in spectrophotometer cells and the spectra were recorded immediately thereafter. The peaks did not shift in wave length, but they did in intensity. The spectra of the CCl_4 extract are examples (Figs. 23 and 24).

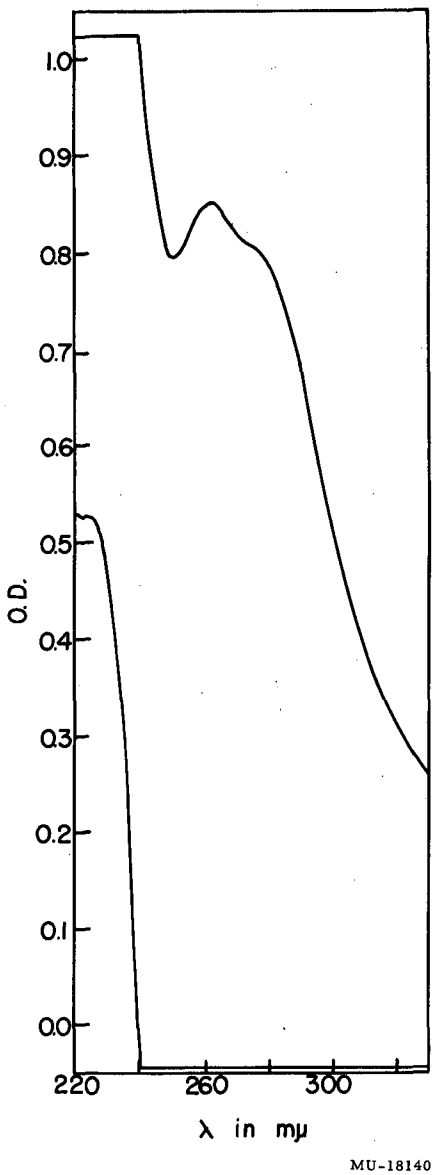


Fig. 18. Spectrum of carbon tetrachloride extract of meteorite.

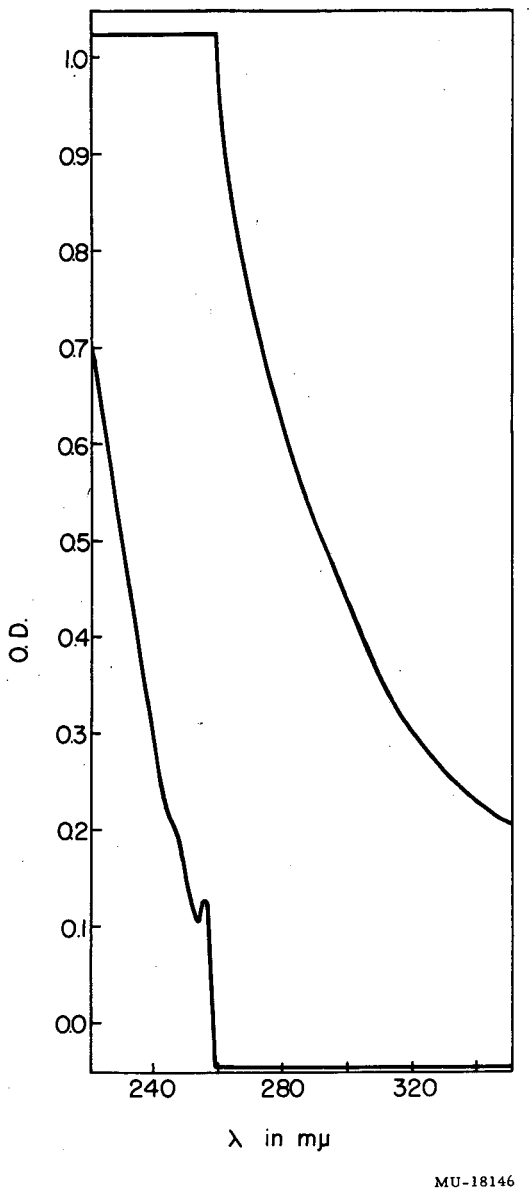


Fig. 19. Spectrum of chloroform extract of meteorite.

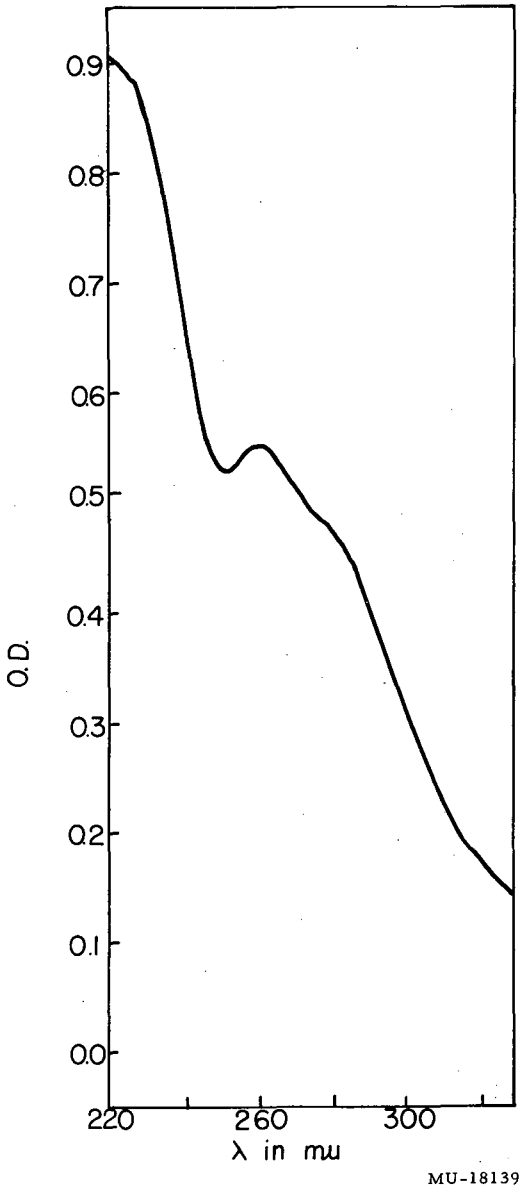
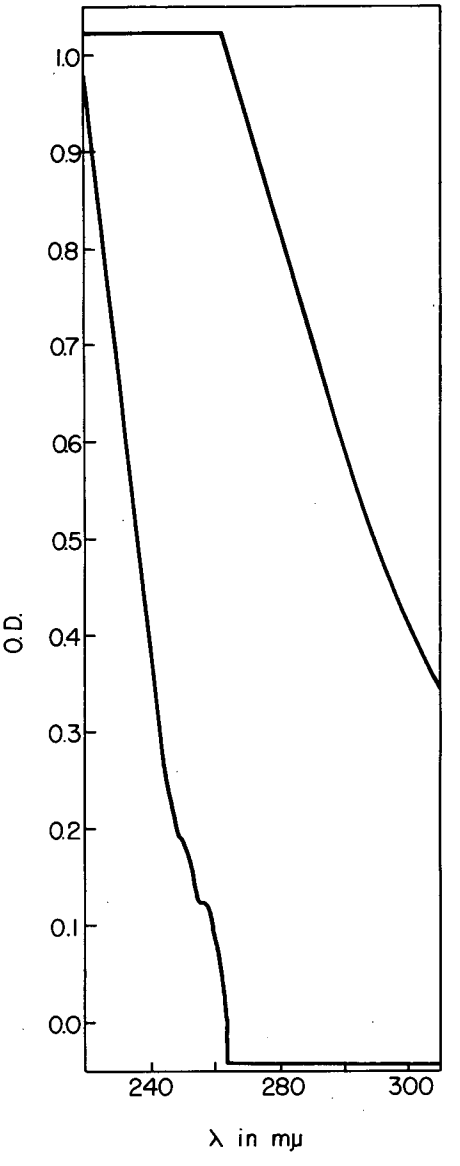


Fig. 20. Spectrum of benzene extract of meteorite.



MU-18144

Fig. 21. Spectrum of ethanol extract of meteorite.

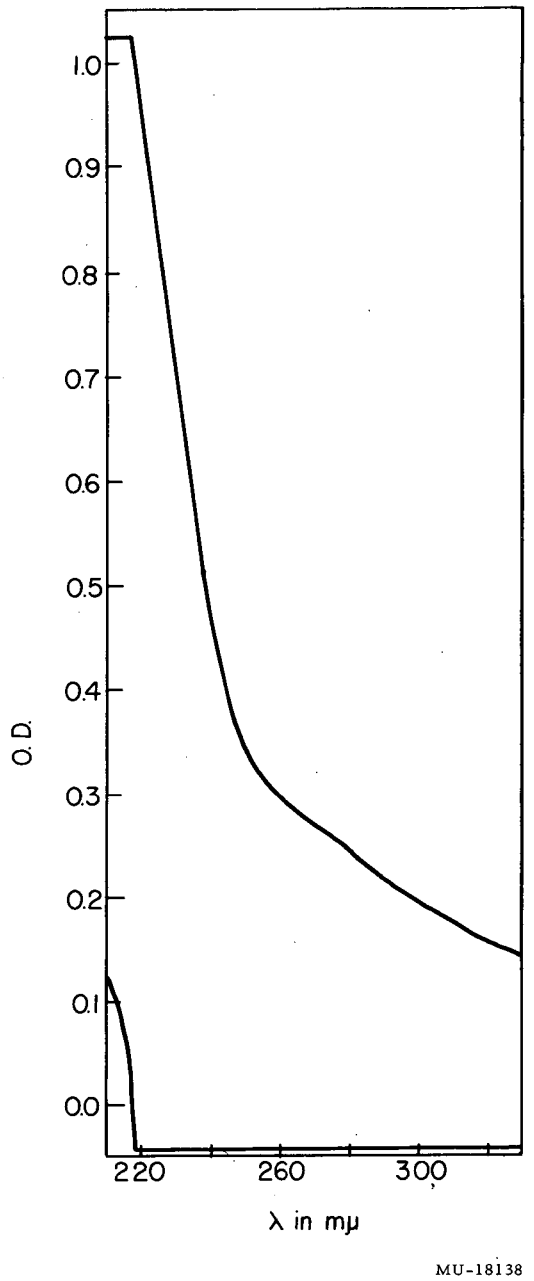


Fig. 22. Spectrum of water extract of meteorite.

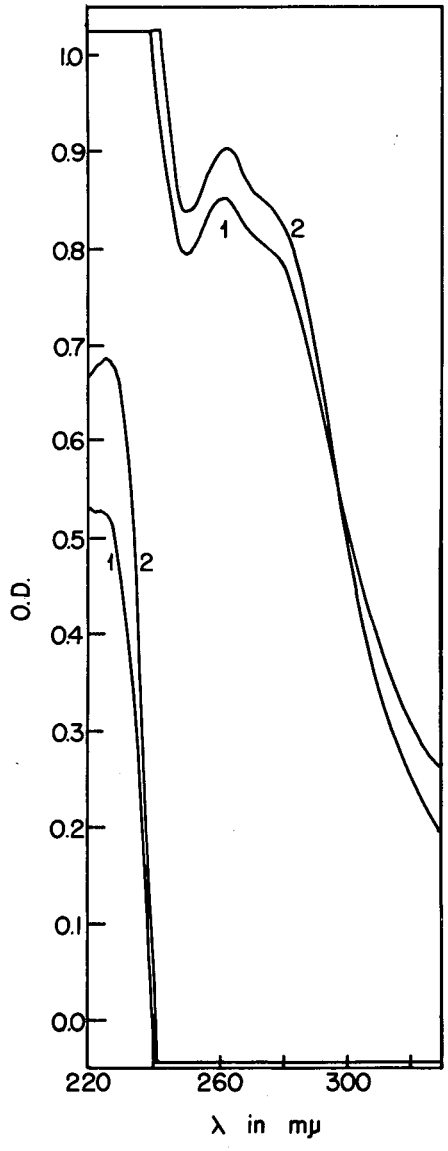
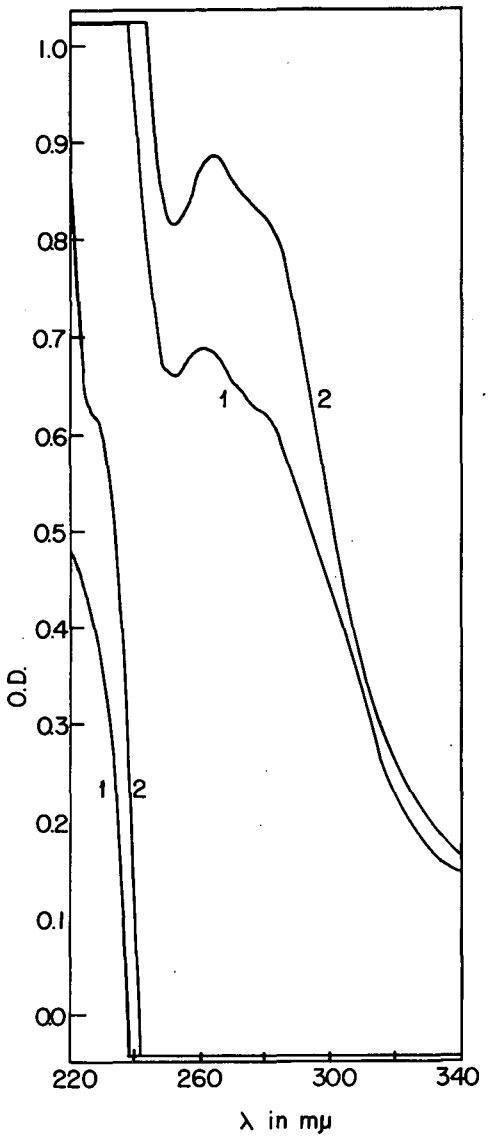


Fig. 23. Spectrum of CCl_4 extract of meteorite (1); extract treated with HCl gas (2).



MU-18143

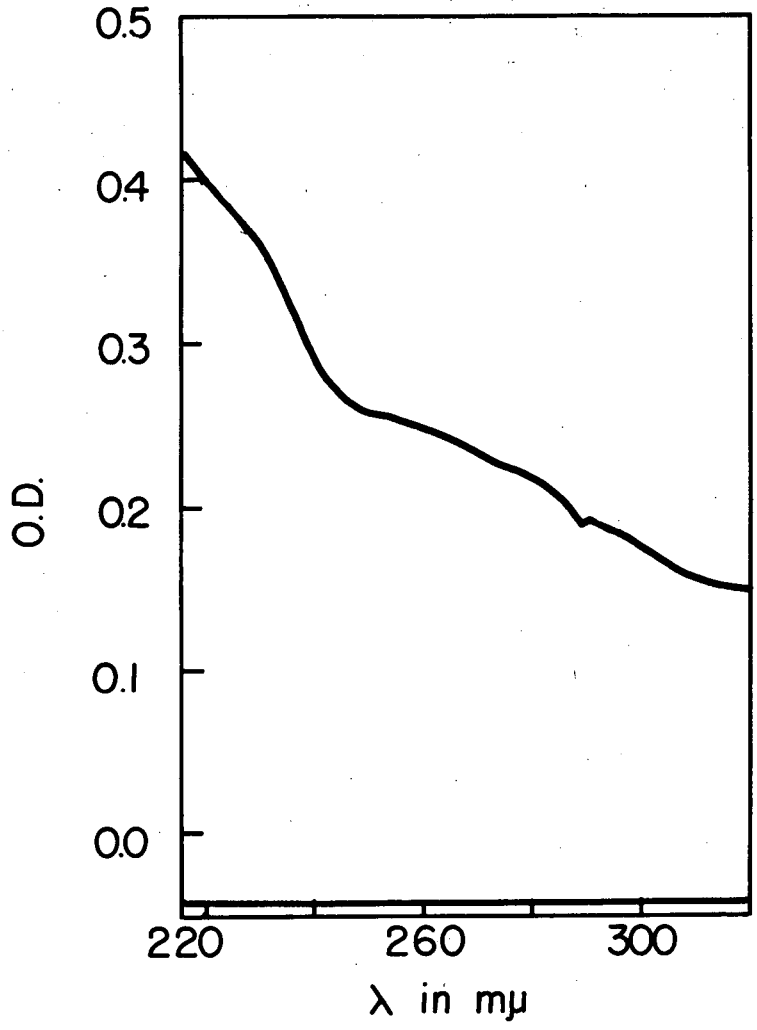
Fig. 24. Spectrum of CCl_4 extract of meteorite (1); extract treated with ammonia (2).

Attempts have been made to separate the heterogeneous material in the extracts into purer components and to isolate material that would give distinguishable peaks in the ultraviolet. This work was carried out with portions of the water extract since there was a plentiful supply of it. According to a carbon-hydrogen analysis, this extract contained 8% carbon; therefore, the first problem would be to separate the carbonaceous material from the salt. To this end, 7.0 mg of the water extract was put in a molecular still. The system was evacuated and the material heated to 300°C. Volatile organic matter was collected on a cold finger, cooled with liquid nitrogen. Methylcyclohexane dissolved 0.8 mg of material off the cold finger. The ultraviolet spectrum of this material showed a flattening out in the region 250 to 270 mμ, but no peak was found (Fig. 25 in contrast to Fig. 22).

A 2-ml portion of the water extract, having an optical density at 260 mμ of 0.92, was shaken with an equal amount of chloroform. The chloroform extracted 33% of the absorption. However, the spectrum of the water extract after the chloroform treatment retained the same shape as it had before the $CHCl_3$ extraction. Moreover, the spectrum of the material extracted by the chloroform was the same as that remaining in the water; i.e., no peak had been extracted. An attempt to fractionate this extract on an ion-exchange column is now under way.

The mass spectra of the extracts most closely resemble, in their general lack of structure, the spectrum of kerogen from oil shale. Therefore, a sample of this oil shale was obtained from the Shell Development Company so that experimental techniques could be practiced on it. A portion of the shale was refluxed for several hours with CCl_4 and the ultraviolet spectrum of the extract was taken. The spectrum had even less of a suggestion of peaks than the spectra of the meteorite extracts; it seemed to be all end absorption. However, the extinction coefficient, expressed in g/ℓ, was of the same magnitude as for the meteorite extracts. A 5-g sample of the oil shale was refluxed with 6 N HCl in order to dissolve the rock and leave the organic matter. After this treatment 97% of the carbon was left in the residue not dissolved by the HCl. However, after combustion of this residue for a carbon-hydrogen determination, 50% of the residue was left, indicating that not all the rock had been dissolved. a 2-g sample from the residue of the HCl digestion was digested further with 48% HF. The solid material left retained 86% carbon. After combustion for carbon-hydrogen analysis there was no residue left, therefore, all the inorganic material had been dissolved; 84% of the carbon in the original oil shale sample was retained through the two digestions.

In summary, the analysis of the organic material in the meteorite indicates the presence of hydrocarbon fragments. There are suggestions of other organic compounds (possibly heterocycles); however, no definite proof of them is provided at this time.



MU-18142

Fig. 25. Spectrum of volatizable material from water extract of meteorite collected on cold finger of molecular still.

We are especially grateful to Dr. E. H. Henderson of the Smithsonian Institution, Washington, D. C., for supplying us with the meteorite and to Dr. D. P. Stevenson of the Shell Development Company, Emeryville, California, for his interest and assistance with the mass-spectrometer analysis. We also wish to thank the following people from the University of California for their kind assistance: Mrs. Juana Acrivos for the ESR Data, Dr. A. Pabst and Mrs. Helena Ruben for the x-ray diffraction pattern, Dr. John Conway for the analysis of the metal elements, Dr. Eugene Huffman for the iron, magnesium, and silicate determinations, and Dr. N. Keith Freeman for the infrared spectra.

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