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BIO-ORGANIC CHEMISTRY QUARTERLY REPORT
June, July, and August 1960

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BIO-ORGANIC CHEMISTRY QUARTERLY REPORT*
June, July, and August 1960

M. Calvin, Director

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

September 22, 1960

FURTHER STUDIES ON THE GENETIC EFFECTS OF D_2O IN DROSOPHILA

Ann M. Hughes

In a previous Quarterly Report[1] it was suggested that the nondisjunction in Drosophila which had been attributed to D_2O might, in part, be caused by the relatively high tritium content of some of the D_2O stock solutions. We have now carried out controlled experiments to investigate this possibility.

The same male stock of Drosophila as was previously used[1] (y/sc^8Y) was grown on four types of media:

Control	(C)	made with H_2O
Deuterium	(D)	made in the same manner as the C, but containing 19% D_2O (by weight),
Tritium	(T)	made in the same manner as the C except that the tritium was added to give an activity of 0.1 μC H^3/g,
Deuterium-tritium	(DT)	made in the same manner as the D, except that tritium was added to give an activity of 0.1 μC H^3/g.

Male flies harvested from these four types of media were individually mated with y/y females grown on normal media. Their progeny were counted and the number of unusual flies (resulting from male nondisjunction) was noted.

The entire experiment was then repeated in exactly the same manner. The results of the two experiments are shown in Tables I and II.

Since the number of unusual females does not vary significantly between the different groups, and since we have some evidence[2] that the phenomenon we are observing is breakage and loss of the Y chromosome, rather than true nondisjunction, we have considered in our combined table (Table III) and in the statistical analysis only the unusual males observed.

*This work was done under the auspices of the U. S. Atomic Energy Commission.

[1] Ann. M. Hughes, in Bio-Organic Chemistry Quarterly Report, UCRL-9208, June 1960, p. 123.

[2] Ann M. Hughes, in Bio-Organic Chemistry Quarterly Report, UCRL-9135, March 1960, p. 58.

Table I

Effect of D_2O on the occurrence of unusual *Drosophila* in the first experiment

	Normal females	Normal males	Unusual females	Unusual males
C	8223	7872	7	15[a]
D	6911	6935	11	16
T	7366	7081	1	7
DT	6872	6804	7	31

[a]Does not include a cluster of 6 found in one creamer.

Table II

Effect of D_2O on the occurrence of unusual progency in *Drosophila* in the second experiment

	Normal females	Normal males	Unusual females	Unusual males
C	8524	8493	4	4
D	7801	7773	6	21
T	9422	9201	1[a]	16[b]
DT	8967	8832	9	41

[a]Does not include a cluster of 5 found in 1 creamer.

[b]Does not include a cluster of 7 found in 1 creamer.

Table III

	Control			Deuterium			Tritium			Deuterium-tritium		
Experiment no.	Total flies	Total unusual males	Unusual males per 1000	Total flies	Total unusual males	Unusual males per 1000	Total flies	Total unusual males	Unusual males per 1000	Total flies	Total unusaal males	Unusual males per 1000
1	16,123	15	0.93	13,873	16	1.2	14,455	7	0.48	13,714	31	2.3
2	17,052	4	0.24	15,601	21	1.3	18,652	16	0.89	17,849	41	2.3

A statistical analysis (χ^2) shows that in the first experiment the difference in occurrence of unusual males in the DT and C groups is significant, while the D or T groups do not differ significantly from the C. We cannot presently explain why the occurrence of unusual males was so high in the control group. This occurrence is not consistent with the normal occurrence of nondisjunction or chromosome loss.

In the second experiment, the occurrence of unusual males in each of the experimental groups (D, T, and DT) is significantly different from that in the C group. Also, the greater occurrence of unusual males in the DT groups in either experiment than in the D or T groups is significant.

Thus it seems probable that both deuterium and tritium are influencing the rate of male nondisjunction or chromosome loss--but whether their effects are synergistic or merely additive cannot be determined at this time. Further work is in progress.

THE CHEMICAL EFFECTS OF IONIZING RADIATION ON NUCLEOTIDES

Cyril Ponnamperuma, Richard M. Lemmon, and Fritz H. Woeller

In a previous report some preliminary experiments on the chemical effects of ionizing radiation on nucleotides were described.[1] The radiation decomposition of uridylic acid in the solid state and in aqueous solution was studied by using the uniformly C^{14}-labeled compound. The distribution of products after irradiation was determined with the aid of paper chromatography.

Further investigations into this subject have involved:

a. The calculation of radiation dosage delivered to the experimental material,

b. The investigation of suitable solvent systems for chromatographic separation of irradiated nucleotides,

c. Analysis for sugars and sugar phosphates,

d. A comparative study of the ultraviolet absorption spectrum of irradiated nucleotides.

Calculation of Radiation Dosage

For the irradiations discussed in this report, the maximum dose was obtained with the Cobalt-60 γ-ray source at a setting of 1.1 in. The dose rate in this position was calculated with the aid of the Fricke ferrous sulfate dosimeter. An irradiated solution of ferrous sulfate was analyzed for ferric ion concentration by direct reading of the absorbency at a wave length of 305 mμ. The dose rate was found to be 6.2×10^6 rads per hour. The two values given in the earlier report[1] for the Co^{60} γ-ray doses have been found to be high by a factor of about five. This was due to an error in calculation.

Investigation of Solvent Systems

For the separation of the products of the irradiated nucleotides by paper chromatography, the following solvent systems were investigated:

a. Isopropanol-HCl [2]

b. Isobutyric acid-NH_3 [3]

c. Isobutyric acid-NH_3-EDTA [4]

---

[1] Cyril Ponnamperuma, R. M. Lemmon, and E. L. Bennett, in Bio-Organic Chemistry Quarterly Report, UCRL-9135, March 1960, p. 27.

[2] J. Smith and R. Markham, Biochem. J. 46, 509 (1950).

[3] M. Lofgran, Acta Chem. Scand. 6, 1030 (1952).

[4] H. Krebs and R. Hems, Biochim. et Biophys. Acta 12, 173 (1953).

d. Methanol-NH_3 [5]

e. Pyridine-H_2O [6]

f. Butanol-urea [7]

g. Butanol-propionic acid [8]

h. Phenol-water [8]

i. Butanol [9]

j. Water (pH 10) [10]

A "trailing" effect on the chromatograms was observed when the acidic solvents were used. In the basic solvent systems, all the material (both unchanged and radiation-altered) traveled with the solvent front. The neutral solvents did not move the nucleotides from the origin. The best results were obtained with two-dimensional chromatograms using isobutyric acid-NH_3-EDTA in one direction, (which separated the purine and pyrimidine derivatives) and butanol-propionic acid in the other (which separated the other breakdown products).

Whatman No. 4 paper, washed in oxalic acid, was used for all separations.

Analysis for Sugars and Sugar Phosphates

The lack of uv absorption in the 0.1% uridylic acid solution irradiated with Co^{60} γ rays at 6.2×10^6 rads established the cleavage of the pyrimidine ring. Under these conditions, it was thought highly probable that ribose or ribose phosphate would be liberated.

To test this possibility, solutions of adenylic, cytidylic, guanylic, and uridylic acid of the same concentration were irradiated at 6.2×10^6 rads. Twenty-five λ of each of these solutions was chromatographed in two dimensions, together with 25 γ each of ribose, ribose phosphate, and deoxyribose as carriers. Autoradiographs were made after a week's exposure of the chromatograms to x-ray film. The chromatograms were then sprayed with an aniline hydrogen phthalate spray, [11] prepared by dissolving 0.93 gram of aniline and 1.66 grams of phthalic acid in 100 ml of water-saturated butanol.

[5] R. Bandurski and B. Axelrod, J. Biol. Chem. 193, 405 (1951).

[6] H. S. Burton, Nature 173, 127 (1954).

[7] C. E. Carter, J. Am. Chem. Soc. 72, 1466 (1950).

[8] Murray Goodman, UCRL-1961, The Separation and Characterization of Phosphorylated Compounds from Green Plants, Sept. 29, 1952, p. 67.

[9] J. Smith and R. Markham, Biochem. J. 45, 294 (1949).

[10] C. Tamm, H. Shapiro, R. Lipschitz, and E. Chargaff, J. Biol. Chem. 203, 673 (1953).

[11] S. M. Partridge, Nature 164, 443 (1949).

One microgram of ribose phosphate, ribose, or deoxyribose could be detected easily by means of the color reaction given by this reagent. The sprayed chromatograms were heated for 5 minutes at 105°C. Superimposing the autoradiographs on the sprayed chromatograms showed no darkening of film corresponding to the areas where the free carrier sugars gave color reactions with the aniline hydrogen phthalate. It must therefore be concluded that when nucleotides are irradiated at this dose level, ribose, ribose phosphate, and deoxyribose are not formed. This confirms the findings of Scholes and Weiss in their experiments with yeast adenylic acid irradiated at 3×10^6 rads in the presence of air.[12]

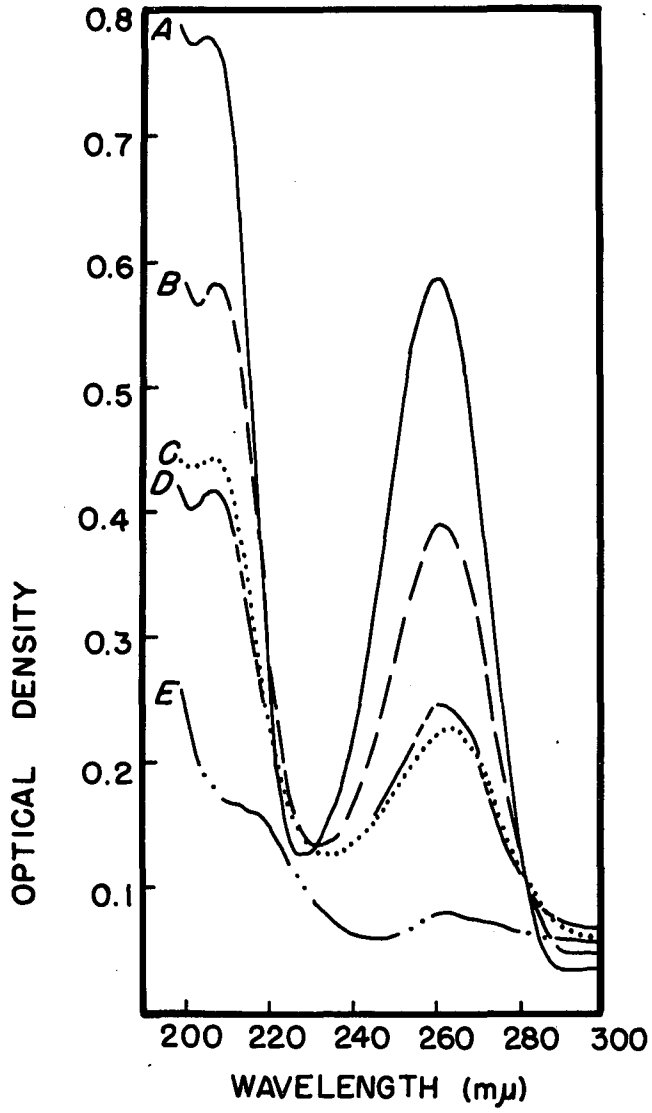
Ultraviolet Absorption Spectra

Figures 1 through 5 show the effect of irradiation on the uv absorption spectra of 0.1% solutions of the nucleotides. All measurements were made at pH 7 with a Cary recording spectrophotometer (Model 14).

In the purine nucleotides, adenylic acid (Fig. 1) and guanylic acid (Fig. 2), the increasing dose of radiation decreased the optical density of the main absorption peak. With the pyrimidine nucleotides, cytidylic acid (Fig. 3) and uridylic acid (Figs. 4 and 5), the optical density decreased up to a dose of 12×10^6 rads. In both cases, however, the absorption increased at 18×10^6 rads. In the cytidylic acid, there was a slight shift of the absorption maximum towards the shorter wave length. But, in the uridylic acid, the maximum was still at 260 mμ. In both cases, the absorbing material could not be detected by paper chromatography, a situation possibly due to loss by volatilization from the paper.

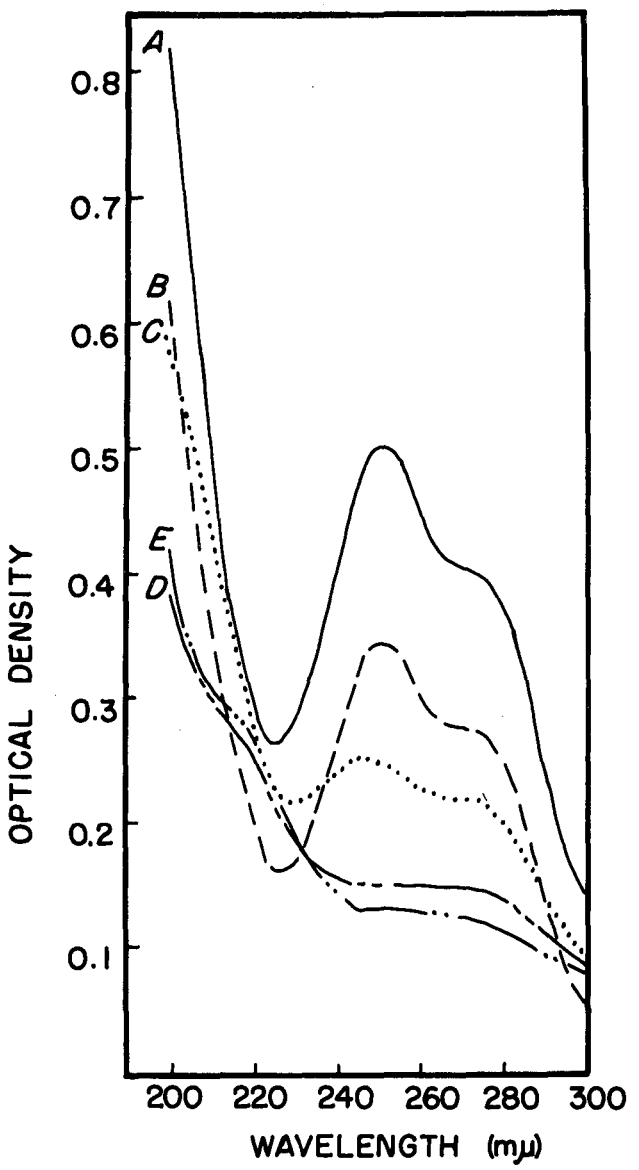
A comparison of the optical density of the different nucleotides after irradiation at 12×10^6 rads shows that under these conditions (aqueous solution at 0.1% concentration) uridylic acid is the most sensitive to irradiation, while cytidylic acid is the least affected. Guanylic acid is less affected by irradiation than adenylic acid. The replacement of the $-NH_2$ group by the -OH group in the 6-position apparently decreases the sensitivity of both purine and pyrimidine nucleotides to radiation.

[12] G. Scholes and J. Weiss, J. Exptl. Cell Research, Supplement 2, 219 (1952).



MU-21329

Fig. 1. Adenylic acid.
A. Unirradiated
B. 1.2×10^6 rads
C. 6×10^6 rads
D. 12×10^6 rads
E. 18×10^6 rads



MU-21330

Fig. 2. Guanylic acid.
A. Unirradiated
B. 1.2×10^6 rads
C. 6×10^6 rads
D. 12×10^6 rads
E. 18×10^6 rads

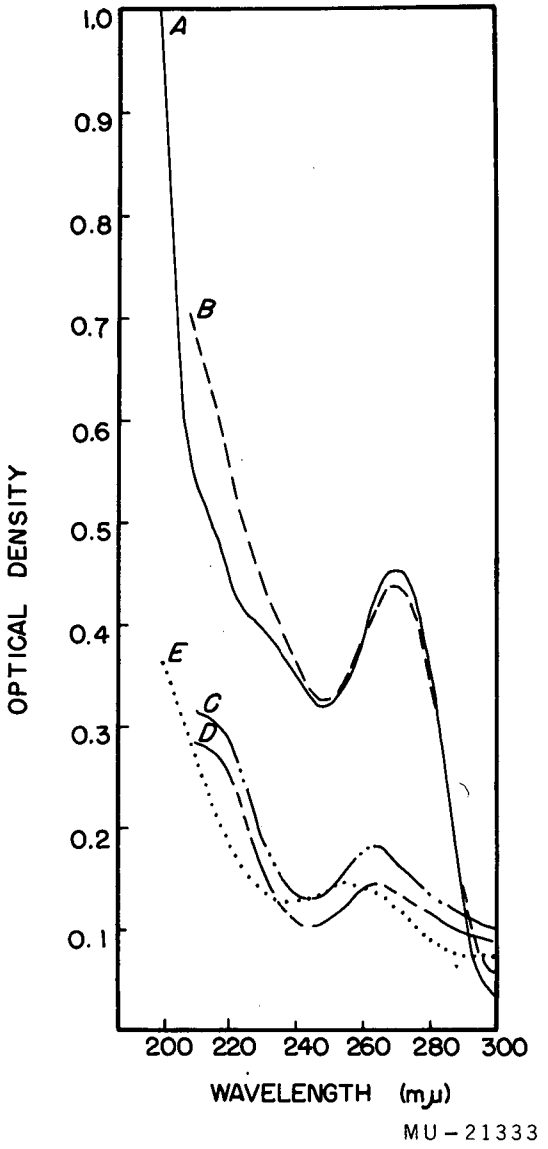
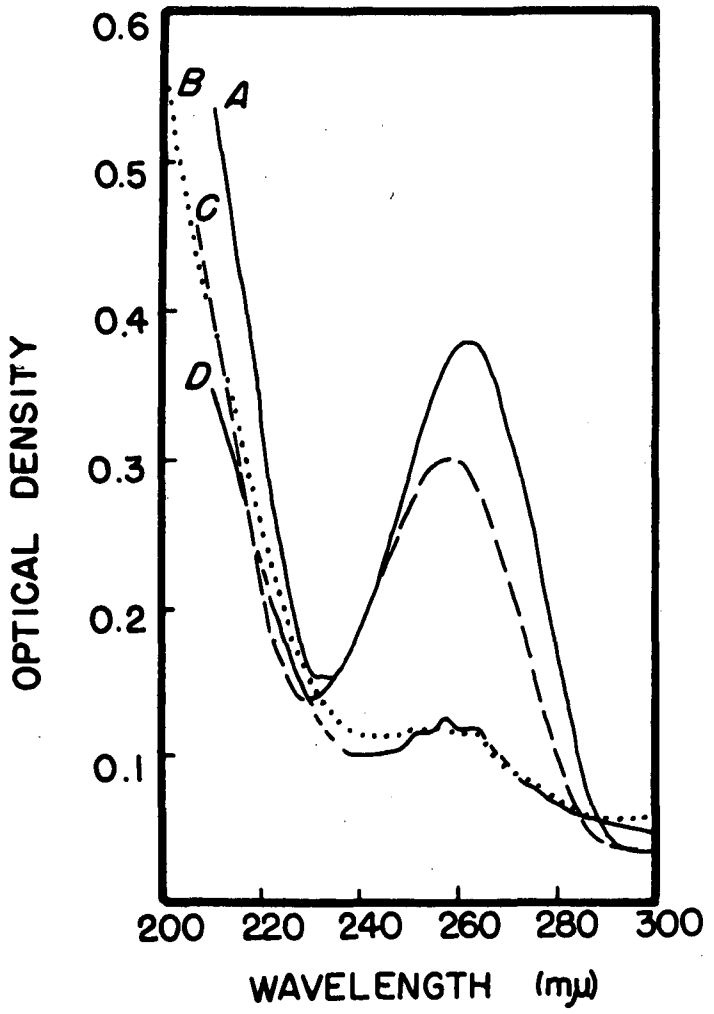
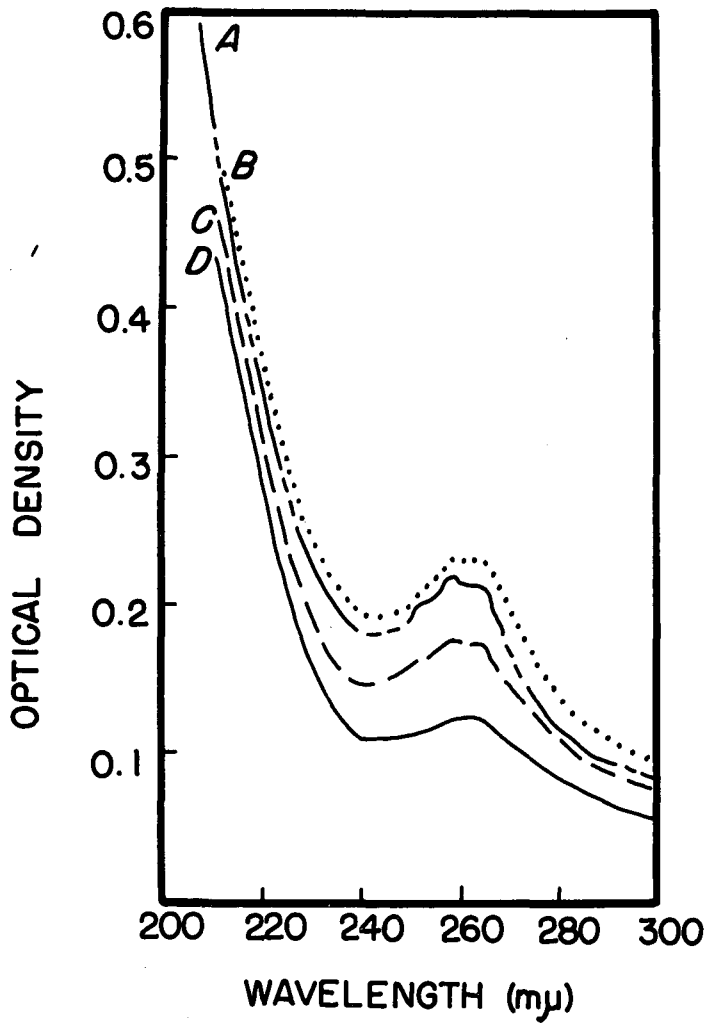


Fig. 3. Cytidylic acid.
A. Unirradiated
B. 1.2×10^6 rads
C. 18×10^6 rads
D. 12×10^6 rads
E. 6×10^6 rads



MU-21331

Fig. 4. Uridylic acid.
A. Unirradiated
B. 0.6×10^6 rads
C. 1.2×10^6 rads
D. 2.4×10^6 rads



MU-21332

Fig. 5. Uridylic acid.
A. 18×10^6 rads
B. 12×10^6 rads
C. 6×10^6 rads
D. 3.6×10^6 rads

EFFECTS OF D_2O ON THE FERTILITY OF MALE BALB/c STRAIN MICE

Paul H. Weaver

In previous experiments the differing effects of D_2O on the fertility of males of outbred and inbred strains of mice were observed.[1,2] This study on the inbred BALB/c strain appears to confirm our tentative conclusion that D_2O causes sterility in some inbred strains more rapidly and completely than in outbred strains.

Each of ten male BALB/c mice was put in a cage with two female BALB/c mice for a control mating. Seven of the males had at least one fertile mating, while three were apparently sterile. The fertile males were then given 30% D_2O in their drinking water in lieu of tap water. After two weeks of D_2O treatment, each male was mated with two female BALB/c mice every week. The males were maintained on 30% D_2O throughout the experiment. The females were examined for vaginal plugs daily. When a plug was found, indicating copulation, the female was removed from the mating cage in order to minimize the effect of D_2O on the developing embryos. All females not exhibiting vaginal plugs by the end of one week were removed from their mating cages, and a new mating group was given each male. Eighteen days after the beginning of their mating week, the females were placed in individual cages to bear the litters. At least two weeks after birth, the litters were counted and sacrificed.

The data, summarized in Table IV, indicate that D_2O produced some sterility in the BALB/c males after 3 weeks' treatment and complete sterility after 4 weeks. The number of litters produced decreased linearly with time, but, as shown by Table V, the size of the litters remained fairly constant. These findings are consistent with our previous observations on inbred strains.

However, the results of the control mating in this experiment suggest that the BALB/c strain is not so fertile and fecund as the Swiss or C_{57} strains. The average litter size of the BALB/c control mating was significantly smaller than that of other strains, and the number of infertile males and females was unusually large. Sperm counts on three BALB/c males after 6 weeks' D_2O treatment revealed an unusually small sperm population in the *vas deferens*. If D_2O-produced sterility is, as we suspect it to be, a genetic abnormality, it is logical to assume that an inbred strain is more susceptible to chromosomal disruption than an outbred strain.

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[1] A. M. Hughes and M. Calvin, Science 127, 1445 (1958).

[2] A. M. Hughes, E. L. Bennett, and M. Calvin, Proc. Natl. Acad. Sci. U.S. 45, 581 (1959).

Table IV

Number of fertile matings					
	Weeks on D_2O before mating				
Male No.	0	2	3	4	5
1	1	0	0	0	0
2	1	1	0	0	0
3	2	1	1	0	0
4	2	0	1	0	0
5	1	1	1	0	0
6	2	2	1	0	0
7	2	1	0	0	0
Total	11	6	4	0	0

Table V

Average litter size				
Weeks on D_2O before mating				
0	2	3	4	5
4.45	6.50	5.75	0	0

DISTRIBUTION OF HEXOKINASE, CHOLINESTERASE, AND LACTIC DEHYDROGENASE IN RAT BRAIN

Edward L. Bennett, J. B. Drori,* Hiromi Morimoto, and Ann Orme

(In collaboration with David Krech and Mark R. Rosenzweig, Department of Psychology and M. Diamond, Department of Anatomy, University of California, Berkeley)

We have been investigating the activity of cholinesterase (ChE) in the rat brain, in search of a correlation between the metabolic functions of the brain and the maze behavior of the rat. A definite correlation of cholinesterase activity with behavior has been demonstrated in two of the strains of rats thus far studied.[1] Furthermore, environmental factors and training have been shown to alter the amount and distribution of ChE in the brain.[2]

In addition to ChE, we have been investigating the relative activity of hexokinase and lactic dehydrogenase (LDH) in the brains of these rats. The methods used for ChE and LDH analyses have been published,[3,4] and are discussed in this report only briefly.

The present study concerns mainly (a) a method for determining the hexokinase activity in rat brain, (b) the relative activity of hexokinase in various areas of the brain, and (c) a comparison of hexokinase activity to that of ChE and LDH.

Our interest in hexokinase stems from the unique role which glucose plays in cerebral tissue as the major energy source. However, two factors complicate the determination of the activity of hexokinase in crude tissue preparations. Glucose-6-phosphate, the product of the hexokinase reaction from glucose, exerts a marked inhibitory action on this enzyme, and it is also metabolized to other products. For these reasons we chose 2-deoxyglucose as the substrate. According to the work of Crane and Sols, the product 2-deoxyglucose-6-phosphate (which is formed by hexokinase from 2-deoxyglucose) does not inhibit the activity of the enzyme and is not further metabolized.[5]

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*Kaiser Foundation Hospital, Oakland, California.

[1] Mark R. Rosenzweig, David Krech, and Edward L. Bennett, Search for Relations between Brain Chemistry and Behavior, Psychol. Bulletin, in press.

[2] David Krech, Mark R. Rosenzweig, and Edward L. Bennett, Effects of Environmental Complexity and Training on Brain Chemistry, J. Comp. Physiol. Psych., in press.

[3] E. L. Bennett, D. Krech, M. R. Rosenzweig, H. Karlsson, N. Dye, and A. Ohlander, J. Neurochem. 3, 144 (1958).

[4] E. L. Bennett, D. Krech, M. R. Rozenzweig, H. Karlsson, N. Dye, and A. Ohlander, J. Neurochem. 3, 153 (1958).

[5] Robert K. Crane and Alberto Sols, J. Biol. Chem. 210, 597 (1954).

We first investigated the effect of variables, including amount of tissue, time of incubation, and initial 2-deoxyglucose concentration, to determine the optimum conditions for our experiments. In order to obtain reasonable differences between samples with and without hexokinase, the incubation conditions must be such that approximately one-half the substrate is utilized. This difference was best obtained by using approximately 15 mg of homogenized brain and 9 μmoles of 2-deoxyglucose in 1.1 ml total volume and incubating at 31°C for one hour. Since, under these conditions, the reaction may not be linear with time or with amount of tissue, tissue from the cerebellum was used as a standard of reference, and other areas of the same rat brain were compared with it.

Experimental Methods

The rats, three S_1 and three S_3, were 125 days old at the time of sacrifice. Well delineated areas of the rat brain were dissected, immediately weighed, and frozen on dry ice. The tissues were stored in a deep freeze until analyzed. For hexokinase analyses, the tissues were homogenized with a Potter-type Teflon-glass homogenizer or a Duall-type all-glass homogenizer with a suitable volume of 0.1 M potassium phosphate buffer, pH 7.0, to obtain a final tissue concentration of 50 mg/ml for all sections except the medulla. This was homogenized in a volume to yield a final concentration of 66.7 mg/ml.

The incubation mixture in these experiments was that used by McLean in studies of hexokinase activity in mammary tissue.[6] It consisted of 0.2 ml tris buffer, pH 7.5 (0.01 M histidine, 0.1 M tris, 0.01 M EDTA, 0.01 M $MgCl_2$); 0.2 ml 0.075 M ATP in 0.04 M $MgCl_2$; 0.3 ml, 0.03 M 2-deoxyglucose; 0.1 ml 0.9 M NaF, which gave a total volume of 0.8 ml. To this was added 300 μl of the homogenized brain sample in 0.1 M potassium phosphate, pH 7.0 which contained (generally) 15 mg of tissue. The mixture was incubated for 1 hr at 31°C. The incubation was stopped by adding 2 ml of 5% $ZnSO_4$, followed by 2 ml of 0.3 M $Ba(OH)_2$. The tubes were centrifuged for 10 minutes and then duplicate 1.0-ml aliquot portions of the supernatant were removed for estimation of the nonutilized 2-deoxyglucose.[6] The colorimetric method of DeMoss and Happel was used to estimate the residual 2-deoxyglucose substrate.[7] The diphenylamine reagent was prepared by dissolving 1 g of diphenylamine (EK White Label) in 100 ml of glacial acetic acid plus 2.75 ml concentrated H_2SO_4. Four ml of the diphenylamine reagent was added to the aliquot portion of the incubation mixture and heated for 50 minutes in boiling water, cooled to room temperature, and read at 515 mμ in a Beckman spectrophotometer. All samples from one incubation were heated simultaneously.

Results

Figure 6 is a representative graph of change of optical density from a blank with no tissue as a function of tissue concentration, using 7.5 to 15 mg of cerebellum. The standard, without tissue, gave a reading of 1.25 density units. At the highest tissue concentration, approximately 60% of the 2-deoxyglucose is utilized, the reaction rate has decreased, and a nonlinear curve

[6] Patricia McLean, Biochim. Biophys. Acta 30, 303 (1958).

[7] R. D. DeMoss and M. E. Happel, J. Bacteriol. 70, 104 (1955).

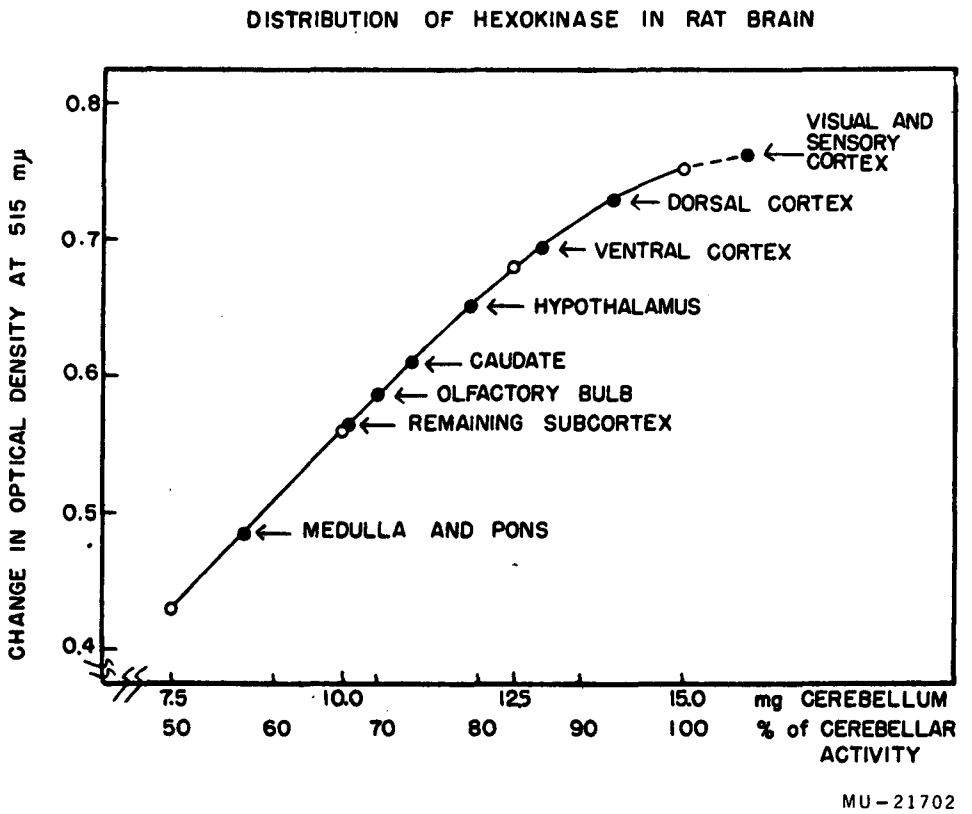

Fig. 6. Distribution of hexokinase in rat brain.
Open circles represent values obtained using 7.5, 10, 12.5 and 15 ml of cerebellum as standard. The values obtained with other brain areas are plotted on this reference curve with solid circles.

as a function of tissue concentration is obtained. The rate of 2-deoxyglucose utilization is approximately 400 μM/g hr. This compares favorably to that reported by Long for rat brain with glucose as a substrate.[8]

For each rat, its own cerebellum served as the standard curve. The activity of the other brain areas from the same rat was plotted and compared with it. These values (averaged from the data obtained from six rats with duplicate incubations and duplicate analyses of each incubation) are also shown on Fig. 6 and are summarized in Table VI. The hexokinase activity of the remainder of the brain and lower centers such as the medulla appears to be significantly lower than in the cortex and cerebellum. Between the area with the lowest activity (the medulla) and the area with the highest activity, a pooled sample of the visual and sensory cortex, there is an approximately twofold difference in activity.

In the course of correlations of rat maze behavior with ChE activity, we have obtained considerable data on ChE activity of various areas of the rat brain and on factors influencing these activities. In Table VII the ChE activities of fifteen areas of the rat brain are summarized, arranged in descending order of activity, and expressed as a percentage of the activity of the cerebellum. The data are averages obtained from 40 rats of two strains and two ages (100 and 125 days). The areas range in weight from 6 to 7 mg to 500 mg. The assays have been made by determination of the rate of hydrolysis of acetylcholine perchlorate under standardized conditions, with a pH stat.[3] In contrast to hexokinase activity, cortical areas tend to have low ChE activity, whereas the subcortical areas tend to be high in ChE activity. In general, there is an inverse relationship between hexokinase activity and ChE activity in nine areas of the brain compared.

A third comparison has been made of ChE or hexokinase activity to LDH activity (Table VII). This has been determined by the rate of reduction of DPN with lactic acid as a substrate.[4] These data are for ten rats, six S_1 and four S_3. No correlations are obvious either between hexokinase activity or LDH activity, or between ChE activity and LDH activity. The range of lactic dehydrogenase activity is more limited than that of hexokinase. A ratio of only about 1.5 exists between the area with the lowest activity (reticular formation) and those with the highest activity of LDH (dorsal cortex, thalamus and caudate). The ratios between the most active and least active areas for hexokinase and ChE activity are 2 and 20, respectively.

Strain differences in ChE activity have been demonstrated; to date we have demonstrated no strain differences in LDH activity, either in this study or in a previous one.[3,4] Insufficient analyses have been made for hexokinase activity to demonstrate the possible presence or absence of strain differences. However, before pursuing this facet further, we hope to develop a more convenient and precise hexokinase assay.

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[8] C. Long, Biochem. J. 50, 407 (1952).

Table VI

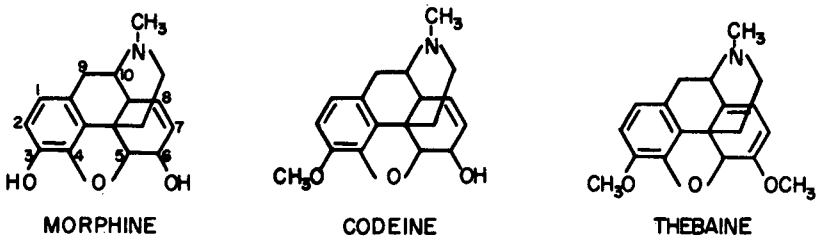
Hexokinase activity of areas of rat brain using 2-deoxyglucose as substrate (Expressed as % of cerebellum activity)

Area	Relative activity
Medulla	57
Remaining subcortex	67
Olfactory bulb	70
Caudate	73
Hypothalamus	79
Ventral cortex	86
Dorsal cortex	93
Cerebellum	100
Visual and sensory cortex	106

Table VII

Comparison of several enzymic activities of areas of rat brain (Expressed as % of cerebellum activity)

Area	ChE	Hexokinase	Lactic dehydrogenase
Olfactory	1850		102
Caudate	1260	73	112
Superior colliculi	650		89
Reticular formation	410	74	
Remainder subcortex	320	67	104
Medulla and pons	290	57	83
Thalamus	280		113
Ventral cortex	237	86	108
Hypothalamus	220	79	90
Olfactory bulb	190	70	99
Inferior colliculi	190		81
Dorsal cortex	155	93	113
Sensory cortex	150	106	106
Visual cortex	128		105
Cerebellum	100	100	100



MU-19255

Fig. 7. Morphine-type alkaloids of P. somniferum.

THE BIOSYNTHESIS OF OPIUM ALKALOIDS

Frank R. Stermitz and Henry Rapoport

It was previously suggested on the basis of comparative rates of $C^{14}O_2$ incorporation that, in Papaver somniferum, thebaine is the first of the octahydrophenanthrene alkaloids to be formed and is converted by successive O-demethylations to codeine and finally morphine.[1,2] Conclusive proof of this interrelationship has now been obtained.

Three plants of P. somniferum were root-fed randomly labeled (C^{14}) morphine, codeine, and thebaine, respectively. At the end of 42 hours, the plants were killed and these three alkaloids isolated from each plant. From the morphine-fed plant only reisolated morphine was active, from the codeine-fed plant isolated codeine and morphine were active, and from the thebaine-fed plant all three alkaloids were radioactive. (Fig. 7).

A 12.7% incorporation of total activity from codeine to morphine was found, whereas the thebaine-fed plant yielded a 7.5% and 5.0% incorporation into morphine and codeine respectively.

It was also established that this incorporation was not due to transmethylation, since degradation of isolated morphine and codeine from the thebaine-fed plant showed a large portion of the activity in the basic carbon skeleton. Indeed, the ratios of methyl group to nucleus activity in the isolated codeine and morphine were only slightly less than these ratios in the thebaine that was fed. Thus, only a minor amount, if any, of transmethylation had taken place. The data regarding the methyl group on the oxygen at position 6 in thebaine was not possible because of the small amount of activity remaining in the isolated compound. Since this is in an enol ether system, the chances for transmethylation would be expected to be greater here than for the other two methyl groups.

Although transmethylation does not appear important, the conversion of thebaine to morphine does involve the release of two one-carbon fragments into the metabolic stream. It will be of interest to learn whether these fragments are merely expired or whether they take part in additional metabolic processes. Because of the large number of alkaloid groups that differ only in respect to the degree of N- and O-methylation, it will also be of value to determine if the demethylation pattern is a general one in alkaloid biosynthesis.

Because of the relatively rapid synthesis and conversion of alkaloids found in this and the previous work,[2] such demethylations could be a significant part of the plant metabolism and perhaps provide one answer to the century-old problem of alkaloid function.

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[1] Frank R. Stermitz and Henry Rapoport, in Bio-Orgainic Chemistry Quarterly Report, UCRL-9041, Dec. 1959, p. 22.

[2] Henry Rapoport, Frank R. Stermitz, and Don R. Baker, J. Am. Chem. Soc. 82, 2765 (1960).

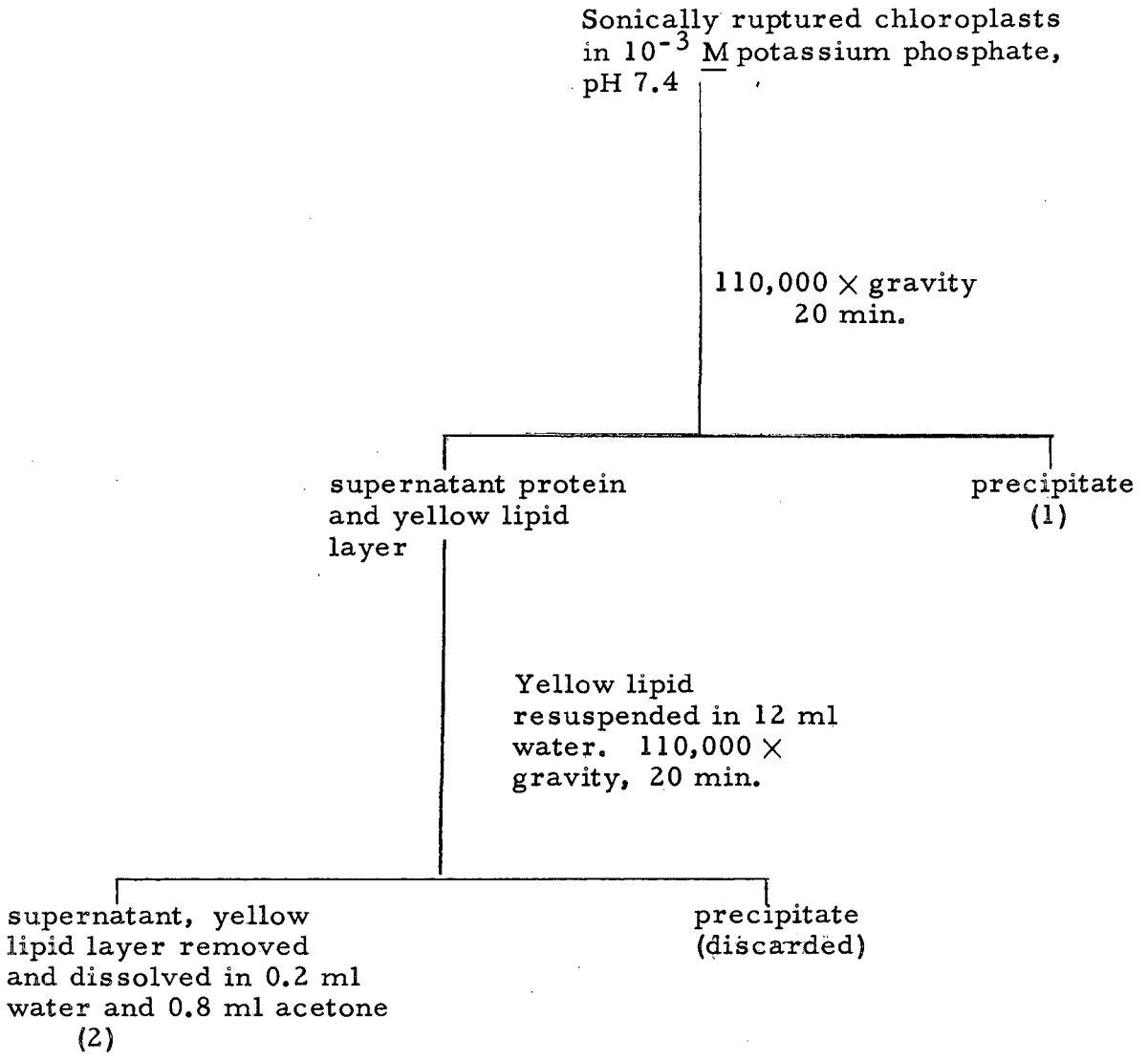

Fig. 8. Preparation of particles with high carotenoid-to-chlorophyll ratios from sonically ruptured chloroplasts.

ISOLATION OF PARTICLES WITH HIGH CAROTENOID TO CHLOROPHYLL RATIOS FROM SPINACH CHLOROPLASTS

Roderic B. Park and Ning G. Pon

Centrifugal fractionation of sonically fragmented spinach chloroplasts in dilute aqueous medium yields three principal fractions: a chlorophyll-containing precipitate, a relatively colorless protein supernatant, and an opaque yellow layer which rises to the surface of the tube. This distinctly yellow layer appears to have a much higher carotenoid-to-chlorophyll ratio than is normally observed in chloroplast fragment preparations.

The preliminary methods used to obtain and purify the carotinoid-rich fractions are given in Fig. 8. The preparation of sonically ruptured chloroplasts is described by Park et al.[1]

The yellow fraction was removed from the centrifuge tube and was found to be completely soluble in 20% methanol, leaving no protein residue. Two properties of this fraction, low density and solubility in organic solvents, suggest that this material is lipid in character.

The absorption spectrum of the yellow fraction (2 in Fig. 8) is compared with the absorption spectrum of the green precipitate (1 in Fig. 8) in Fig. 9. The difference spectrum lacks the 663-mμ chlorophyll peak and demonstrates characteristic carotenoid absorption in the range 400 through 500 mμ. Better purifications of the yellow fraction will show whether the chlorophyll we presently see in the fraction is a contaminant.

Electron microscopic examination of the yellow fraction has shown that these particles are the "osmiophyllic granules" seen in osmium-fixed thin sections of higher plant chloroplasts.[2] Osmium fixation of the yellow fraction followed by chromium shadowing gives the picture shown in Fig. 10. We hope that further efforts in characterization of these particles will explain their function within the chloroplast.

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[1] Roderic B. Park, Ning G. Pon, Karel P. Louwrier, and Melvin Calvin, D-Threose 2, 4-Diphosphate Inhibition of 3-Phosphoglyceric Acid Photoreduction by a Sonically Ruptured Spinach Chloroplast System, Biochim. Biophys. Acta 42, 27 (1960).

[2] Roderic B. Park and Ning G. Pon, Correlation of Structure with Function in *Spinacea oleracea* Chloroplasts, J. Mol. Biol. (in press).

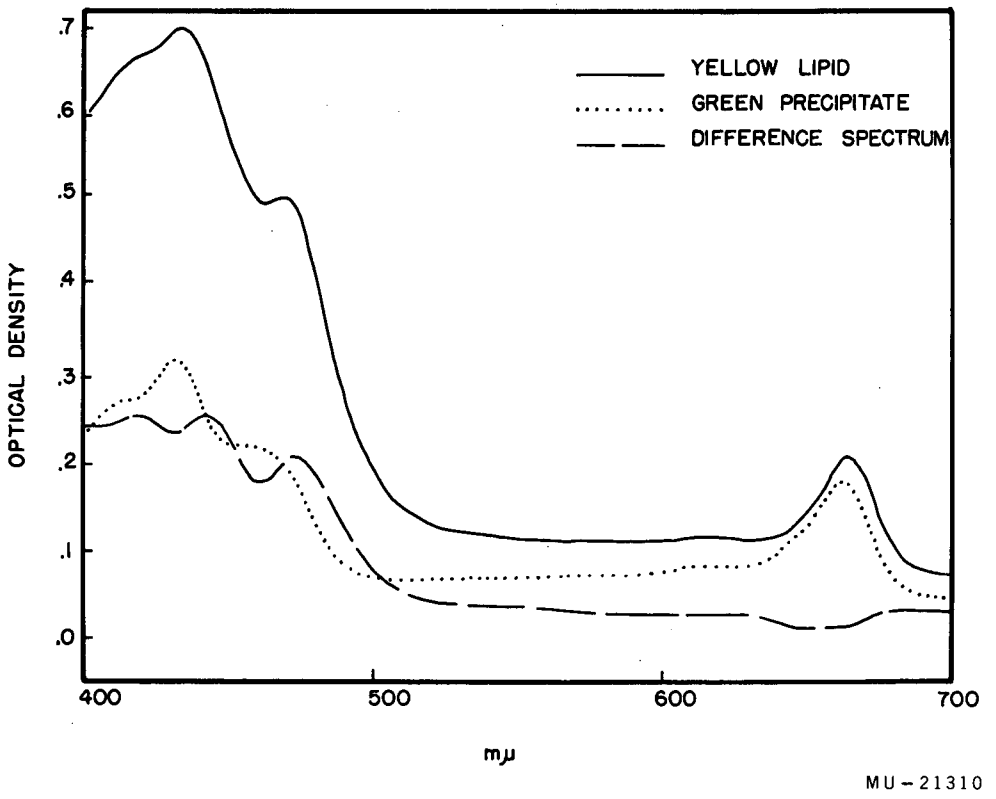

Fig. 9. A comparison of the absorption spectrum in 80% acetone of the green precipitate and yellow lipid obtained from sonically ruptured spinach chloroplasts.

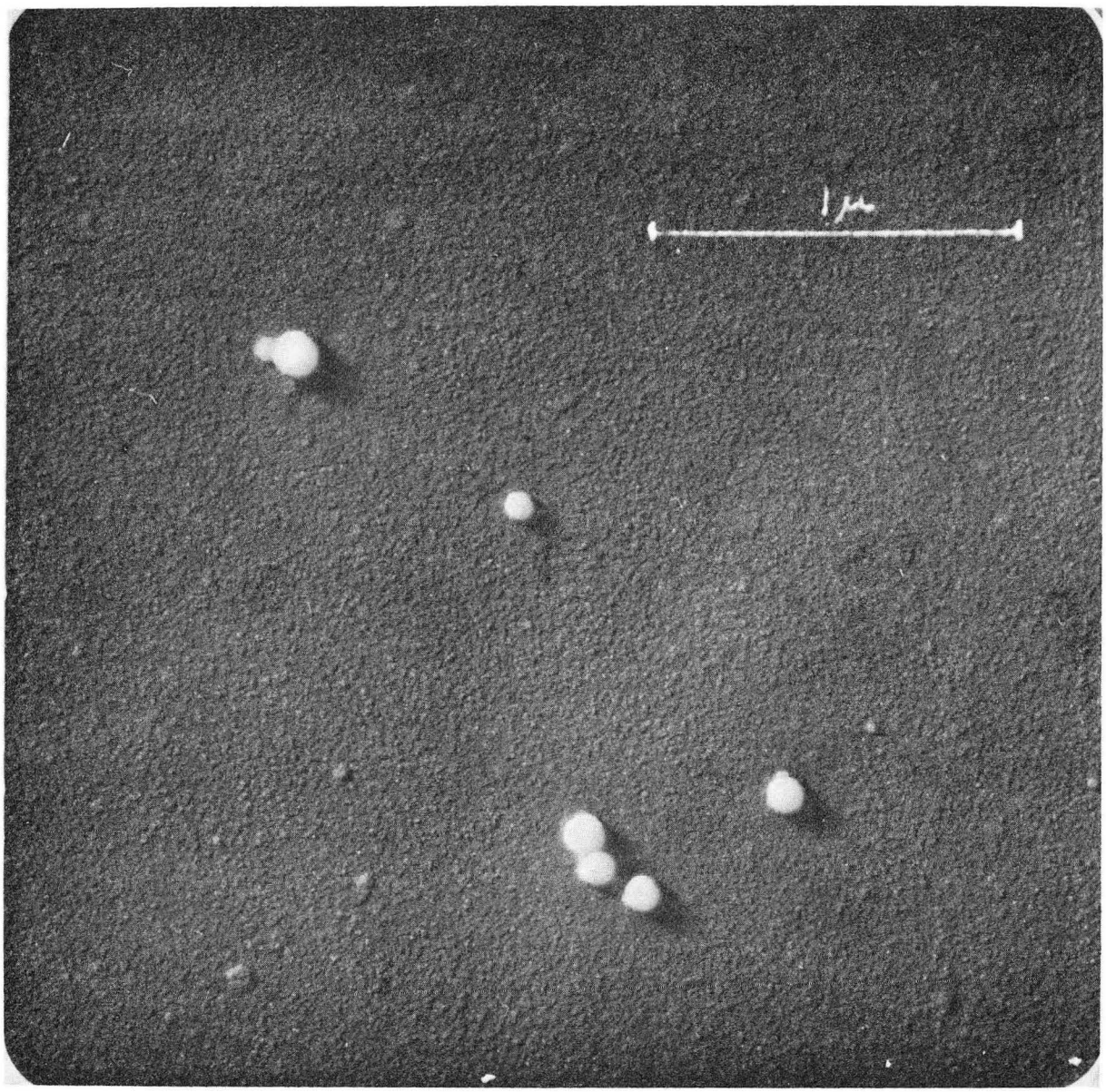

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Fig. 10. An electron micrograph of the yellow lipid particles after osmium fixation and chromium shadowing. Large particles are the lipids. No polystyrene particles are present.

THE HILL REACTION ACTIVITY OF SMALL CHLOROPLAST FRAGMENTS

Ulrich Heber

Electron microscopic studies on chloroplast fragments in this Laboratory have demonstrated the presence of 200-A particles in the lamellar structures of the chloroplast.[1] Calculation shows that these particles contain roughly the same number of chlorophyll molecules as the photosynthetic unit first proposed by Emerson and Arnold.[2, 3] In order to find whether these particles are actually photosynthetic units capable of oxygen evolution in the presence of an oxidant and light (Hill reaction), it was necessary to separate them from the lamellar structure.

Destruction of the lamellar structure was accomplished by ultrasonic treatment and reaction with the detergents digitonin, desoxycholate, and dodecylsulfate. After destruction of the lamellar structure, the particles obtained were separated by hanging-curtain electrophoresis. The Hill-reaction activity of the preparations indicated the extent to which enzymatic activity of the particles had been destroyed by the separation procedures.

Results

We found that the best method of disintegration was prolonged ultrasonic treatment in the presence of the reducing agents, glutathione or cysteine. Without these compounds, enzyme activity is rapidly lost during sonication.

It is also possible to render the insoluble lamellar structure of chloroplasts somewhat soluble by the addition of digitonin or desoxycholate to the chloroplast suspension. Addition of more than 2.5 moles of digitonin or desoxycholate per mole of chlorophyll causes a heavy loss of enzymic activity. The addition of one mole of dodecylsulfate per mole of chlorophyll causes nearly complete loss of Hill-reaction activity. Inactivation of the Hill reaction by the above-mentioned chemicals is apparently irreversible.

Enzymatically active preparations obtained by the above methods were separated on the hanging curtain. If the preparations remained active during destruction of the lamellar structure, the separation pattern on the hanging curtain was the same no matter which method of destruction was applied. Enzymatic activity was retained after prolonged separation (12 to 20 hours) in the electric field if

(a) the buffer pH was less than 8.0;

(b) the buffer contained a protective agent such as methanol or sugar;

(c) the separation was done at low temperature.

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[1] Roderic B. Park and Ning G. Pon, Correlation of Structure with Function in *Spinacea oleracea* Chloroplasts, J. Mol. Biol. (in press).

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

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

With these precautions, it is possible to get a separation of the desintegrated insoluble chloroplastic matter on the hanging curtain, as indicated by the following criteria:

(a) The proteins migrate in two peaks. Only the larger protein peak contains chlorophyll and the protein/chlorophyll ratio of subfractions obtained from the larger peak is not constant.

(b) The maximum Hill-reaction activity of the eluants does not correspond to the maximum chlorophyll concentrations. This may be due to inhibition by certain proteins rather than to the separation of an unusually active particle

Electron microscopy of the fractions eluted from the hanging curtain are still inconclusive, but suggest that much of the material is larger than 200 A in diameter.

FACTORS AFFECTING THE PERMEABILITY OF CHLORELLA CELLS TO METABOLITES OF INTEREST

John M. Turner

It has been found in this Laboratory that Chlorella pyrenoidosa is unable to utilize the carbon of externally supplied dicarboxylic and amino acids under normal physiological conditions. If the cell wall of this organism could be rendered permeable to such metabolites, however, this would provide a useful research tool for the study of intermediary metabolism.

The chemical composition and structure of the cell wall of Chlorella have been investigated by Northcote, Goulding, and Horne.[1] The wall was found to be 210 A thick, and shown to be a two-component structure in which microfibrils (α-cellulose, plus polymers of galactose, mannose, arabinose, and rhamnose) 30 to 50 A in diameter are enclosed in a granular matrix (hemicellulose plus glycoprotein and some lipid).

Several approaches to the modification of the cell-wall structure are apparent, with the distinction between cell-wall destruction and selective damage to the permeability barrier kept in mind.

The Use of Enzyme Preparations

Culture filtrates of Myrothecium verrucaria, grown on cellulose powder, were incubated with washed Chlorella cells in acetate buffer, pH 5. No effect on the algal cells could be detected by using morphological appearance (thickness of cell wall, swelling, etc.) as criteria. Incubation

[1] D. H. Northcote, K. J. Goulding, and R. W. Horne, Biochem. J. 70, 391 (1958).

was carried out for up to 24 hr, both at room temperature and 30°C. It was found that culture filtrates produced no detectable amounts of reducing sugars when incubated with cellulose powder under similar conditions, and it was decided to try commercially available cellulase preparations.

In a number of experiments using cellulase and hemicellulase, both singly and together, these preparations caused no detectable effect on Chlorella suspensions. Morphological appearance and the possible release of compounds absorbing at 675 mμ (chlorophyll) into the suspending medium were used as criteria.

A considerable effort would be required to prepare enzymes capable of removing the microfibrillar or matrix components of Chlorella cell wall, because their purification, specificity, optimal conditions for activity, etc., have been little studied.

Ultrasonic Treatment

Use of a Raytheon sonic oscillator (9 kc/s) gave negative results, but the more powerful "Crystalab Ultra-sonerator", operating at 1500 kc/s, caused the appearance of substances absorbing at 675 mμ in Chlorella suspension supernatants. Optical densities increased with time of sonication up to 60 sec. This suggested cell wall damage, and possible release of protoplast material, although no cell damage could be detected with an optical microscope.

To examine the effect of sonication on permeability, algae were fed $C^{14}O_2$ in a regular photosynthesis experiment. Samples of these cells were treated for various lengths of time in the Ultra-sonerator. The untreated control sample was retained. All samples were centrifuged, and the supernatants autoradiographed. Results showed that the untreated cells had released only glycolic or glyceric acid into the suspending medium. Sonically-treated cells had released at least thirteen distinct compounds, the autoradiographs showing the usual spectrum of labeled compounds formed during photosynthetic $C^{14}O_2$ fixation. It was found that maximum release of labeled compounds had occurred after approximately 5 sec sonication, at which time the release of chlorophyll-like substance into the suspending medium was only approximately 30% of that observed after 60 sec. This suggested that increased permeability was independent of cell disruption. Further experiments showed that the photosynthetic ability of Chlorella cells was not affected by sonication for lengths of time up to approximately 10 sec.

When labeled phosphoglyceric acid or malate (eluted from chromatograms) was incubated with sonically treated algae under normal conditions for photosynthesis, no incorporation of the former could be detected, but some fumarate was formed from malate by the treated cells. Only 2 to 3% was converted, however. Fumarase activity was more apparent in the dark and increased with the time of incubation.

The Effect of Freezing and Thawing

Freezing to the temperature of a dry ice slurry, followed by thawing at room temperature, was found to release labeled compounds from algae

preincubated with $C^{14}O_2$. Photosynthetic ability was extinguished by this treatment, however.

Freezing to -18°C also extinguished the photosynthetic ability of thawed Chlorella suspensions. When freezing had been preceded by ultrasonic treatment, however, most of the CO_2 fixation was retained (see Table VIII).

Further experiments showed that preliminary sonication virtually abolished the release of chlorophyll-like substances into suspension media when algal suspensions were frozen and thawed.

When algae suspensions were thawed immediately after freezing at -18°C some retention of the photosynthetic ability was observed although preliminary sonication increased the extent of retention. Freezing and thawing alone caused a loss of 20% of photosynthetic ability compared with untreated cells, whereas preliminary sonication caused a loss of only 7%.

Preliminary experiments show that labeled aspartate is incorporated to a small extent by sonically treated cells, and to an even lesser extent by frozen-thawed cells. This was determined by autoradiography; the appearance of labeled compounds other than aspartate was used as a criterion.

Conclusions

The techniques described suggest that Chlorella cells may be rendered permeable to dicarboxylic and amino acids by either sonic treatment, freezing and thawing, or both. The small degree of incorporation observed is probably due to concentration gradient effects. In order to demonstrate extensive incorporation, the concentration of external labeled compounds should be increased. This could probably be achieved by reducing the total volume of reaction mixtures, the density of the cell suspension being correspondingly increased. The possible effect of depleting Chlorella of intracellular metabolites, by CO_2 starvation, will also be investigated.

Table VIII

Radioactivity, counts/min, in sample of whole suspension. (Approximately 10% cell suspension in pH 7.4 buffer. Photosynthesis stopped by addition of MeOH to give final concentration 80%).

	Untreated cells	Frozen and thawed cells	Sonicated then frozen-thawed cells	Frozen-thawed then sonicated cells
Untreated sample	15,000	19,000	15,000	5,000
Sample and acetic acid	15,000	500	14,000	250

THE ELECTRON SPIN RESONANCE (ESR) OF PHOTOSYNTHETIC SYSTEMS, PHOTOSYNTHETIC PIGMENTS, AND MODEL SYSTEMS

Alex F. H. Anderson and Melvin Calvin

Introduction

A logical step in the investigation of the unpaired electrons induced by light in photosynthetic systems and detected by ESR technique is a study of ESR signals in the extracts from these systems. Additionally, one might attempt to build a model system, using the compounds in the extract, which agrees with our knowledge of the microstructure of the photosynthetic system, e.g., chlorophyll is thought to exist in the chloroplast as monolayers associated with protein.[1]

Sogo, Jost, and Calvin[2] made preliminary experiments on methanol extracts of Chlorella and Rhodospirillum rubrum, relatively pure photosynthetic pigments, and mixtures of these pigments. The signals from these pigments differed from those in the biological material. The pigments gave signals that were dependent on the gases adsorbed on their surface, and their line width was half that of the biological material.

S. S. Brody et al.[3] have investigated the ESR of solutions and microcrystals of chlorophyll. The crystals had a dark signal, and a photoinduced signal of quantum yield of 7%. The solutions of chlorophyll had no dark signal, but a photoinduced paramagnetism of 3% quantum yield was obtained.

Krasnovskii et al.[4] have studied the photo-oxidation and photoreduction of chlorophylls a and b, pheophytins a and b, hematoporphyrin, and magnesium phthalocyanine with ESR. Molecular oxygen did not give unpaired electrons when it was introduced into illuminated pyridine and alcoholic solutions of the pigments. However, the pigment molecules were photoreduced in the presence of ascorbic acid, and the ESR spectrum was assigned to the free radical of ascorbic acid. No signal could be found for the pigment-free radicals.

All the investigations carried out point to the difficulty of manipulating the compounds involved, and carrying out the measurement under conditions that would give some measure of reproducibility and lead to an unambiguous interpretation of the spectra obtained.

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[1] H. J. Trurnit and C. Colmano, Biochim. Biophys. Acta 30, 447 (1958); M. Calvin, Brookhaven Symp. in Biol. 11, 160 (1958); M. Calvin in Proceedings McCollum-Pratt Symposium on Light and Life, (Johns Hopkins Press (1960)) (in press) (UCRL-9170).

[2] P. G. Sogo, M. Jost, and M. Calvin, Radiation Research, Suppl. 1, 511 (1959).

[3] S. S. Brody, G. Newall, and T. Castner, J. Phys. Chem. 64, 554 (1960).

[4] N. N. Bubnov, A. A. Krasnovskii, A. V. Umrikhina, V. R. Tsepalov, and V. Ya. Shylyapintokh, Biofizika 5, 121 (1960); UCRL-Trans. 560.

The work reported here is in the nature of "sighting experiments" designed to get some feeling for the manipulation of the extracts, and--from the crude measurements--suggestions for a more detailed study on ESR signals in extracts of biological material.

Preliminary Experiments on Chlorella Extracts

The methanol extract was prepared from Chlorella. A sample of 250 ml of Chlorella culture was centrifuged to remove the culture medium. The wet-packed algae (about 5 cc) were washed twice with distilled water. They were then extracted with 100 cc of absolute methanol at room temperature, and the insoluble material centrifuged off. The methanol extract was made into a sample suitable for testing in the ESR spectrometer[2] in three ways.

The first method was to evaporate the extract down under vacuum, scrape the sticky material out of the flask, and apply it to a silvered copper rod. The second method was to redissolve the methanol extract in petroleum ether, place the solution in a quartz tube (sample tube in Fig. 12), and then evaporate the solvent, leaving a film of extracted material about 0.5 mm thick on the lower 1.5 cm of the tube. The third procedure was to treat the 100 cc of methanol extract with 50 cc of petroleum ether in a separatory funnel, and add just sufficient distilled water so that two phases were obtained. Most of the highly colored pigments were transferred to the petroleum ether (the methanol-water phase was pale yellow). The petroleum ether was separated and a film deposited as the second procedure. The separations are summarized in Fig. 11.

Electron Spin Resonance of the Extract Films

The methanol extract was applied to a metal rod as a paste. When applied it was "doped" with unknown quantities of water from a paint brush. The resultant samples gave signals which had similar form (i.e., line width of about 6 gauss), but were not reproducible in height and rates of decay.

The films prepared from petroleum ether (non-phase-separated) and deposited on quartz gave higher yield of free spins, and were more reproducible. The gas phase in contact with the surface of the film was controlled by using the apparatus in Fig. 12.

Influence of Water Vapor (Oxygen-Free) on Methanol Extracts Deposited as Films from Petroleum Ether (Non-Phase-Separated)

The film was deposited, as described, on the quartz ampule and then evacuated with a vacuum pump to 10 microns pressure for several hours to remove traces of methanol, petroleum ether, water, and adsorbed oxygen. The sample was protected from the light when necessary by placing a layer of heavy brown paper round the ampule. After the sample was placed in the ESR spectrometer, the visible light from a projection lamp was shone on the sample. In the absence of water vapor, no dark signal, and only a very small light-induced signal, was found. The sample kept at room temperature

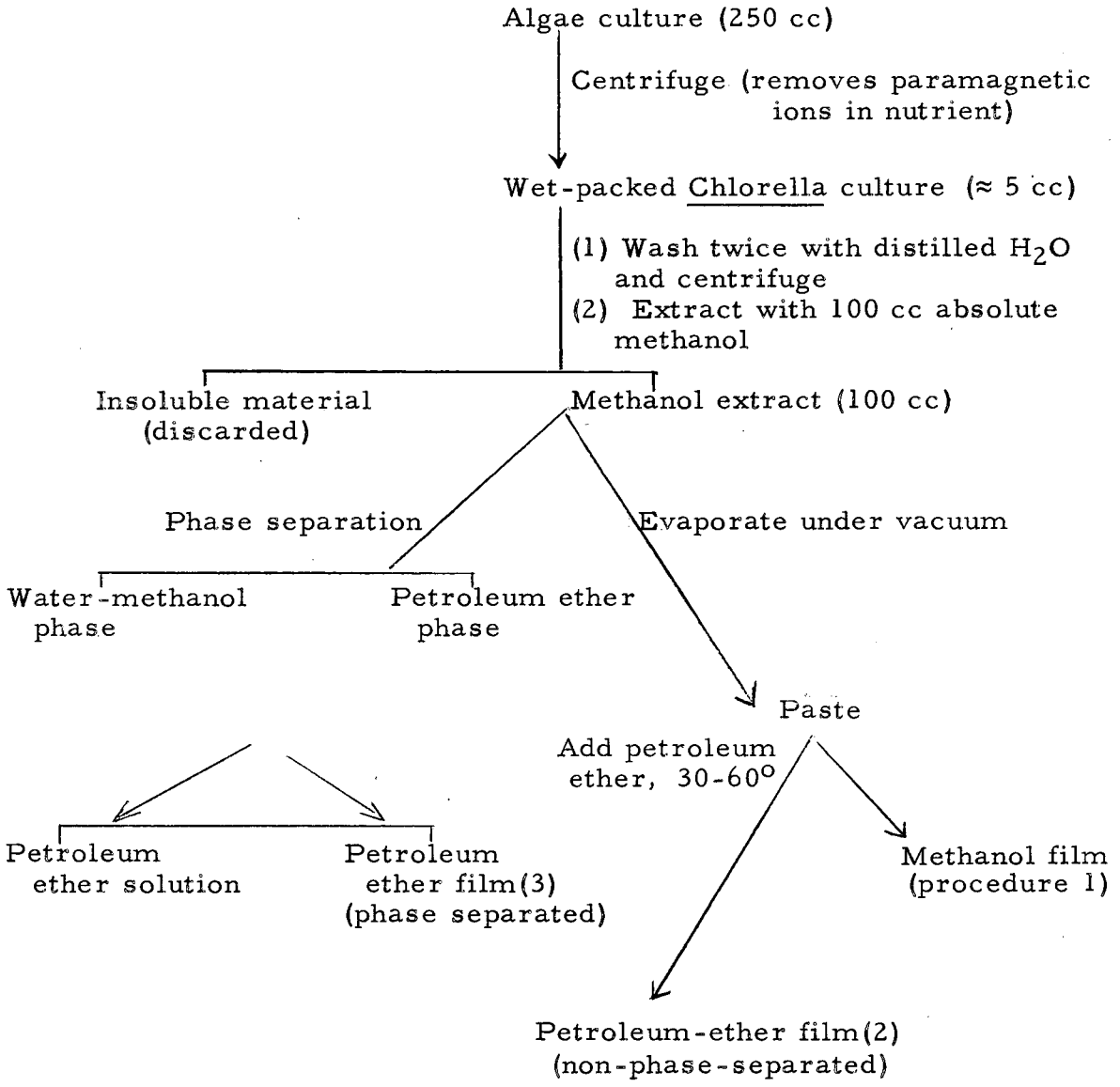

Fig. 11. Chlorella extract preparation.

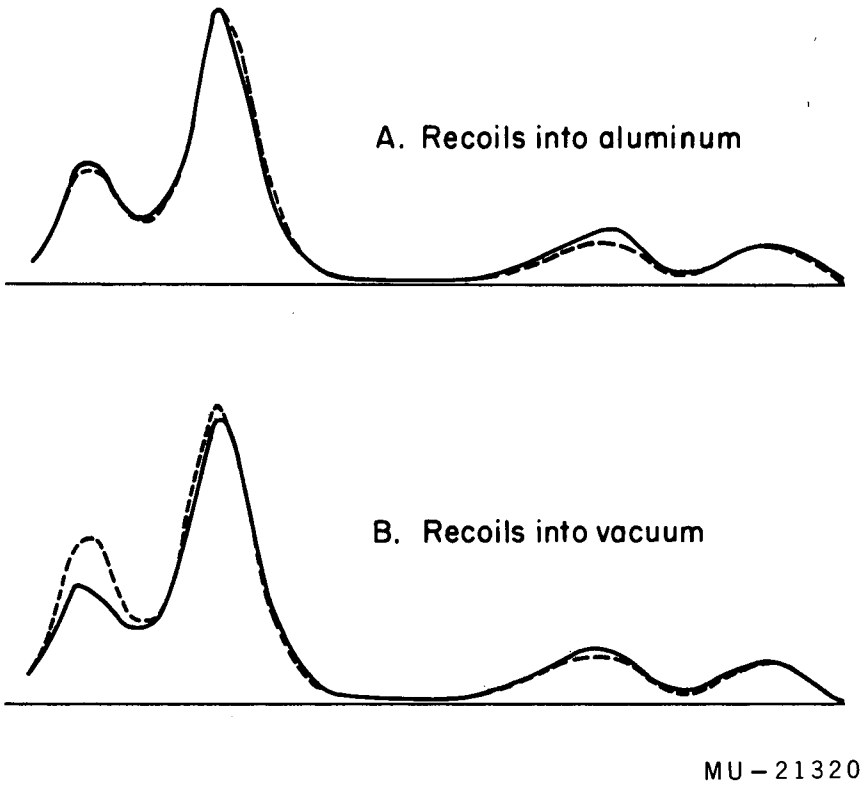

Fig. 12. Apparatus for film preparation and water vapor control.

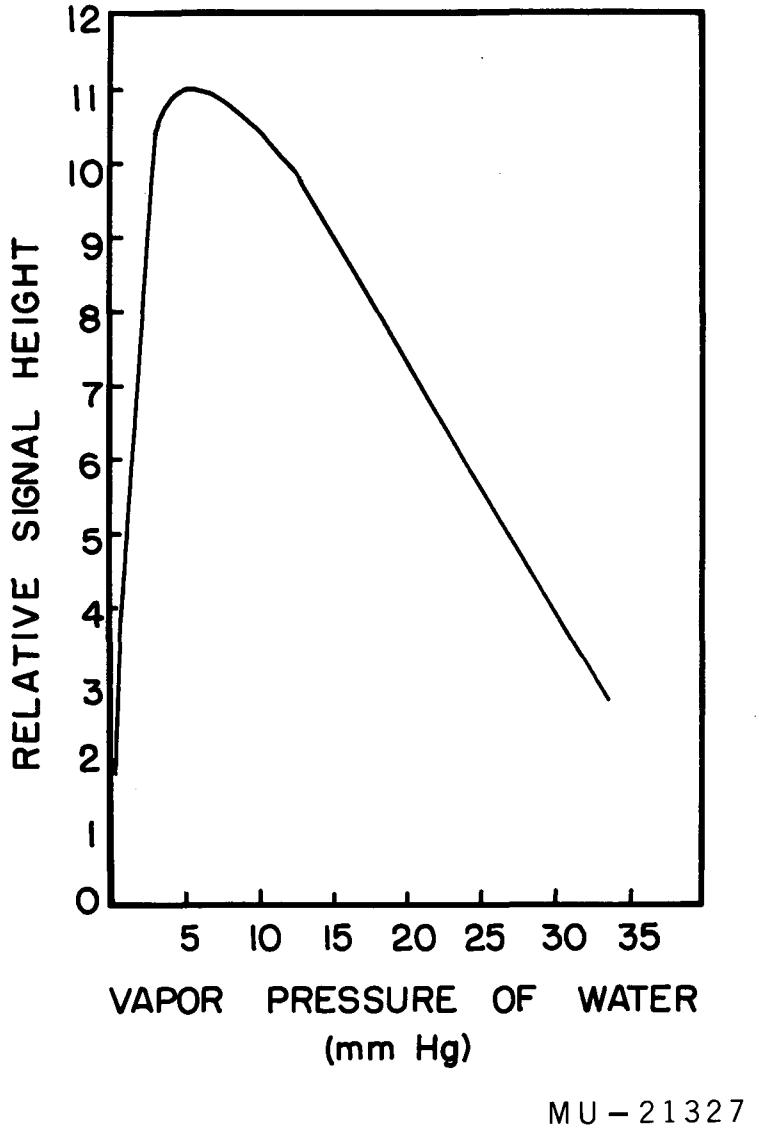

Fig. 13. Steady-state signal height as a function of water vapor.

was then exposed to water vapor at a definite pressure. The water in A (Fig. 12) was solidified by means of liquid nitrogen, and the whole apparatus evacuated for several hours to remove any traces of oxygen. The oxygen-free (boiled, distilled) water was then allowed to come to the temperature of water-ice bath, and the sample exposed to this vapor pressure of water for 1 hour. Stopcocks C and D were then closed and the sample was tested for dark-and light-induced ESR signals. The experiment was repeated several times at increasing vapor pressures of water, each obtained by altering the temperature of the water bath around A.

In each case a small dark signal was observed. Two observations can be made on the light-induced signals: first, the steady-state concentration of photoinduced spins, as a function of water vapor pressure, and, second, the rate of decay of the spins after the light is turned off. Since the line width remained constant, the concentration of spins was taken to be proportional to the amplitude difference between the peaks of the differentiated absorption curve.

Figures 13 and 14 present these data. It is seen in Fig. 13 that the steady-state population of unpaired spins goes through a maximum below 4.7 mm Hg water vapor pressure. For a given method of producing the samples the decay rate (slope of curves in Fig. 14) is relatively independent of water vapor pressure. It is noted, however, that for the single film evaporated from petroleum ether with phase separation, both the equilibrium amplitude (value at t = 0) and the rate of decay are markedly different.

Production of Spins in Extract Solutions

It is of interest to find out whether or not solutions of the extracted pigments produce signals under the influence of light. The methanol solutions could not be tested because the dielectric constant of methanol is such that the microwaves are strongly absorbed. However, the methanol-extracted material can be transferred to petroleum ether and these solutions can be investigated in the ESR apparatus.

A petroleum ether solution was put in the quartz tube and the light-induced signals were observed as the petroleum ether was evaporated. Only when solid material was present did signals appear. The effects of adding oxygen, water, and compounds such as quinones were not studied.

Discussion

The results show that water strongly influences the production of spins and that the nature of the film is important. The chemical analysis of the extracts is in progress, by use of techniques of column chromatography and spectroscopic analysis. A balance has been constructed for the preparation of chlorophyll monolayers with the intention of adding the separate compounds from the extract and studying their photochemical properties, both spectroscopically and in the ESR apparatus.

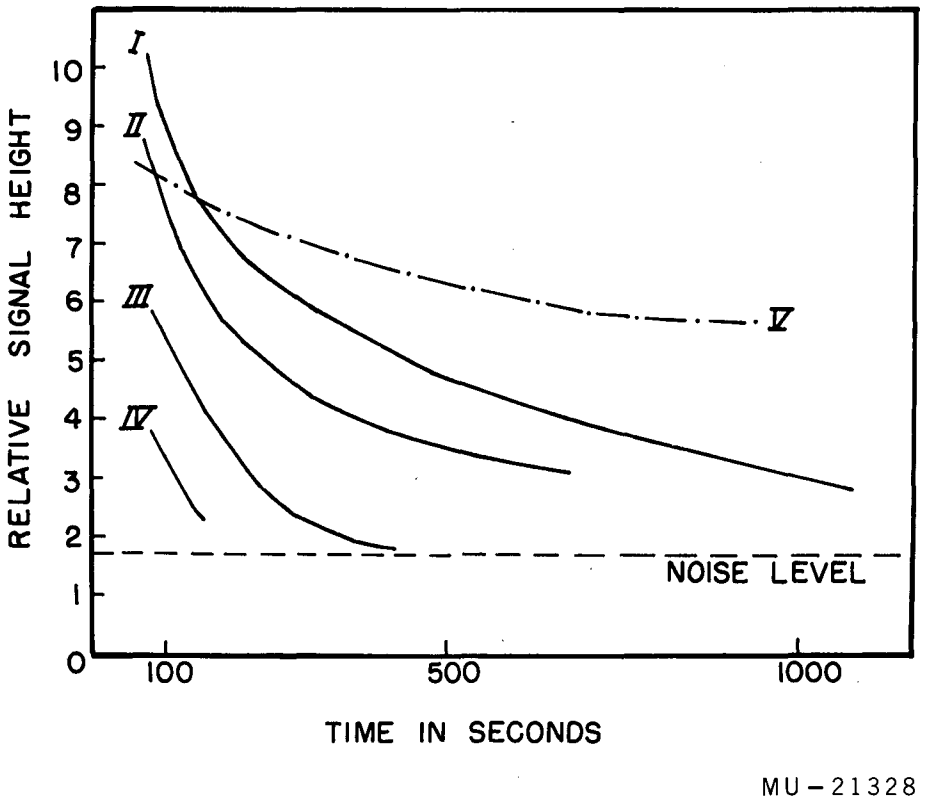

Fig. 14. Decay rates as a function of water vapor pressure.

ELECTRON TRANSFER BETWEEN ORGANIC MOLECULES

John W. Eastman and Melvin Calvin

Halogenated quinones, as electron acceptors, were combined with the following donors: the aromatic hydrocarbon, perylene; and the arylamines, N, N, N', N'-tetramethyl-p-phenylenediamine and N, N-dimethyl aniline.

Perylene

A low concentration of unpaired electron spins was present in the crystalline 1:1 complex perylene:o-chloranil.[1] The concentration depended upon the method of preparation and exhibited a time dependence.

The electron spin resonance (ESR) absorption by the solid was investigated at elevated temperatures. At 90°C the ESR absorption reached a maximum in 2 hours, whereas at 25° C the maximum occurred only after 5 days.

At any given time during the reaction of the complex, the ESR absorption could be reversibly eliminated by dissolving the complex in nitrobenzene. As the solid was dissolved or precipitated, the ESR absorption disappeared or reappeared.

No change in the concentration of unpaired electrons was produced by pulverizing the crystals to a fine powder.

Perylene and o-chloranil react to produce unpaired electron spins which are present only in the solid. There is an imperfection in the solid which contains unpaired electrons. The physical environment of this thermally introduced imperfection is being investigated by saturation characteristics of the ESR absorption.

The width of the ESR absorption increased as the microwave power saturation of the resonance increased. Therefore, the observed width of the line is a result of local molecular interactions and not a result of failure to resolve hyperfine structure.

An increase in the ESR absorption was observed when the solid was illuminated at temperatures above 80° C. Only near-infrared radiation was effective in producing the increase (Fig. 15).

No photoinduced ESR absorption could be detected in ethylene dichloride solutions between -150° and +50° C.

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[1] John W. Eastman and Melvin Calvin, in Bio-Organic Chemistry Quarterly Report, UCRL-9041, Dec. 1959, p. 40.

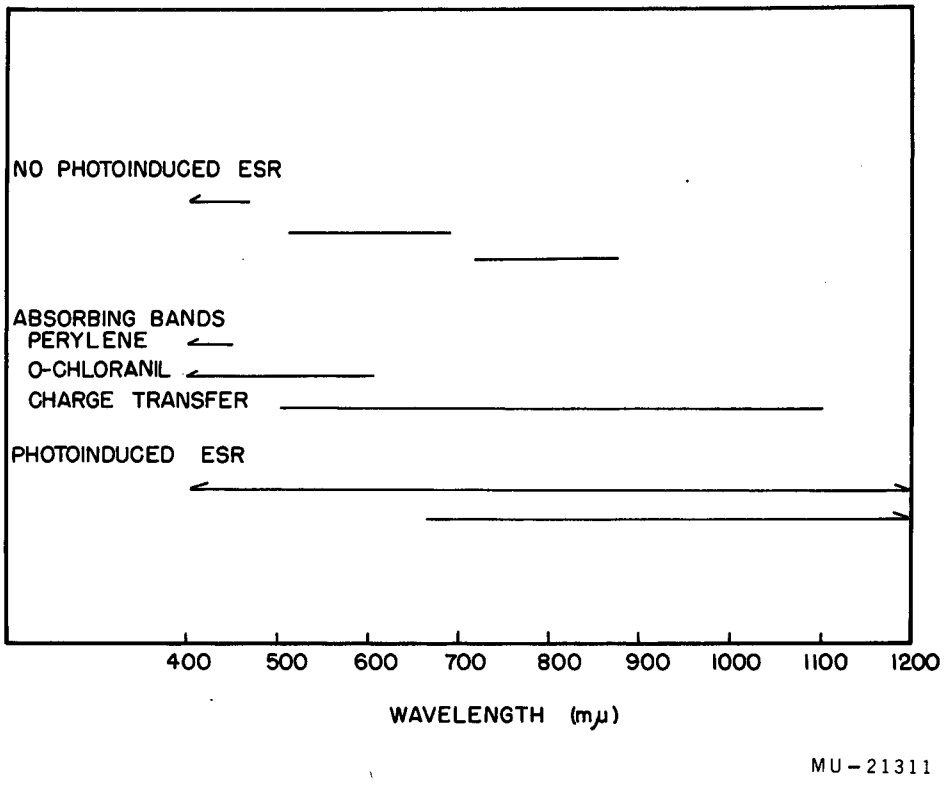

Fig. 15. Comparison of wave lengths. Photoinduced ESR absorption at 80 to 90° C and optical absorption by the crystal perylene:o-chloranil.

N, N, N', N'-tetramethyl-p-phenylenediamine

The solid complex TMØD:p-chloranil contains approximately 11% free radical.[2] When the freshly prepared solid was dissolved in acetonitrile, two distinct ESR absorptions were observed (Fig. 16a). A narrow single line, assigned to the chloranil semiquinone, was superimposed on a set of thirteen triplets, because of the TMØD positive ion. The semiquinone resonance decreased rapidly, and the $TMØD^+$ resonance increased proportionately (Fig. 16b).

Likewise, when the freshly prepared solid was dissolved, the optical absorption by the semiquionone at 450 mμ decayed and the absorption by the positive radical ion at 600 mμ increased.

After these rapid changes were complete, additional reactivity was observed, but the product was unknown. Both the initial rapid reaction and the slower one were independent of the presence of air.

Table IX summarizes the reactions of TMØD (D) with p-chloranil (A), according to our interpretation. In the nonionizing solvent, ethylene dichloride, a charge-transfer complex (AD) is formed. An unknown product (X) is formed in one hour. In acetonitrile a two-electron transfer occurs, producing the dinegative quinone ion and the TMØD positive ion radical. Intermediate in the reaction is the semiquinone (Fig. 16). After several days, further reaction produces an unknown product.

It appears certain that the solid complex TMØD:p-chloranil contains free radicals stabilized within the crystal. The free-radical concentration depends upon the time taken and solvent used to prepare the solid.

N, N-dimethyl aniline

The free-radical intermediates in the reaction of quinones with dimethyl aniline[1] were studied.

The ESR absorption by the radicals was asymmetric. The asymmetry did not change appreciably at the freezing point of the solution, but was somewhat greater at -150° C. The radicals are therefore not completely free to rotate, even in liquid dimethyl aniline.

When tetrafluoro-p-benzosemiquinone was produced in ethanol, five lines, each separated by 4 gauss, were observed by ESR.[3] However, when tetrafluoro-p-benzoquinone (pQF_4) was dissolved in dimethyl aniline, only one line, 4 gauss wide, was observed. Either the ESR absorption is not due to the semiquinone, or the structure collapses to one line.

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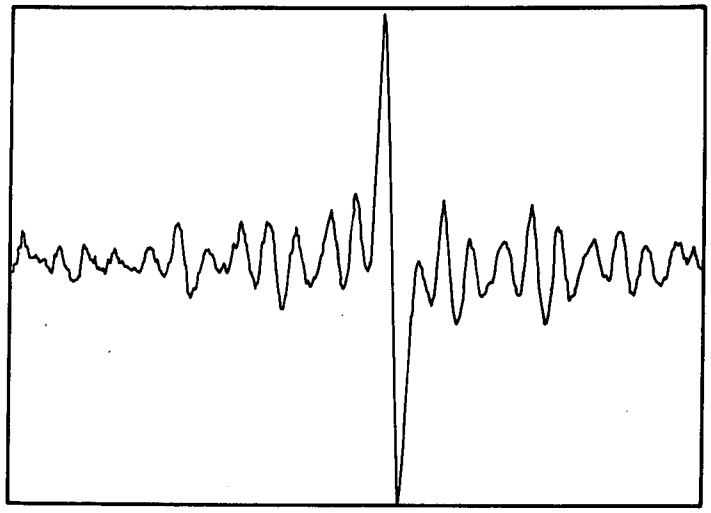
[2] D. Bijl, H. Kainer, and A. C. Rose-Innes, J. Chem. Phys. 30, 765 (1959).

[3] D. H. Anderson, P. J. Frank, and H. S. Gutowsky, J. Chem. Phys. 32, 196 (1960).



A

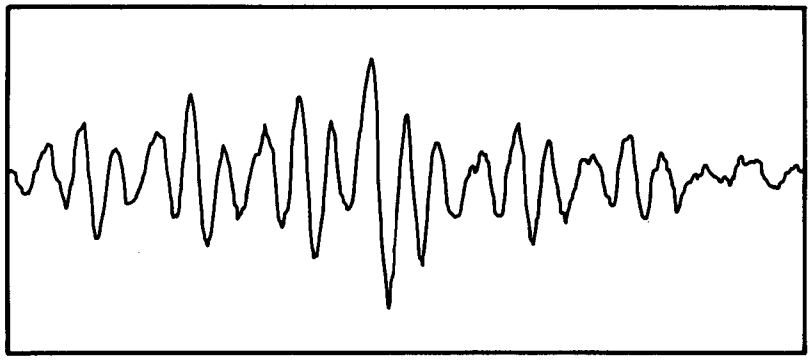


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Fig. 16. Derivative of ESR absorption. TMØD:p-chloranil in acetonitrile. Reaction time: (a) 25 min, (b) 1 hour.

Table IX

Reaction of TMØD (D) with p-chloranil (A)

Solvent and condition	Reaction	Time for complete reaction
Ethylene dichloride	$A + D \rightleftarrows AD$	< 1 min
air	$A + D \rightarrow X$	60 min
Ethylene dichloride	$A + D \rightleftarrows AD$	< 5 min
N_2	$A + D \rightarrow X$	60 min
Acetonitrile	$A + D \rightleftarrows AD$	< 1 min
air	$A + 2D \rightarrow A^{=} + 2D^{+}$	60 min (longer reaction not studied)
Acetonitrile	$A + D \rightleftarrows AD$	< 1 min
high vacuum	$A + 2D \rightarrow A^{=} + 2D^{+}$	30 min
	$A + D \rightarrow X$	10 days

Table X

Comparison of g values

Quinone	Quinone + dimethyl aniline in dimethyl aniline I	Air oxidation of hydroquinone in ethanolic KOH II	Quinone + sodium iodide solid[a] III	Difference I - II or I - III
pQF_4	2.0054[b]	2.0048 ± 0.0006[c]		0.0006
$pQCl_4$	2.0056	2.0058[d]		-0.0002
			2.0044 ± .0008	0.0012
$pQBr_4$	2.0086		2.0068 ± .0010	0.0018
$oQCl_4$	2.0058			
$oQBr_4$	2.0094		2.0077 ± .0008	0.0017

[a] H. Kainer, D. Bijl, and A. C. Rose-Innes, Nature 178, 1462 (1956).

[b] ± 0.0002.

[c] See Reference 3.

[d] J. E. Wertz and J. L. Vivo, J. Chem. Phys. 23, 2441 (1955).

The g values of the radicals observed in this experiment are compared in Table X with those observed by other workers for semiquinones. If one assumes that the g values for the solid salts (Column III) are lower because of radical interaction with the positive ion, then the relative g values measured in this experiment are in excellent agreement with those of other workers for semiquinones. It is concluded that semiquinones are indeed produced when the quinones are dissolved in dimethyl aniline and that the ESR spectra has collapsed to one line in pQF_4.

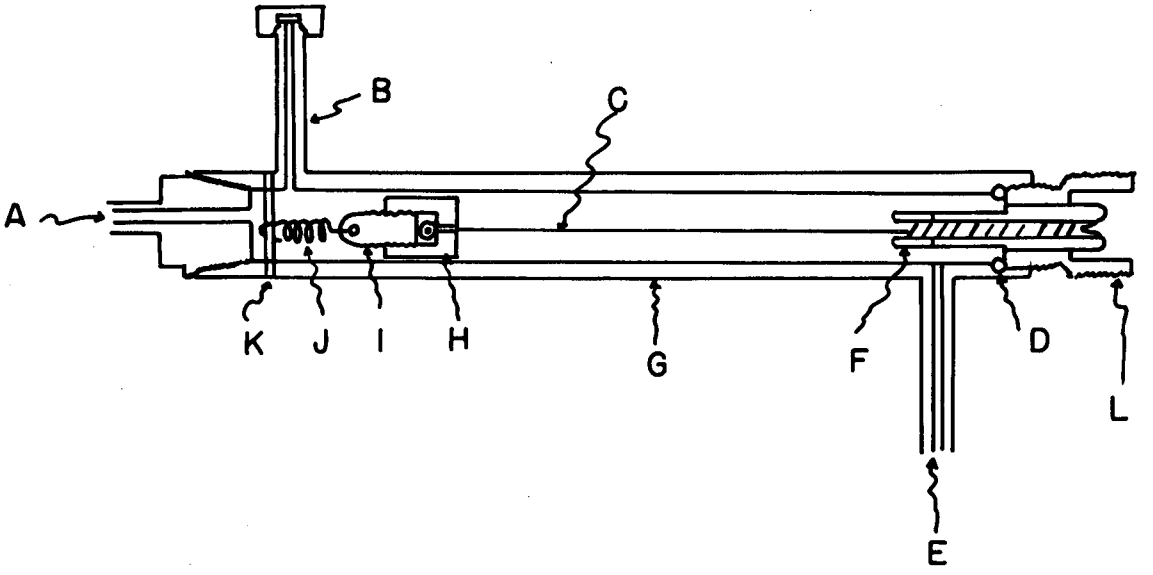
Saturation of the ESR absorption by the pQF_4 radical showed that the width of the absorption increased with increasing power saturation, and with decreasing temperature. Therefore there is no unresolved hyperfine structure as one expects from the narrowed line shape.

The temperature dependence of the ESR did not obey Curie's Law. This indicates the presence of some equilibrium which produces fewer radicals as the temperature is decreased.

The following model is proposed to explain the physical data. Semiquinones are produced in local reactive cages. Several quinone molecules react with the dimethyl aniline and are held in a cage of locally high concentration. Reacted dimethyl aniline prevents the semiquinones from diffusing and undergoing further reaction themselves. Perhaps the semiquinones are in equilibrium with dimers.

When various solvents were added to the reaction mixture, the ESR absorption was destroyed.

When the reaction mixture was centrifuged no change in the radical concentration was observed. If one assumes a solution viscosity of twice the viscosity of dimethyl aniline, and particle density of 1.1 times the density of dimethyl aniline, then from the conditions of centrifugation, the maximum particle diameter for uncharged particles left in solution would be 200 mμ.



MU-21334-A

Fig. 17. Proportional counting tube used in gas chromatography.

A. Connection to chromatography unit.
B. Inlet for auxiliary counting gas
C. 2-mil stainless steel wire soldered to receptacle at one end and to washer at the other.
D. Teflon gasket
E. Same connection as outlet of chromatography unit.
F. Teflon sleeve
G. Brass counter tube
H. Grooved teflon
I. Nylon
J. Weak spring
K. Pin
L. UG-496/U receptacle (modified)

A PROPORTIONAL COUNTER USED FOR DETECTION OF RADIOACTIVE COMPONENTS IN GAS CHROMATOGRAPHY

Irville M. Whittemore

Wolfgang and Rowland[1] and Wolfgang and Mackay[2] have described proportional counters for use in conjunction with gas chromatography to detect radioactive effluent materials. The proportional counter enables one to determine total activities in various components of radioactive mixtures that can be separated by the gas chromatograph. Combination of the mass and radioactivity measurements enables one to calculate specific activities of components without the necessity of trapping components. Trapping may still be done if desired because the components are still separated and not destroyed by being passed through the proportional counter.

A counter similar to that described in Refs. 1 and 2 has been constructed here with certain modifications. The main purpose of these modifications was to eliminate difficulties we encountered with the teflon-to-metal standard taper seal in the earlier counter design. The modified counter is shown in Fig. 17, which indicates the general type of construction. The UG-496/U receptacle was modified by machining off the mounting flange and placing a suitable thread on the unthreaded side. A matching thread was placed on the counter barrel with provision for making a gasket seal. Unless an adapter is used, this type of construction is limited to counters of about 1/2 inch diameter or less, but this is not an important restriction. The connection is tight and makes a good ground contact to the counter body. As the anode wire termination is in what would be the active volume of the counter, it is covered by a teflon sleeve to lower the field gradient in its vicinity sufficiently to keep pulses from occurring there. In other respects the counter is similar to that described earlier.[1, 2]

Wolfgang and Rowland[1] recommend the use of helium as a carrier gas with the addition of an equal amount of methane to the carrier stream after the separation of components and before the entrance of the gas into the counting tube. This makes a suitable counting-gas mixture; helium alone is poor. Pure methane has been our choice as both a carrier gas and a counting gas. The loss of sensitivity in the mass trace (as compared to the use of pure helium) is offset in most instances by the convenience and stability of having only one gas to handle. Plateaus are longer although they occur at somewhat higher voltages. If the highest possible mass sensitivity is desired, helium can be used and methane can be added subsequently.

Our counter was found to be unstable at temperatures above about 60°C. This proved to be a surface effect of the brass tube which could be overcome in two ways. Coating the inside of the tube with Aquadag (an aqueous graphite suspension) has proved effective in stabilizing the counter to temperatures as high as 180° C, but this type of coating introduces some

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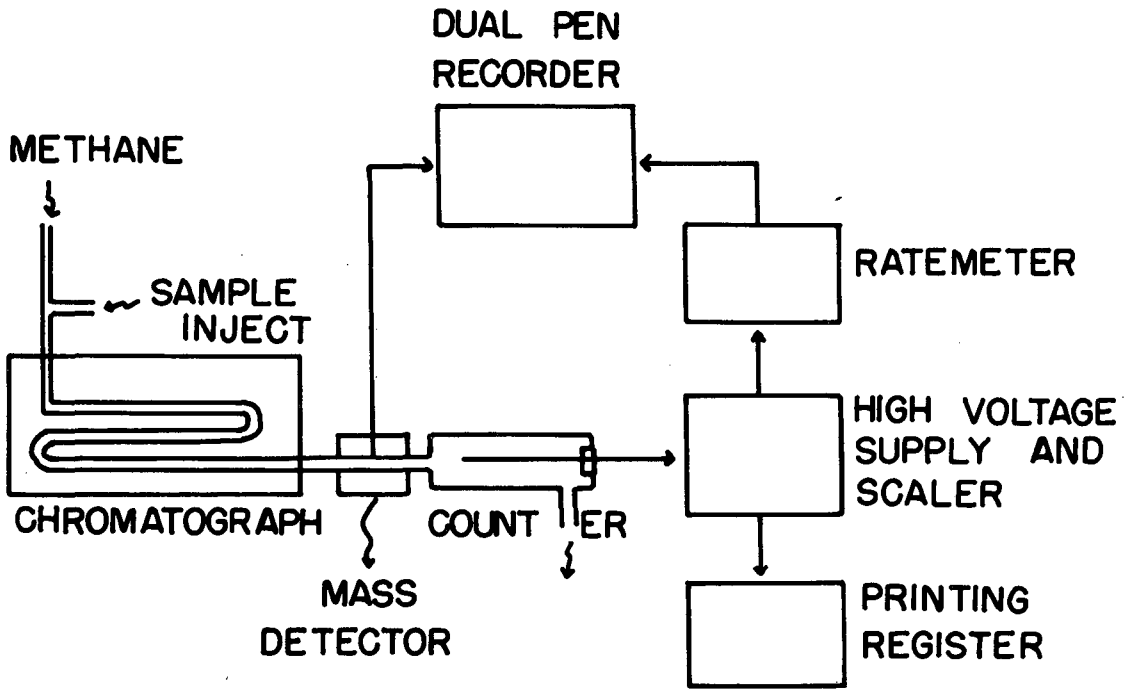
[1] Richard Wolfgang and F. S. Rowland, Anal. Chem. 30, 903 (1958).

[2] Richard Wolfgang and C. F. Mackay, Nucleonics 16, No. 10, 69 (1958).



MU-21335

Fig. 18. Apparatus used for detection of radioactive components in gas chromatography.

chemical problems. The coating is lost in decontamination procedures and, in fact, may be responsible for the retention of some fraction of the components coming through the counter. The Aquadag used by us is in a basic suspension and may retain some of this basicity after it is coated on the counter walls and hence react with acidic components emerging from the chromatograph. As the purpose of the graphite seemed to be to raise the work function of the brass,[3] the tube was gold plated on the inside. Although testing has not yet been completed, it appears that the untreated gold surface is effective in thermally stabilizing the counter.

The block diagram of the apparatus is shown in Fig. 18. This is similar to that described by Wolfgang and Rowland[1] with the addition of one feature. A Tracerlab printing timer (Model SC5A) was modified to act as a printing register. The printer is set to print out the number of register pulses recorded on the scaler in certain intervals (e.g., each minute). Thus, a minute-by-minute record of the counting rate of the tube is printed in a quantitative fashion. This eliminates the necessity of integrating the area under the activity peaks or of recording the counting data in some manual way. The number on the tape, multiplied by the scaling factor, gives the number of counts recorded by the tube each minute. In addition to aiding the quantitative determination of activity, the tape enables one to detect small peaks with more reliability than can be achieved through sole dependence on the trace derived from the ratemeter output.

[3] D. H. Wilkinson, Ionization Chambers and Counters, (Cambridge Univ. Press, Cambridge, 1950), p. 236.