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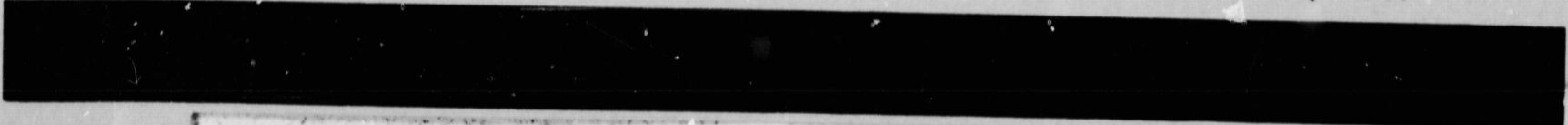
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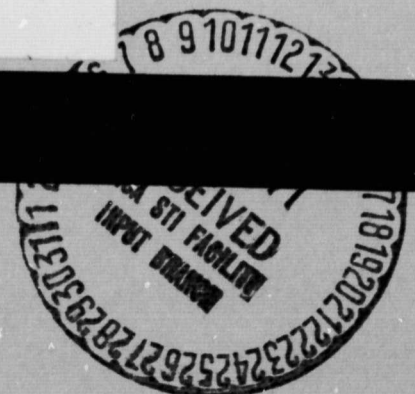
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LONG WAVELENGTH ULTRAVIOLET PHOTOPRODUCTION

OF AMINO ACIDS ON THE PRIMITIVE EARTH

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Abstract

Amino acids are produced under possible primitive Earth conditions by irradiation of gas mixtures with long wavelength ultraviolet light, representing the most abundant useful energy source for prebiological organic synthesis. H_2S is the initial photon acceptor in this work; superthermal H photodissociation products appear to initiate reactions leading to amino acid synthesis with an overall quantum yield $\sim 5 \times 10^{-5}$.

A wide range of experiments (1) has shown that amino acids, and other molecules of importance for the origin of life on the primitive Earth, can be produced readily from a variety of gases, and with a very wide range of energy sources, provided only that the net conditions are reducing. Experiments have been performed utilizing silent and corona discharges, high temperatures, α-,β-,γ- and X-rays, shock waves, acoustical energy, and short wavelength ultraviolet light (uv). As long ago as 1959, Miller and Urey (2) presented a table which showed that uv at wavelengths $\lambda < 2500$ Å provided to the primitive Earth more than 100 times more energy than all non-electromagnetic energy sources combined; and from models of the evolution of the Sun, Sagan (3) calculated that some 4×10^9 years ago the uv photon flux at $\lambda < 2600$ Å was $\sim$ 100 times greater than that in the vacuum uv. The shape of the Planck distribution is very steep on the Wien edge, and one might have anticipated that the longest wavelengths which could accomplish organic photochemistry on the primitive Earth would have been studied extensively. Curiously this has not been the case. The reason is not hard to find: the proposed primary constituents of the primitive reducing atmosphere, hydrogen, methane, ammonia, water, ethane and perhaps CO and N_2 are all entirely transparent at $\lambda > 2400$ Å -- and, except for a very weak absorption by ammonia, all are essentially transparent at $\lambda > 2000$ Å. What is needed is a plausible far uv photon acceptor for the primitive earth.

In this work we consider hydrogen sulfide as such a candidate photon acceptor. In tables of cosmic abundances S ranks with such atoms as Mg and Si, coming just after H, He, O, N, and C; it is a pervasive constituent of volcanic effluvia even today; and under primitive reducing conditions sulfur should have been almost entirely in the form H_2S. Holland (4) calculates on equilibrium grounds that H_2S was a prominent constituent of the primitive atmosphere, with an abundance comparable to that of ammonia and water vapor. H_2S has a broad absorption continuum beginning at about 2700 Å and continuing down to the vacuum ultraviolet (5). Absorption coefficients are large enough that 6 cm-atm of H_2S absorbs (1 - 1/e) of the incident 2537 Å radiation; thus reaction vessels of a few ℓ capacity are adequate for such simulations. The dissociation energy of the HS-H bond is 85-95 kcal/mole (6). The energy of a 2537 Å photon producing a photodissociation event leaves an excess energy of 18-28 kcal/mole; 97% of this excess energy is converted into translational kinetic energy of the H atom, an elementary

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consequence of the conservation of linear momentum. Thus 2537 Å photodissociation of H_2S produces hot H atoms which are superthermal by 17.5 to 27.2 kcal/mole. This is only about 20% of the bond dissociation energy of CH_3-H, NH_2-H and OH-H, but collisional perturbations of the state function of the larger molecule by hot H atoms of this energy is known, e.g., for hydrocarbons, to provide the activation energy for further reactions (6). Accordingly investigations were initiated into the long wavelength uv photochemistry of simulated primitive atmospheres containing H_2S. Other plausible long λ uv photon acceptors can be imagined; and we will report separately successful experiments on the production of amino acids in which formaldehyde is the photon acceptor.

We report here the results of a series of experiments performed over several years; it was initiated at the Smithsonian Astrophysical Observatory and Harvard University and completed at Cornell University. The basic form of the apparatus is shown in Figure 1. A 4 to 13 liter quartz or pyrex reaction vessel (cf. Table 1) is provided with a spectrosil or quartz well, fused on to it such that a mercury line emission source inserted into the well can irradiate gases in the reactior vessel in cylindrical geometry. In some experiments the source is water-cooled and thermally insulated from the reaction vessel by a vacuum dead space. With water cooling the attenuation of the 2537 Å line is negligible, but the attenuation of the 1849 Å line is essentially complete. In other experiments dry nitrogen was used to cool, and in such cases both 1849 Å and 2537 Å irradiation occurred. Copper-constantan thermocouple gauges were inserted in various places in the reaction vessel to monitor the temperature history of the experiment. Irradiated gases were circulated through a liquid water trough by a slightly modified version of the greaseless solenoid pump of the Watson design (7).

Besides H_2S and the equilibrium vapor pressure of H_2O above the liquid, these experiments employed ammonia, methane, and, in all but one case, ethane. Ethane is known both experimentally (8) and by equilibrium thermodynamic calculations to be produced in reducing atmospheres of cosmic composition (9); and photochemical calculations for the Jovian planets indicate that large quantities of ethane may result from the short wavelength ultraviolet photodissociation of methane (10). In the only other experiments on long wavelength uv irradiation of primitive atmospheres which we know of, a Hg sensitization experiment in which mercury vapor was the photon acceptor

(11), glycine, alanine and perhaps α-amino butyric acid were produced -- but only in the presence of ethane; in making amino acids it is evidentally desirable to begin with preformed C-C bonds. In all of our experiments gases were transferred to the reaction vessel through a spiral tube kept at -23° C to -64° C to prevent small quantities of Hg vapor or any trace of stopcock grease from passing into the experimental portion of the vacuum system.

With the precursor gases, one or two Hg resonance lines, the gas temperature (12), and the time history of irradiation as variable conditions, five experiments were performed, as outlined in Table I. Experiments 1 and 2 were extremes: Expt. 1 was ethane-free, 2537 $\overset{\circ}{\text{A}}$ only, cool and brief; Expt. 2 added ethane and the 1849 $\overset{\circ}{\text{A}}$ line, was performed hot and for an extended period. In Expt. 3, 1849 $\overset{\circ}{\text{A}}$ radiation was removed, the gas temperature was dropped and the irradiation time shortened. Expt. 4 was a hot radiation-free control; Expt. 5 a lengthy room temperature 2537 $\overset{\circ}{\text{A}}$ radiation run. These experiments and their results are now described in somewhat more detail.

Experiment 1: The purest commercially available precursor gases were procured and purified by repeated fractional distillations through constant temperature baths until found to be tensiometrically homogeneous. Purity was also checked with a PE Model 620 high-dispersion doublebeam spectrophotometer. Analysis of the gas sample after 14 hours' irradiation with a CEC Model 10C 180° sector mass spectrometer revealed the presence of H_2. The quantity of non-condensable gases at -196° C (H_2, N_2, or possibly some CO) collected at the end of the experiment indicates that several hundred cc of this fraction comes from CH_4, NH_3, or H_2O. Fractional distillation of the gas products at the end of the experiment and subsequent infrared spectroscopy revealed no new molecules with permitted vibration-rotation spectra. There seems little doubt that photolytic hot hydrogen atoms from H_2S did initiate chain reactions. Mass spectra of an evaporated aliquot of the aqueous solution revealed the presence of polymeric sulfur, S_1, ..., S_8,... (Fig. 2). The reaction vessel was removed from the vacuum system and rinsed with water. The presence of elemental S was confirmed by a melting point analysis. After repeated washings and dryings the quantity of S collected indicated that at least 22% of the initial H_2S was photo-dissociated in the experiment. After 14 hours the color of the aqueous solution was a pale yellow. Paper chromatography of this solution performed immediately after the experiment revealed no ninhydrin-positive

features; ion exchange chromatography on a Beckman Model 120C amino acid
analyzer several months after the experiment also gave negative results.

Experiment 2: Here ethane is added, 1849 Å radiation is   introduced,
and the temperature of the reaction vessel is raised to 400° C. In addition
the gas phase photolysis products were isolated from the liquid water bath
for the first 15 hours of the experiment. Finally, 2 mc of $C^{14}H_4$ and 1 mc
of $C^{14}H_3-C^{12}H_3$ were added to the gas mixture, bringing the amino acid detectiv-
ity into the picomole range by autoradiographic paper chromatography. The
temperature of the aqueous solution was varied with heating tapes between
70° and 90° C for the first 9 days of the experiment, providing significant
quantities of water vapor in the reaction vessel; and then dropped to 25° C
for the last 3 days.

A deep orangish-brown viscid (polymeric) material appeared within minutes
of turning on the Hg discharge, and continued to develop throughout the exper-
iment. Mass spectra of this dark liquid performed on an AEI Model MS-902 mass
spectrometer revealed peaks well above the background at m/e = 188, 220, 260
and 280, among others. No attempt to interpret this data is made here. The
liquid was examined for amino acids by 2-dimensional chromatography on
Whatman #1 chromatographic paper. The solvents were 10 methanol: 10 n-butanol:
5 water: 2 ammonia, and 10 acetone: 10 n-butanol: 5 water: 2 ammonia. The
paper chromatograms were examined by ninhydrin spray and by autoradiography
with Kodak Medical X-ray Film Type NS-54T after one month's exposure. In
addition a Hitachi Model KLA-3B amino acid analyzer was employed. Identifi-
cations were confirmed by adding standard chromatographically pure solutions
of candidate amino acids to the experimental liquid. Amino acids found by
both autoradiography and ion exchange chromatography are listed in Table 2.
This represents the first successful synthesis of the sulfur-containing amino
acid cystine;   we discuss the synthesis of sulfur-containing amino acids
under prebiological conditions in more detail elsewhere (15). One ninhydrin-
positive spot was eluted from the paper and analyzed with a mass spectrometer.
Its peak mass number and cracking pattern are consistent with those of urea.

After two decades of experience with prebiological organic chemistry, ex-
perimenters rarely check to see whether the molecules they discover could be
produced by microorganisms metabolizing other experimental products. To
check this remote possibility, we mixed 0.25 ml solution from Expt. 1 with
0.2 ml solution from Expt. 2. This mixture was divided into two parts, the
first immediately analyzed with an amino acid analyzer; the other after

standing one month. The concentration of all amino acids remained fixed with-
in experimental error, except for serine which declined in abundance signifi-
cantly after one month; but serine is (with threonine) the most thermolabile
amino acid known (16).

Experiment 3: This experiment differs from Expt. 2 in that 1849 Å rad-
iation is not employed, and the gas phase photolytic products are not isolated
from the liquid solution at any time in the experiment. The liquid solution
again turned a deep viscid orange-brown. Alanine, glycine, serine, and
cysteine were observed.

Experiment 4: This control experiment on the absence of uv was performed
by constructing the entire reaction vessel of pyrex borosilicate glass which
is uv-opaque. In contrast with previous experiments, the liquid medium here
remained colorless throughout the experiment. Also in contrast with the pre-
vious three experiments, the residual vapor pressure over a liquid nitrogen
trap at experiment's end was only 10 mm Hg, indicating no evolution of H_2.
Both paper chromatography and the amino acid analyzer gave negative results
for amino acids.

Experiment 5: This experiment was a room temperature 2537 Å irradiation.
After seven hours of photolysis, 2 mc of $C^{14}H_4$ were introduced. After two days
of photolysis, additional H_2S was introduced into the reaction vessel. Drops
of brownish liquid were observed forming during the course of the experiment,
but the coloration of the liquid medium at the end of 25 days of photolysis
was a slightly greenish yellow. The quantity of residual gas (mainly H_2) not
condensable at -196° C after 2 days was calculated to be several l.

Evaporites from the liquid solution were analyzed on a Beckman Model
120B amino acid analyzer. In addition 0.5 ml was dried under N_2 and hydro-
lyzed for 22 hours in 5.7 N HCl at 105°C. It was then dried under vacuum and
also introduced into the amino acid analyzer. From the first sample 0.006
μmole/ml of alanine was detected. The acid hydrolysis fraction however
yielded 0.077 μmole/ml of alanine, some 13 times more. Glycine, serine,
cystine and aspartic and glutamic acids were also detected in the acid
hydrolysis fraction. This result strongly suggests that amino acids in such
experiments are not primarily made as free amino acids, but rather in some
other form -- possibly nitriles or polypeptides -- from which they are
released on hydrolysis.

By C^{14} counting of the products of this experiment, we find that more
than 2% of the initial methane ahs gone into liquid or solid products, estab-

lishing that chain reactions initiated by 2537 Å photolysis of H_2S eventually lead to CH_4 dissociation and participation in subsequent reactions. Hydrocarbon sulfides such as $(C_2H_5S)_2$ and $(C_2H_5)_2S$ are known gas phase intermediaries in such experiments (17).

Energetics: We can perform a crude calculation of the energetics and quantum yields in such experiments from the results of Expt. 2, where a total $\sim$ 1 μmole/cc or $\sim 10^{19}$ molecules of amino acids were formed. The Hanovia SC 2537 and S654 A lamps employed emitted $\sim 1.6 \times 10^{13}$ ergs at 2537 Å during the experiment. We neglect the much smaller amounts of 1849 Å and other line and continuum emission. From the initial proportion of H_2S employed, the 5 cm mean light path through the reaction vessel, and the 2537 Å absorption coefficient of H_2S (5), we calculate that $\sim$ 10% of the radiation emitted at the beginning of the experiment was absorbed by H_2S; and rather less towards the end of the experiment. Thus $\sim 6 \times 10^6$ amino acid molecules were produced per erg absorbed. We compare this efficiency number with the 5×10^{10} per erg found in comparable shock experiments (18). Thus it appears that shocks are $\sim 10^4$ times more efficient than uv for prebiological organic synthesis. But $\lambda < 2600$ Å uv was at least 10^3 times more abundant on the primitive Earth. If we allow for the facts that (a) only free amino acids and not the products of acid hydrolysis were analyzed in Expt. 2; that (b) at $\lambda < 2537$ Å the H_2S photolytic products are more energetic than at 2537 Å; and that (c) other photon acceptors -- e.g., HCHO -- may be effective at $\lambda < 2537$ Å, we conclude that overall uv and shock energies were of comparable importance in the prebiological synthesis of amino acids. A previous conclusion (18) that shocks were more important relied on earlier uv quantum yields from Hg sensitization experiments (11). The quantum yield per 2537 Å photon found in our experiment is $Q \sim 5 \times 10^{-5}$, about an order of magnitude more than found in (11). Assuming all $\lambda < 2600$ Å photons were absorbed by H_2S on the primitive Earth, and that no subsequent destruction of amino acids occurred, this Q and the photon flux for the early Sun (3) imply ~ 200 Kg cm^{-2} of amino acids produced. Destruction of course must have occurred, but a very high concentration of organic compounds and conditions quite congenial for the origin of life seem to be suggested.

Conclusions: The principal conclusions of this work follow: a) Hydrogen sulfide is an acceptable long wavelength photon acceptor for prebiological organic chemistry. The 1849 Å line is not necessary for the production of amino acids, but initial ethane is. Ethane is a likely product

of electrical discharge and short wavelength uv irradiation of methane. Temperatures higher than plausible mean surface temperatures are not required.
(b) A quantum yield $Q > 5 \times 10^{-5}$, and an efficiency number $\lesssim 6 \times 10^{6}$ amino acid molecules per erg are implied. Over 10^{9} years of uv irradiation of the early Earth this is the equivalent of 200 Kg cm^{-2} of amino acids produced, a huge number suggesting congenial conditions for the origin of life. (c) Cystine and therefore perhaps other sulfur-containing amino acids can be produced in simulated prebiological conditions (19).

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Table 1

Summary of Experiments

Variable	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Volume (in cc) and composition of reaction vessel	3780, Quartz	4195, Quartz	4189, Quartz	5673, Pyrex	12686, Pyrex
Precursors (quantities in cc)	CH_4 1128; NH_3, 1150; H_2S, 1150; liquid H_2O, 10	CH_4, 591; C_2H_6, 2010; NH_3, 1000; H_2S, 549; liquid H_2O, 15	CH_4, 612; C_2H_6, 2024; NH_3, 1004; H_2S, 542; liquid H_2O, 16	CH_4, 801; C_2H_6, 2738; NH_3, 1366; H_2S, 726, liquid H_2O, 21	CH_4, 1638; C_2H_6, 6055; NH_3, 3035; H_2S, 1678[a]; liquid H_2O, 50
Emission line	2537 Å	2537 Å, 1849 Å	2537 Å	None	2537 Å
Gas temperature	20° C	400° C	215-360° C	300-400° C	50° C
Trap temperature	-23° C	-23° C	-64° C	-64° C	-64° C
Irradiation time	14 hrs.	(2537 + 1849), 2 days; 2537, 1/2 day; (2537 + 1849), 9 days.	30 hrs.	11 days	25 days
Amino acids detected in liquid solution	None	Ala, gly, ser, glu, asp, cys	Ala, gly, ser, glu, asp, cys	None	Ala, ...[d]
Other products	$S_1, \ldots, S_8, \ldots$	$S_1, \ldots, S_8, \ldots$ and unidentified high mass number products	Unexamined	Unexamined	Unexamined
Appearance of liquid solution	Pale yellow	Deep viscid orange-brown	Deep viscid orange-brown	Colorless	Greenish yellow; brown droplets formed in glassware

[a] After 2 days' irradiation an additional 989 cc of H_2S was introduced into the system.

[b] Liquid water was isolated from gas phase photolytic products for first 15 hrs. of this experiment.

[c] Only amino acids confirmed both by C^{14}-autoradiography and an amino acid analyzer are listed.

[d] Yield after acid hydrolysis is ten times greater and includes gly, ser, glu, asp and cys.

Table 2

Amino Acid Yields (in μmole/ml) from Expt. 2

ala	gly	cys	ser	glu	asp
0.976	0.092	0.042	0.010	0.010	0.005

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(1) See, e.g., S.L. Miller, J. Amer. Chem. Soc., 77, 2351 (1955); P.H. Abelson, Ann. N.Y. Acad. Sci., 69, 274 (1957); C. Ponnamperuma and N.W. Gabel, Space Life Sci., 1, 65 (1968); D.H. Kenyon and G. Steinman, "Biochemical Predestination" (McGraw-Hill, New York, 1969); M. Calvin, "Chemical Evolution" (Oxford University Press, New York, 1969); N.H. Horowitz, F.D. Drake, S.L. Miller, L.E. Orgel, and C. Sagan, Chapter 5 of "Biology and the Future of Man," P. Handler, ed. (Oxford University Press, New York, 1970).

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(12) The most reasonable estimate for the temperature of the primitive Earth is something around room temperature, but there may have been localized times and places when surface temperatures were higher; upper atmospheric temperatures were of course much higher. Methane is not successfully attacked by radicals and hot hydrogen until it is brought to a temperature of some 300°C (13). Accordingly in some experiments temperatures of 300°-400°C were employed. Amino acids have been produced by heat alone from a reducing atmosphere, but temperatures of 900° to 1100°C are required (14); we show below that temperatures of 300° to 400°C alone will not produce detectable amino acids.

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Figure Captions

Figure 1:   Schematic diagram of a typical experimental setup. Gases are
     irradiated by cooled uv source at upper right and circulated by
     greaseless solenoid pump at upper left through water both at lower
     left.

Figure 2:   Mass spectra of an evaporated aliquot of aqueous solution from
     Expt. 1 performed on a CEC Model 10C 180° sector mass spectrometer.
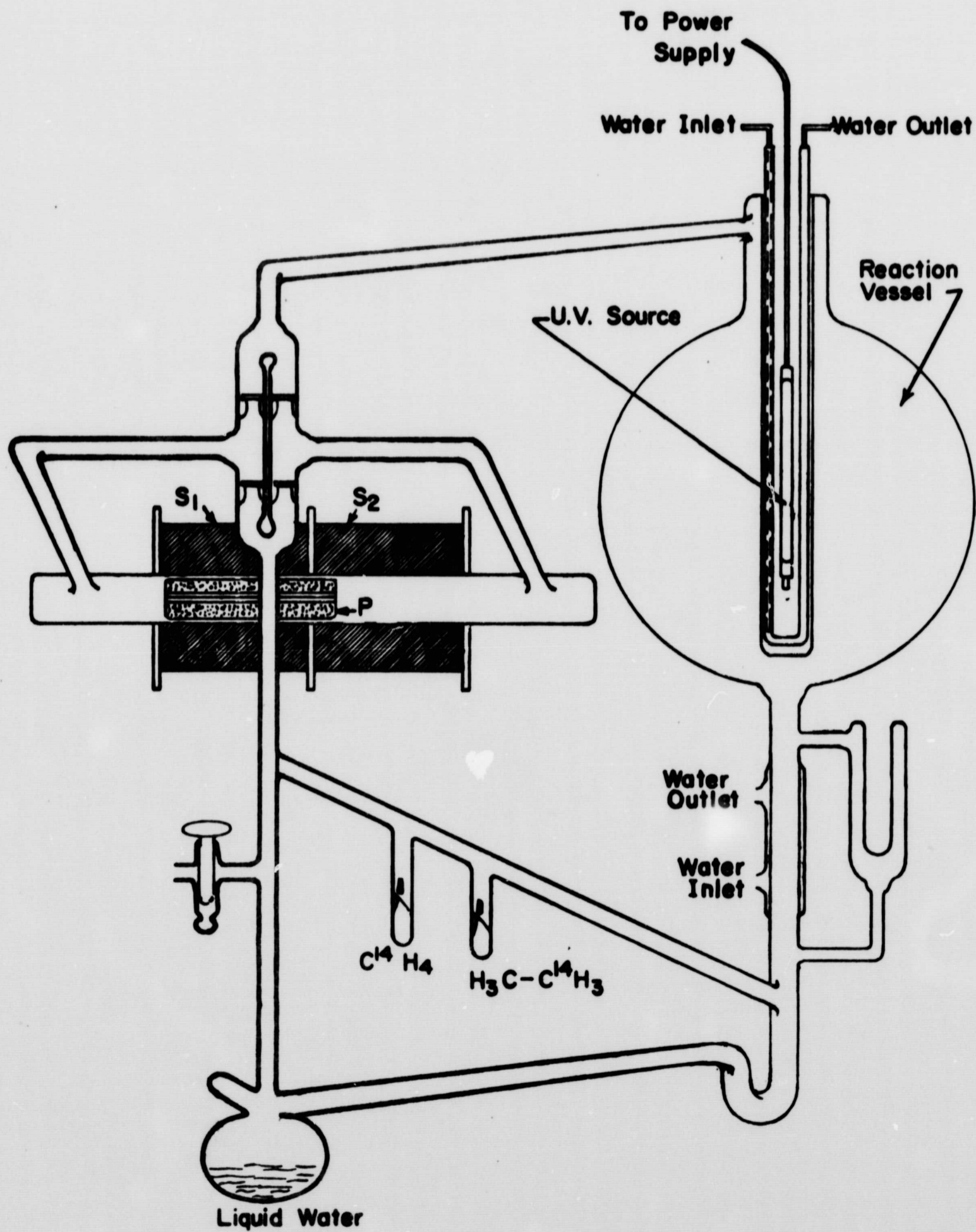   Lines are seen for polymeric sulfur to S_8, each with its satellite
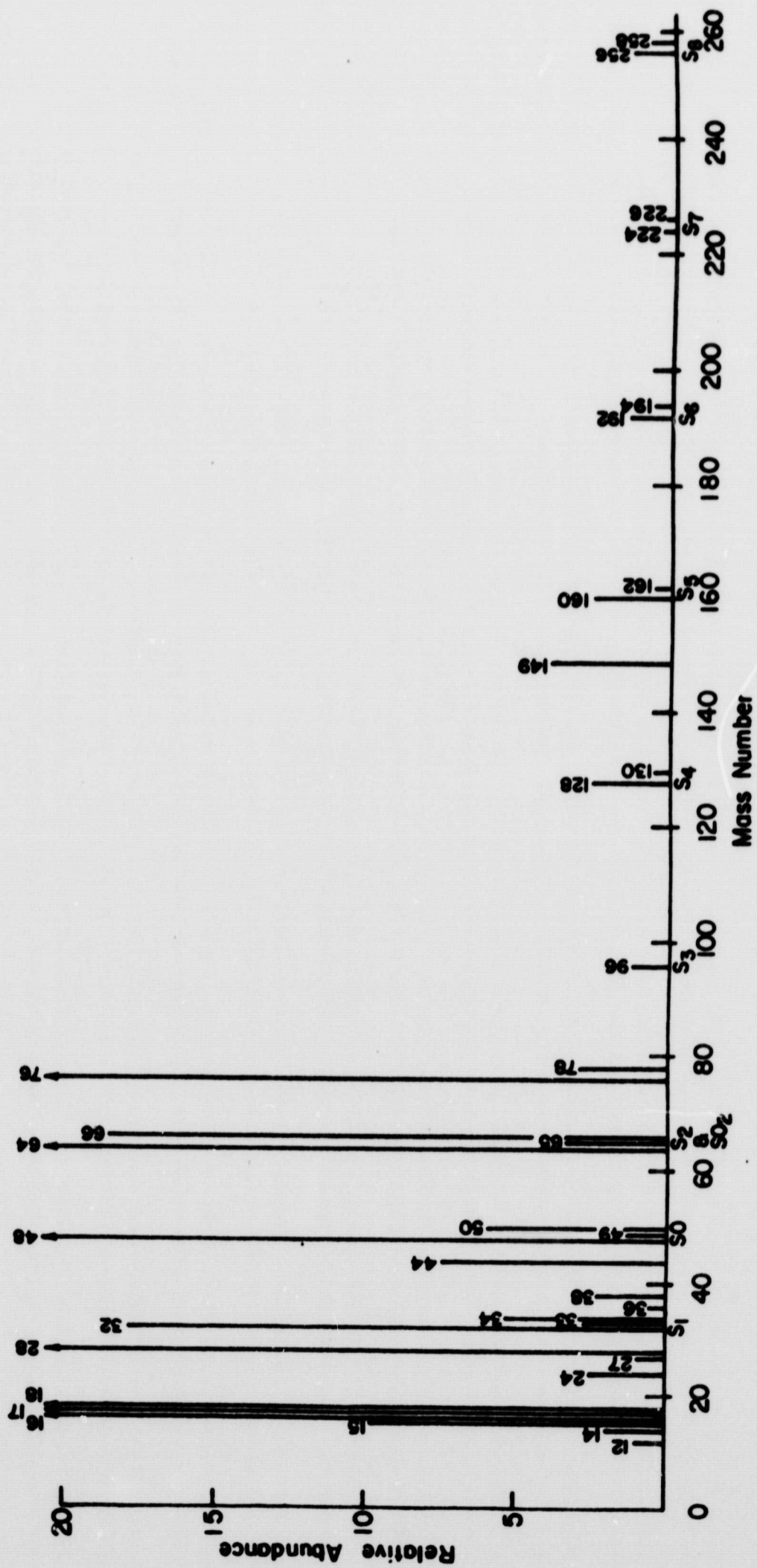 S^{34} line.

To Power
Supply

Water Inlet — Water Outlet

U.V. Source

Reaction
Vessel

S_1

S_2

P

Water
Outlet

Water
Inlet

$C^{14}H_4$

$H_3C-C^{14}H_3$

Liquid Water

Figure 1

Figure 2.