THE PHOTOOXIDATION OF TRYPTOPHAN BY VISIBLE LIGHT IN THE PRESENCE OF METHYLENE BLUE

By

JE HYUN KIM

Bachelor of Science

Seoul National University

Seoul, Korea

1953

Submitted to the faculty of the Graduate School of
the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
August, 1962

THE PHOTOOXIDATION OF TRYPTOPHAN BY VISIBLE LIGHT IN THE PRESENCE OF METHYLENE BLUE

Thesis Approved:

L M Henderson
Thesis Adviser

Franklin R. Leach

Dean of the Graduate School

ACKNOWLEDGMENTS

The author is sincerely grateful to Dr. L. M. Henderson for his constant encouragement and kind guidance during the course of this experiment. He also wishes to express appreciation to Dr. Franklin R. Leach and Dr. R. A. Mitchell for discussions and for reading the manuscript.

The author would like to thank Mr. George V. Odell for helpful discussions of these experiments.

He is indebted to the Department of Biochemistry for facilities and financial support during this study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (CONTINUED)

INTRODUCTION

Two approaches have been used to study the effect of radiation on compounds of biological importance. One involves exposure of the living organism to the radiation and the other is the exposure of model compounds which may yield products similar to those formed in the living cell. Using the second approach in most instances biological activity was lost during the early stages of photooxidation. Thus Oster et al. (1) observed that tobacco mosaic virus was inactivated by ultraviolet light although no chemical, serological or physical changes were detected, and crystallization of the virus was still possible. There is now much evidence that loss of biological activity of proteins is associated with the destruction of the easily oxidizable amino acid residues, such as tryptophan.

In spite of the biological importance of the photooxidation of tryptophan, little work has been done to clarify the nature of the reaction, or to identify the photooxidation products. This thesis presents the results of a study of the photooxidative degradation of tryptophan labeled specifically with C^{14} in different positions of the molecule. Attempts to isolate and characterize the major degradation products are also described.

LITERATURE REVIEW

A. The Importance of Sensitizers in Photooxidation by Visible Light.

Photosensitized biological processes were observed by Raab in 1899 (2) who found that a very dilute solution of acridine killed paramecia in six minutes in direct sunlight, but had no effect during long periods in darkness. Many workers have studied the lethal effects of light on bacteria (3, 4, 5, 6), fungi (7, 8, 9) and bacteriophage (10) as well as the destructive effects upon their toxins (11) using methylene blue and other dyes as sensitizers. Jodlhaver and Tappeiner found that certain enzymes and toxins were destroyed by a light dependent reaction, but only in the presence of oxygen. Moreover, it was found that the oxidation occurred without a photosensitizer when ultraviolet light was used. With visible light, however, the photosensitizer was needed (12, 13). The hemolysis of red cells is similarly sensitized by eosin or rose bengal, and prevented by the addition of tryptophan (14). Hematoporphyrin, a product of the decomposition of hemoglobin, acts as a photosensitizer causing marked hemolysis.

B. Biological Effects of Photooxidation on Vertebrates.

In whole vertebrates the effects are more complex and irregular. Obviously, in multi-cellular organisms the only part which can be directly affected is the outer surface, since the penetrating power is limited.

Thus, in mammals the primary effects are found in the skin, but

they may be accompanied by marked secondary effects in distant organs, e.g., the circulatory, respiratory and central nervous systems. The problem is complicated by the fact that the symptoms vary with conditions of irradiation and dosage of sensitizer. Hausmann (15) described the following groups of symptoms for white mice sensitized with hematoporphyrin under different conditions: (a) under extreme conditions of irradiation or dosage of sensitizer the animal may go into a coma in a very few moments and die shortly after, (b) under less severe conditions an acute form occurs in which the animal scratches vigorously at first and waltzes about, the ears soon become inflamed and animal blinks the eyelids and attempts to avoid the light; shortly afterwards he becomes weak and dyspneic; death may occur in a few hours, (c) in sub-acute conditions, severe edema of the skin appears, and (d) with still less severe conditions the ears become necrotic and may slough off completely. The hair may fall out in a ring shaped arc around the eyes.

Sheard et al. (16) have observed that animals fed exclusively on buckwheat may develop serious symptoms (fagopyrismus) which occurred only when the animals were exposed to sunlight. The animals jumped around, probably because of sensory stimulation from skin irritation. They develop severe skin eruptions, and the results are often fatal. Only white animals or animals having white spots are affected. This is because the skin of the dark areas is protected by the absorption of light by the pigmented hair.

A wide variety of other physiological effects and sensitizers have been enumerated by Blum (17). In his review he mentioned inhibition of clotting of the blood in the presence of light and methylene blue, stimulation of nerves, contraction of isolated skeletal muscle bathed by the

photosensitizer solution, and production of arrythmias in isolated turtle or mammalian heart perfused with photosensitizing solutions and exposed to light.

C. The Site of Photooxidative Attack.

Numerous investigators have suggested that the proteins comprise the site of photooxidative attack in the cell. Schmidt and Norman (18) have observed that hemolysis by eosine and sunlight could be prevented by the presence of tyrosine and tryptophan or by proteins containing these amino acids. No protection was observed with other amino acids or with gelatin which contains neither tyrosine nor tryptophan. They therefore suggested that these easily oxidizable amino acids in the protein molecule are the point of attack in the cell. Weil and Buchert (19, 20) found that in the presence of methylene blue the photooxidation of the histidine and tryptophan residues of β-lactoglobulin was very rapid and occurred with oxygen uptake equal to that found with isolated amino acids. Oxidation of cysteine was slower and that of tyrosine began only after the complete oxidation of tryptophan and histidine. These changes in β-lactoglobulin were accompanied by a marked shift in absorption toward shorter wave lengths as well as a general rise in absorption over the entire ultraviolet range. There was also a considerable decrease in solubility at the isoelectric point. No additional amino groups were detectable at the end of the experiment, indicating that the peptide bonds remained intact. Gelatin and salmine, which contain little or no tryptophan, histidine, tyrosine, cysteine or cystine, took up only minute amounts of oxygen when irradiated.

Recent experiments on crystalline enzymes have shown essentially the same thing. When lysozyme and chymotrypsin were irradiated with

visible light in the presence of methylene blue, oxygen was taken up by the solution, tryptophan was oxidized and loss of enzymatic activity occurred. This finding indicates that tryptophan is the site of photo-oxidation. Tyrosine seems to be oxidized at a slower rate (20, 21, 22). Shugar (23) compared the behavior of crystalline lysozyme and ribonuclease (which contains no tryptophan) when irradiated with visible light in the presence of riboflavin. He found that only lysozyme was inactivated, which again emphasizes the importance of tryptophan for photosensitized inactivation. Weil et al. (24) also studied the photooxidation of crystalline ribonuclease in the presence of methylene blue, and found that complete inactivation of the enzyme occurred when three of the four molecules of histidine had been oxidized.

The experiments of Lieben (25) demonstrated the decomposition of tryptophan and tyrosine in the presence of visible light and hematoporphyrin or rose bengal. Carter (26) found that of a large number of compounds examined only ring compounds containing a hydroxy or amino group were susceptible to photooxidation by visible light in the presence of sensitizers (methylene blue or fluorescent dyes).

More detailed study of the action of visible light on susceptible substances in the presence of sensitizer is desirable to understand the many physiological phenomena described above. Weil has suggested on the basis of spectrophotometric evidence that the oxidation of the pyrrole ring of tryptophan was faster than that of the benzene ring (27).

Although no evidence for hydrogen peroxide formation has been found, formation of organic peroxides, not susceptible to the action of catalase, during tryptophan and tyrosine irradiation is probable (28, 29).

Photooxidation studies employing tryptophan labeled with C^{14} in

specific positions of the ring or side chain may be expected to provide data which would elucidate the mechanism of this process.

MATERIALS AND EXPERIMENTAL METHODS

A. Materials.

DL-Tryptophan obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio, was used in unlabeled experiments. The DL-tryptophan-7a-C^{14} (266 μc per mmole) was synthesized by Henderson et al. (30) and the DL-tryptophan-5-C^{14} (290 μc per mmole) was synthesized by Mathur and Henderson (31). The DL-tryptophan-α-C^{14} (233 μc per mmole) was obtained from Tracerlab, Inc., Waltham, Massachusetts.

Methylene blue (double zinc salt) was obtained from Allied Chemical and Dye Corporation, New York, New York. The known compounds used as standards in paper chromatography were obtained commercially and used without further purification.

Scintillation solution was prepared with 6 g of 2,4-diphenyloxazole (Packard Instrument Company, Inc.), 0.3 g of 1,4-bis-2-(5-phenyloxazoyl)-benzene (Packard Instrument Company, Inc.), 900 ml of sulfur-free toluene (Eastman Organic Chemicals, New York) and 600 ml of absolute ethanol.

B. Methods.

1. Photooxidation Techniques

a. Manometric studies

Oxygen uptake was measured during the photooxidation of tryptophan using Warburg apparatus. In the earlier experiments 10 μmoles of DL-tryptophan and 100 μmoles of potassium phosphate buffer (pH 8.5) (total volume 1.5 ml) were placed in the Warburg flask which also contained

in the center well 0.1 ml of 20 per cent potassium hydroxide. After equilibrating for 10 minutes at 37°, 0.5 ml of 0.02 per cent methylene blue was tipped in from the side arm, and the flask was exposed to light from a 150 watt Westinghouse spot light. The lamp was located approximately 40 cm from the liquid level of the solution in the Warburg flask.

In the studies of the photooxidation process DL-tryptophan-α-C^{14}, DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14} were used. At the end of the reaction the amount of $C^{14}O_2$ in the center well was determined as described in Part 2 of this section. Aliquots of the photooxidized mixture were removed at various times by tipping the reaction mixture into the side arm, and then withdrawing a sample with a micropipette. Other experiments were conducted using different concentrations of tryptophan, methylene blue and phosphate buffer as described in the following sections. In some experiments no potassium hydroxide was placed in the center well, $C^{14}O_2$ was swept out with air and absorbed in Hyamine X-10.

b. Larger scale photooxidation experiments

In these experiments, 0.5 μmoles of DL-tryptophan-7a-C^{14} and 25 μmoles of unlabeled DL-tryptophan, 5 mmoles of potassium phosphate buffer (pH 8.5), 5 mg of methylene blue solution were placed in a 250 ml beaker in a total volume of 71 ml and the beaker was exposed to light for 16 hours in a Precision Shaking Incubator. In another experiment, 10 μmoles of DL-tryptophan, 2 mg of methylene blue and 2 mmoles of potassium phosphate buffer (pH 8.5) in a total volume of 28 ml were introduced into an Erlenmeyer flask and exposed for 8 hours to two spot lights. The distance from light source to the sample was 23 cm. The intensity of the light measured with a Bertram Chrostar light meter

was 13. Afternoon indirect light from distant landscape objects was 11 on the same meter.

c. Very large scale photooxidation experiments

Very large scale experiments were performed in an attempt to obtain pure photooxidation products. Two hundred μmoles of DL-tryptophan, 16 mmoles of potassium phosphate buffer (pH 8.5) and 40 mg of methylene blue in a total of 560 ml were introduced into a Roux culture bottle. The bottle was placed in a Precision Shaking Incubator and illuminated by two Westinghouse spot lights located at a distance of 25 cm.

2. Products Formed During Photooxidation

In the first experiment Darco G-60 was used to remove the methylene blue as described by Weil. Tryptophan and kynurenine were identified in the filtrate by examination of paper chromatograms (32) under ultraviolet light and after ninhydrin spraying (Table I).

To check on the adsorption characteristics of Darco G-60 several tryptophan metabolites and other amino acids were treated with this adsorbent. Tryptophan, kynurenine, 3-hydroxykynurenine, 3-hydroxyanthranilic acid and methylene blue were adsorbed but alanine, aspartic acid and serine were not. In subsequent experiments the charcoal treatment of the reaction mixture was omitted and the methylene blue was not removed.

Twenty μl of photooxidation products were removed from the side arm of the Warburg flasks at specified intervals, and spotted on Whatman No. 1 paper. The chromatograms were developed by the descending technique using the organic phase of freshly prepared n-butanol:acetic acid:water solvent. In some cases 20 per cent potassium chloride solution (33),

methanol:n-butanol:benzene:water = 4:2:2:2 (34), 80 per cent phenol, ethyl ether:88 per cent formic acid:water = 5:2:1 (35), and ethyl cellosolve:ammonia:water = 80:5:15 (36, 37) were also used.

The chromatograms were examined using a short-wave ultraviolet light (Model SL 2537, Fisher Scientific Company) and a long-wave ultraviolet light (Model 3660, Fisher Scientific Company). Color tests were made using 0.2 per cent ninhydrin in 10 per cent v/v acetic acid in water (38), Ehrlich's reagent (39, 40), methyl red (41), Nessler reagent, diazotized p-nitroaniline (42), dipping reagent consisting with dimethylaminobenzaldehyde in 100 ml of concentrated HCl:acetone = 10:90 (43) and ammoniacal silver nitrate (44).

The paper strips were scanned with a Radio-Chromatogram Scanner, Model 1620 A, C-100B, Nuclear-Chicago, connected to Rectilinear Galvanometric Recorder of Texas Instruments Inc., or with a Model RSC-Scanner of Radiological Service Company Inc. Radioactivity in each peak was determined from the total area under the curve using a planimeter.

The oxygen consumed during photooxidation was measured using manometric techniques as previously described and the amount of radioactive $C^{14}O_2$ released was determined with the vibrating reed electrometer. Filter paper in the center well of the Warburg flask was removed after 8 hours illumination and the center well was washed twice with a small amount of distilled water. The paper and washings were introduced into a wet combustion tube and an excess of perchloric acid added. The radioactive $C^{14}O_2$ released was swept with carrier carbon dioxide into a 250 ml ionization chamber (serial No. 3095; Applied Physics Corporation, Pasadena, California) and the radioactivity determined (45) using a vibrating reed electrometer, Model 31, Applied Physics Corporation. In

the experiments using Hyamine X-10, 0.5 ml of Hyamine solution from the gas washing bottle was removed at specified times and added to 10 ml of scintillation solution for counting in a Tricarb Liquid Scintillation Spectrometer.

3. Attempted Isolation and Purification of Products

Cation exchange resin beds were prepared as follows: Dowex-50W-X8(H^+), 100 to 200 mesh, was sedimented in deionized water and the fine particles were removed by decantation. The washed resin was packed without pressure in a column (1.4 x 56 cm) to a height of 8 cm and washed with 50 ml of 5 N hydrochloric acid. This column was used in investigating the products from the manometric study. Large scale experiments were performed on a 5.6 x 44 cm column packed with Dowex-50W-X8(H^+) resin. The resin was washed with 1 liter of 5 N hydrochloric acid. In small scale experiments a Dowex-50W(H^+) column (4 x 50 cm) was used.

In all cases, the columns were washed with distilled water until free of chloride ion. After introduction of the sample on the column, it was developed with water and hydrochloric acid. The concentration of hydrochloric acid was increased gradually as shown in Fig. 8.

Dowex-2-X8(Cl^-), 50 to 100 mesh, was sedimented in the same manner as the Dowex-50W(H^+) resins. The column (1.4 x 14 cm) was packed with Dowex-2(Cl^-) resin without pressure for the separation of the first peak from Dowex-50W(H^+). For small scale experiments a column (4 x 50 cm) was packed with Dowex-2(Cl^-) resin without pressure. Conversion to the formate form was accomplished with 2 M sodium formate until free of chloride ion followed by washing with a volume of 2 N formic acid equal to one-third the volume of sodium formate used (46, 47). These columns

were washed with deionized water just before use. The sample was placed on the column and eluted by gradient elution with water and formic acid as described under Results. The eluants from Dowex-50W(H^+) and from Dowex-2 (formate) columns were collected in 5 ml fractions, and 0.1 ml of every third fraction of eluant added to 10 ml of scintillation solution for counting in the Tricarb.

The peak radioactive fractions from the Dowex-50W(H^+) column and from the Dowex-2 (formate) column were dried under reduced pressure or lyophilyzed. The dried materials were dissolved in a small volume of 50 per cent alcohol, spotted on Whatman No. 1 paper and developed using n-butanol:acetic acid:water.

In further attempts to obtain purified photooxidation products paper chromatograms were developed using n-butanol:acetic acid:water and the piece of filter paper containing the photooxidation product was cut out and sandwiched between two glass plates resting in a petri dish. The photooxidation product was eluted from filter paper with water by capillary attraction (48).

RESULTS

A. Oxygen Uptake and Carbon Dioxide Release.

Table I shows the composition of the reaction mixtures used in the manometric studies. The rate of oxygen uptake of the manometric studies are summarized in Fig. 1.

As shown in Tables I and II when Warburg flasks were illuminated with one lamp located 40 cm from sample, oxygen uptake was 0.6 moles per mole of tryptophan (Exp. 1). Under the same experimental conditions, but using a greater intensity of light more oxygen uptake was obtained (Exp. 3 and 4). When the concentration of tryptophan and methylene blue were changed, large differences of oxygen uptake were not observed (Exp. 5-16), but a smaller number of reaction products was found by paper chromatography in Exp. 14, 15 and 16 than in Exp. 5, 6 and 7.

B. Rate of $C^{14}O_2$ Formation from DL-Tryptophan-α-C^{14}, DL-Tryptophan-5-C^{14} and DL-Tryptophan-7a-C^{14}.

Weil et al. (19) observed that the absorption maximum at 280 mμ, which is due to conjugation of the ethylenic linkage in the pyrrole ring with the benzene chromophore disappeared completely after the uptake of 2 moles of oxygen per mole of tryptophan. He also noted the formation of a new maximum at 260 mμ which indicated that the photooxidation up to this point had been confined to the pyrrole ring, inasmuch as the benzene chromophore alone should have a maximum at about 260 mμ. After photo-oxidation which resulted in 4 moles of oxygen uptake per mole of tryptophan

TABLE I

COMPOSITION OF REACTION MIXTURES IN MANOMETRIC EXPERIMENTS

Experiment Number	Tryptophan μmoles	Potassium Phosphate Buffer pH 8.5, μmoles	Methylene Blue μg	Total Volume ml	20% KOH in center well ml
1	10	100	100	2	0.1
2	10	100	100	2	0.1
3	5	100	100	2	0.1
4	5	100	100	2	0.1
5, 6, 7	5	50	50	0.7	0.1
8, 9, 10	5	50	50	0.7	0.1
11, 12, 13	5	50	50	0.7	0.1
14, 15, 16	2.5	50	50	0.7	*

*Apparatus was swept continually with air and $C^{14}O_2$ trapped in Hyamine X-10.

TABLE II

EXPERIMENTAL CONDITIONS, OXYGEN UPTAKE AND CARBON DIOXIDE EVOLVED IN MANOMETRIC EXPERIMENTS IN PHOTOOXIDATION OF TRYPTOPHAN

Experiment Number	Type of Light Source	Distance from Sample cm	Exposure Time (min)	O_2 Uptake (moles/mole of tryptophan)	CO_2 Evolved (moles/mole of tryptophan)
1	one lamp[1]	40	520	0.6	0
2	one infrared lamp	30	480	1.17	0.26
3	one lamp	30	480	2.13	0.23
4	two lamps	30	480	3.9	1.25
5, 6, 7	two lamps	30	480	3.6	----
8, 9, 10	two lamps	30	480	3.6	----
11, 12, 13	two lamps	30	480	3.5	----
14, 15, 16	two lamps	30	480	3.37	[2]

[1]Westinghouse spot light

[2]Hyamine X-10 used to absorb CO_2

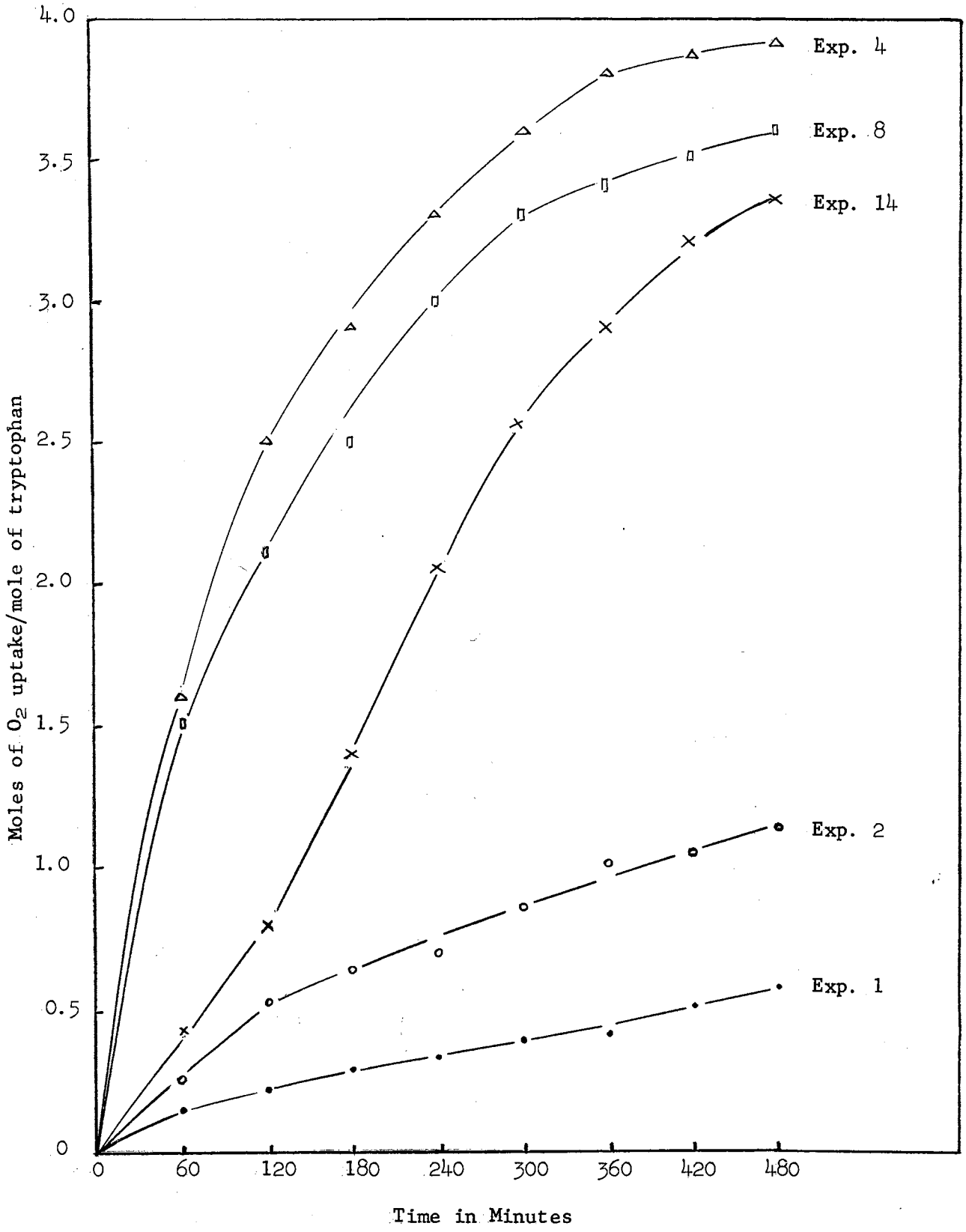


Fig. 1. Rate of Oxygen Uptake in Manometric Experiments.

there was no minimum at 240 mμ and no maximum at 280 mμ, indicating that all the tryptophan was destroyed. When phenylalanine was photooxidized under the same condition as tryptophan, no oxygen uptake and carbon dioxide evolution were observed. From this evidence he concluded that the benzene nucleus of tryptophan was not involved in photooxidation.

The experiments presented here show that at 3.37 moles of oxygen uptake per mole of tryptophan, large amounts of $C^{14}O_2$ from the beginning of the reaction and reached a plateau after 4 hours. On the other hand DL-tryptophan-5-C^{14} and 7a-C^{14} started to evolve $C^{14}O_2$ sometime after the reaction started and the rate of evolution rapidly increased with time. This indicated that the benzene nucleus is resistant to photooxidation, but once it was ruptured it is rapidly degraded.

Fig. 2 shows the rate of $C^{14}O_2$ appearance in Exp. 14, 15, 16 and duplicate experiments at a later date gave very similar results.

C. Photooxidation Products and Column Experiments.

1. Small Scale Experiments

The photooxidation products formed (Exp. 5) were separated by paper chromatography. Chromatograms were developed 18 hours during which the solvent front moved approximately 34 cm. The position of the spots were located using ultraviolet light (366 mμ and 2537 mμ) and also by spraying with ninhydrin solution. The results are shown in Fig. 3.

The paper strips were scanned with a Radio-Chromatogram Scanner. Fig. 4 shows the results of the scanning of the reaction mixture after 2 hours of oxidation of tryptophan-α-C^{14}.

Fig. 5, 6 and 7 show the amount of radioactivity found in the photooxidation products from DL-tryptophan-α-C^{14}, DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14}. All of the spots which had high levels of radioactivity

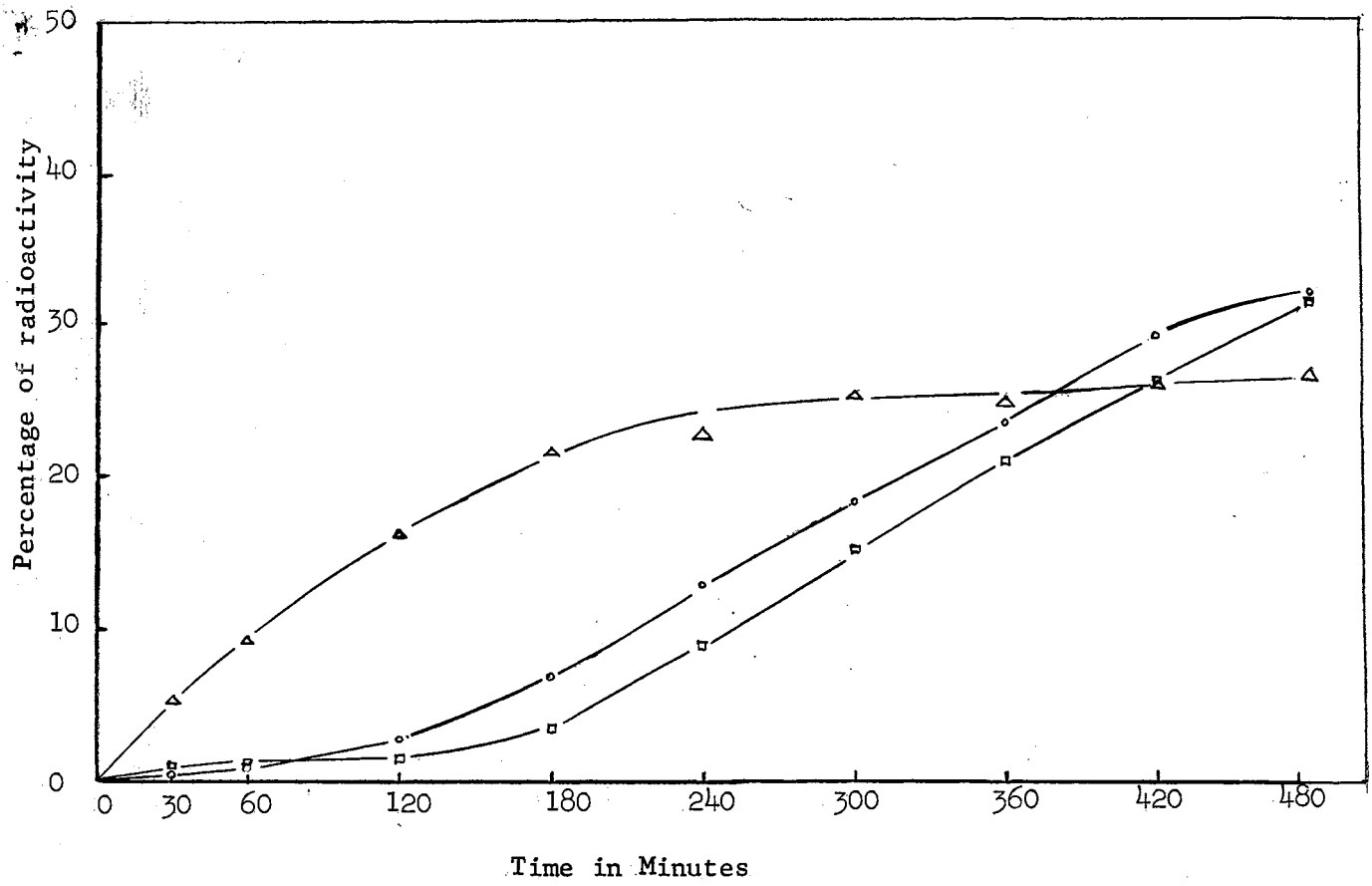


Fig. 2. Percentage of $C^{14}O_2$ from DL-tryptophan-α-C^{14}, DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14} at Different Times of Photooxidation.

△ - DL-tryptophan-α-C^{14}
▫ - DL-tryptophan-5-C^{14}
○ - DL-tryptophan-7a-C^{14}

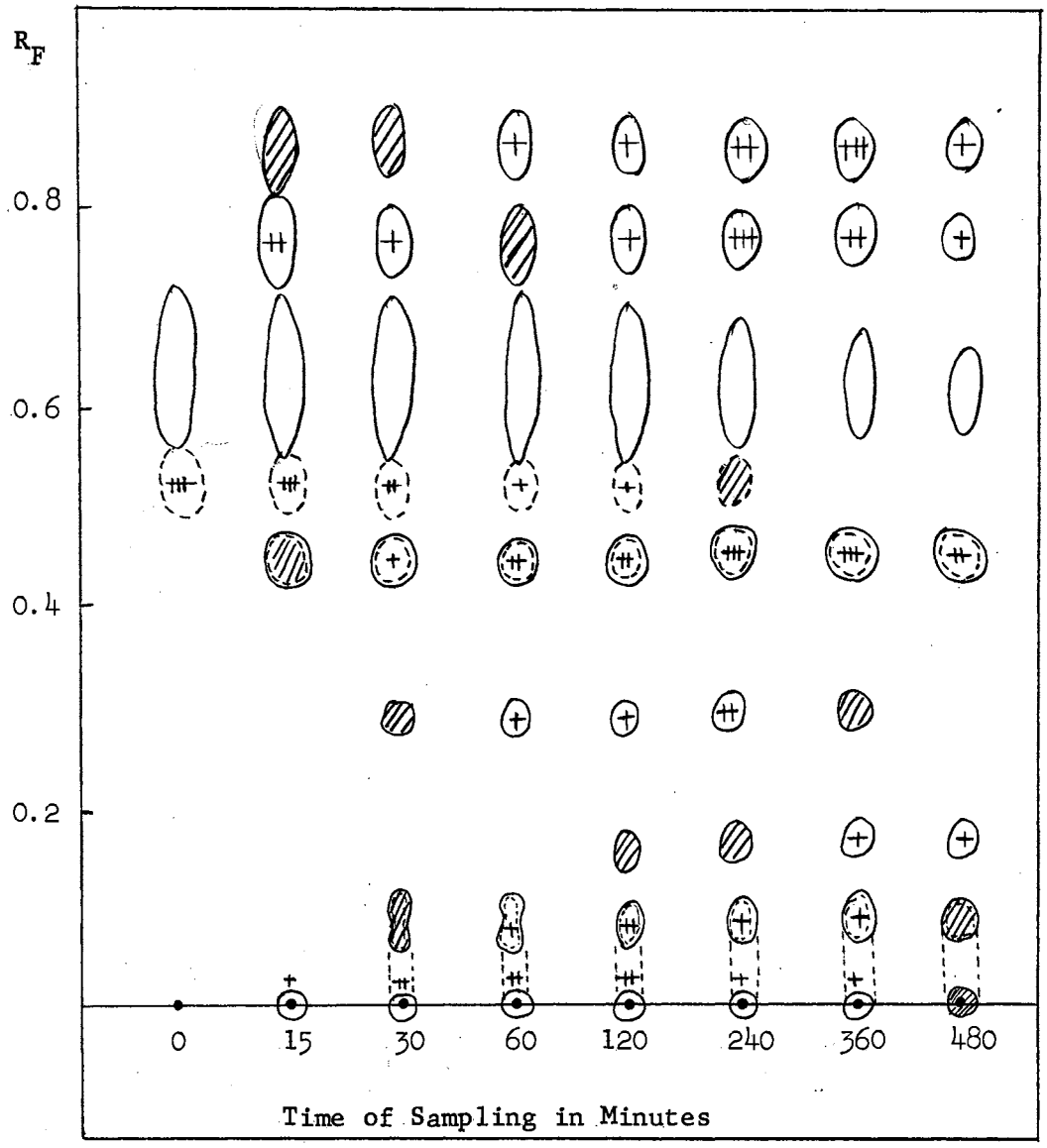


Fig. 3. Photooxidation Products from DL-tryptophan-α-C^{14} Obtained at Different Times.
—Fluorescent spot; --- , ninhydrin positive spot.
Intensity of Color was Given + < ++ < +++ in Increasing Order and Shading Indicates Weak Spot.
R_F value 0.65 was methylene blue.

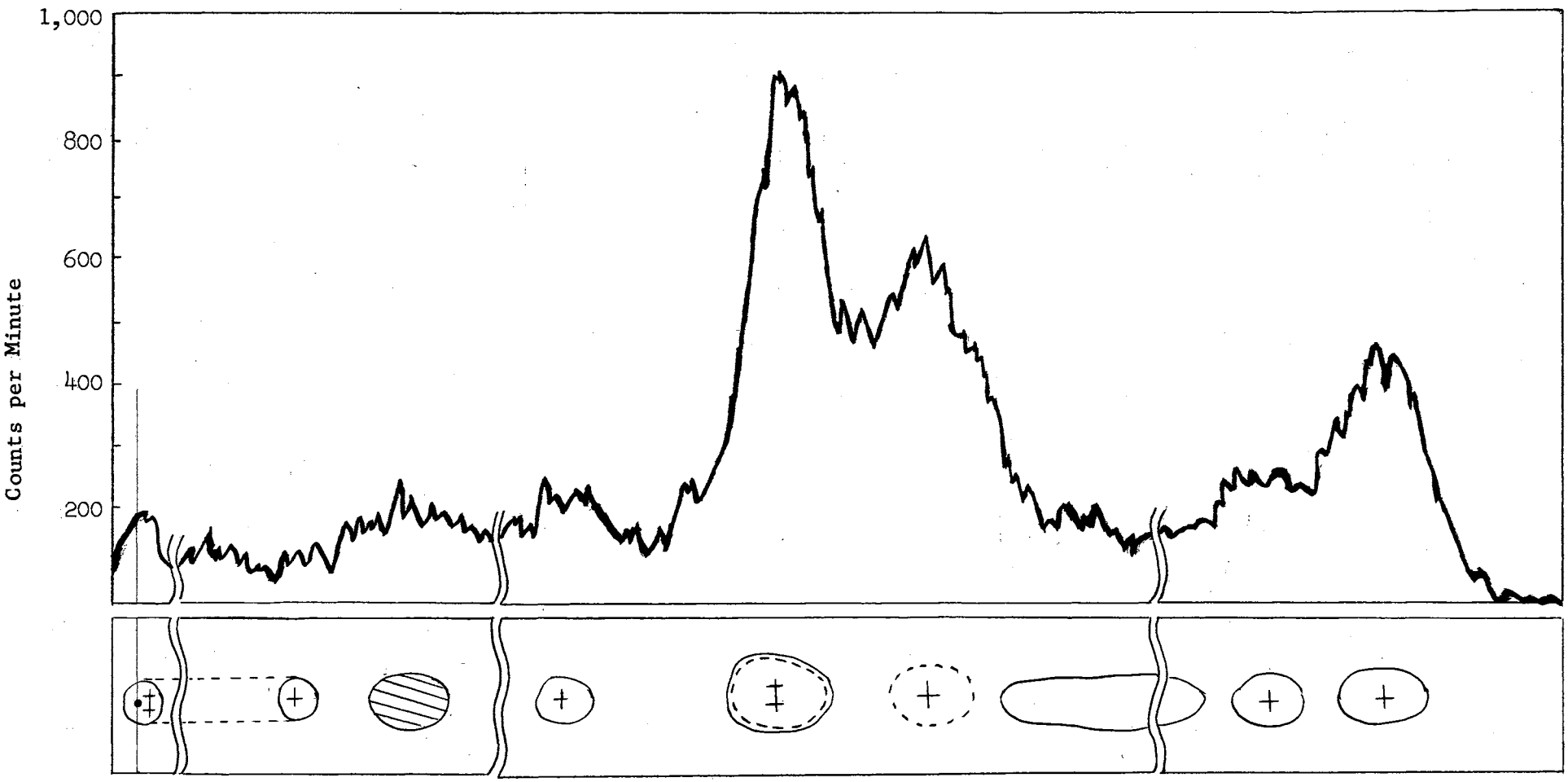


Fig. 4. Location of C^{14}-labeled Photooxidation Products on Paper Chromatograms. The Sample was Obtained from DL-Tryptophan-α-C^{14} which was Illuminated for 120 Minutes (Exp. 5). R_F Value of Each Spot Are Given in Fig. 3. --- , Ninhydrin positive spot; —— , fluorescent spot. Intensity of Color was given + < ++ < +++ in Increasing Order and Shading Indicates Weak Spot.

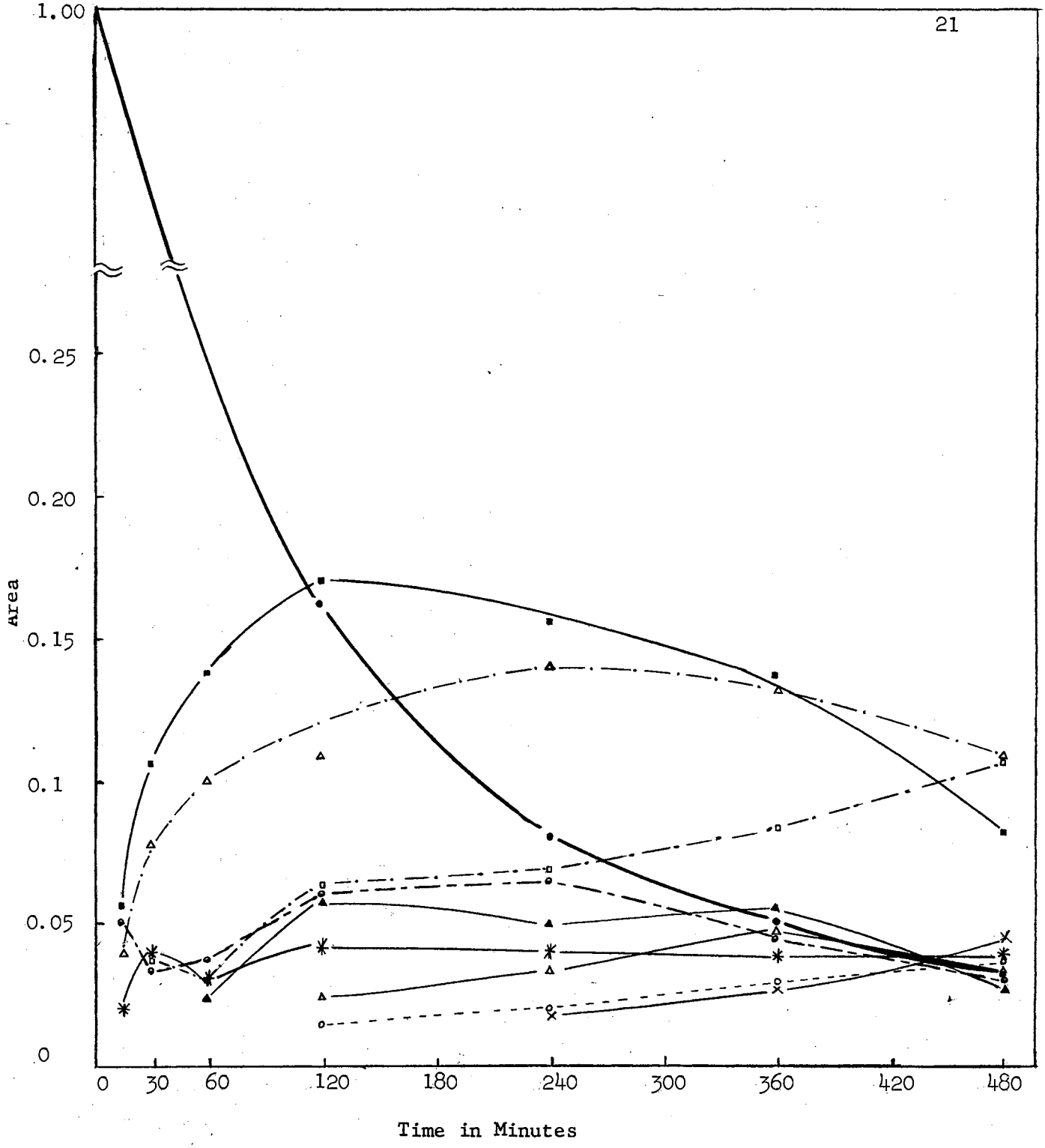


Fig. 5. Distribution of the Radioactivity from DL-Tryptophan-α-C^{14} in Products Separated by Paper Chromatography after Varying Period of Photo-oxidation. ●—●, tryptophan; ■—■, kynurenine; ×—×, R_F 0.05; △—△, R_F 0.10; o---o, R_F 0.17; □-·-□, R_F 0.20; ▲—▲, R_F 0.35; ✱—✱, R_F 0.70; ⊖---⊖, R_F 0.80; △-·-△, R_F 0.90.

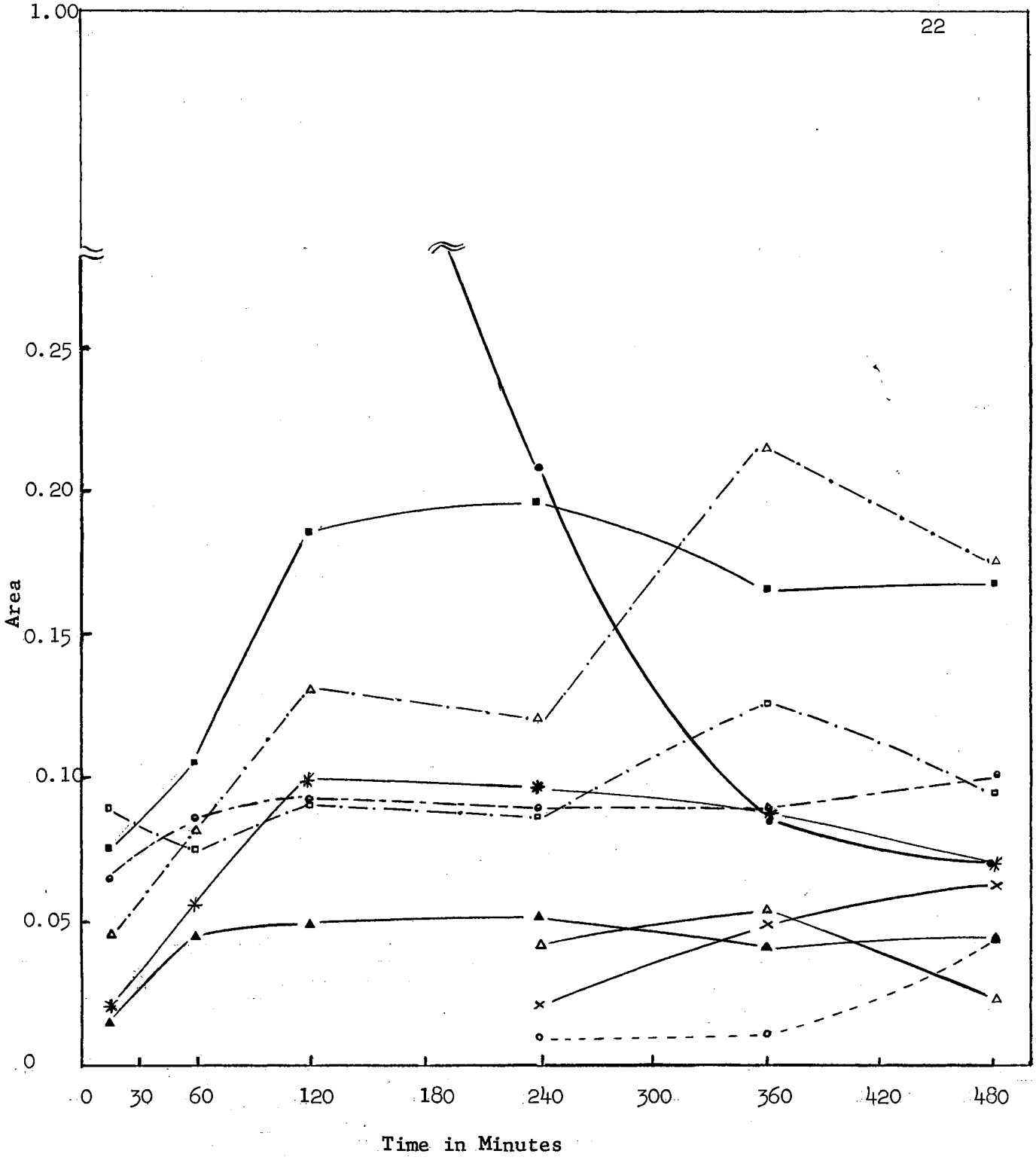


Fig. 6. Distribution of the Radioactivity from DL-Tryptophan-5-C^{14} in Products Separated by Paper Chromatography after Varying Periods of Photo-oxidation. •——• , tryptophan; ▪—▪, kynurenine; ×—×, R_F 0.05; △—△, R_F 0.10; ○---○, R_F 0.17; □-·-·□, R_F 0.20; ▲—▲, R_F 0.35; ✱—✱, R_F 0.70; ⊖---⊖, R_F 0.80; △-·-△, R_F 0.90.

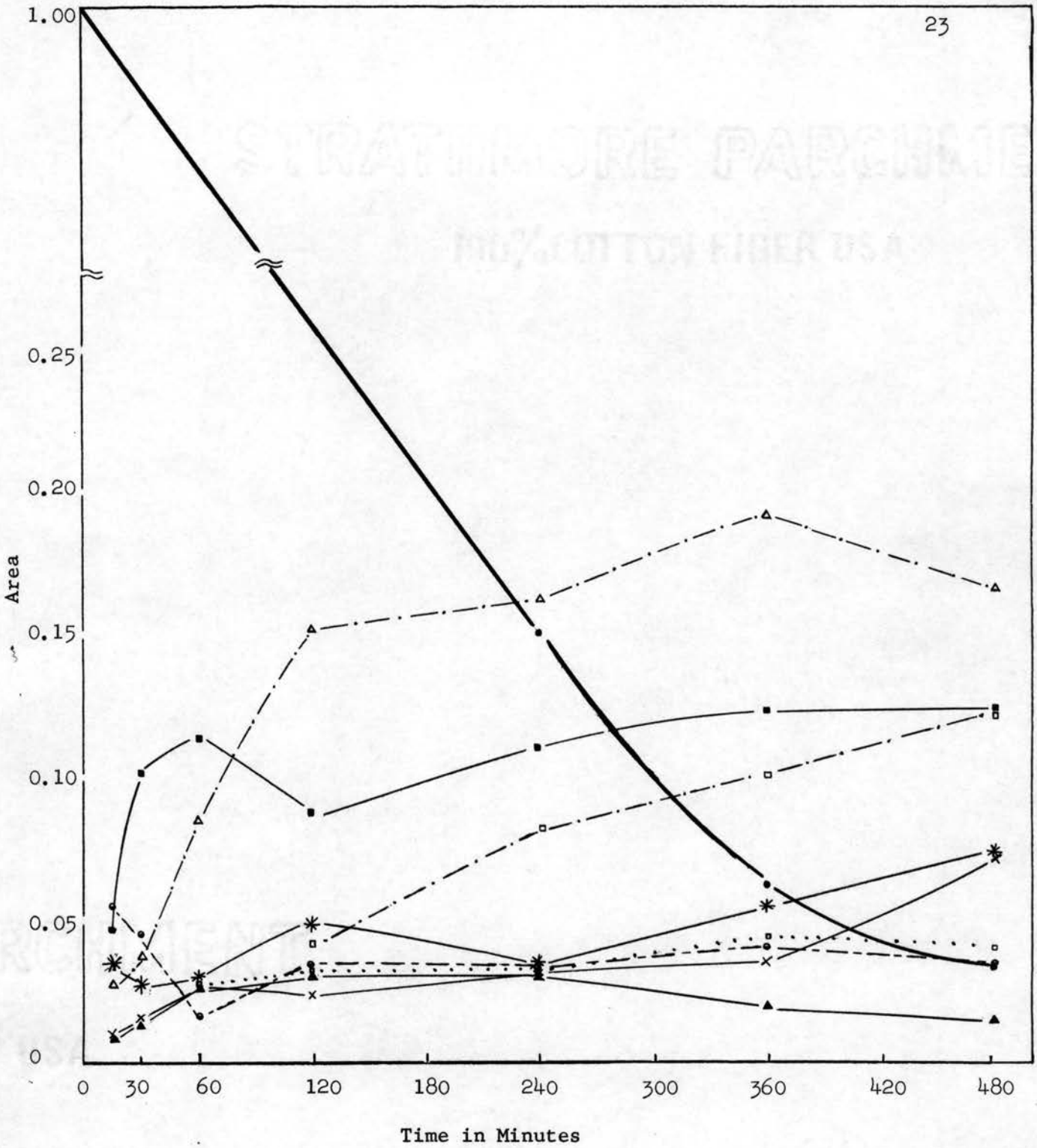


Fig. 7. Distribution of the Radioactivity from DL-Tryptophan-7a-C^{14} in Products Separated by Paper Chromatography after Varying Periods of Photo-oxidation. •——•, tryptophan; ▪—▪, kynurenine; x——x, R_F 0.05; ▫-·-▫, R_F 0.20; ▫····▫, R_F 0.27-0.30; ▲—▲, R_F 0.35; ✱—✱, R_F 0.70; ⊖---⊖, R_F 0.80; △-·-△, R_F 0.90.

fluoresced strongly when irradiated with light of 366 mμ or 253.7 mμ.

Two radioactive spots at R_F 0.10 and R_F 0.17 were obtained from both DL-tryptophan-α-C^{14} and -5-C^{14}, but neither of these fluorescent spots was radioactive in the DL-tryptophan-7a-C^{14} experiments. The significance of this will be discussed later. DL-Tryptophan-7a-C^{14}, however, produced a radioactive compound with an R_F value of 0.27-0.30. This compound was not radioactive when produced from tryptophan-5 or -α-C^{14}. In all three cases, kynurenine was found in early stages of the photooxidation reaction and this result is in agreement with the proposal of Weil (27). The disappearance of a substance with an R_F value of 0.86 was accompanied by an increase of a substance with an R_F value of 0.05. This suggests that the low R_F value substance was directly derived from the high R_F value substance. Earlier work (48) dealing with the ultraviolet light catalyzed degradation of tryptophan indicated that aspartic acid, alanine, serine and tryptamine were products. In these experiments negligible radioactivity was found at the R_F values corresponding to these four compounds.

In an attempt to isolate photooxidation products, the contents of the Warburg flask from the experiment with DL-tryptophan-7a-C^{14} (Exp. 7) were passed through a Dowex-50W(H^+) column which was developed as described in Experimental Methods. The distribution of radioactivity in each fraction was determined on a small aliquot using the Tricarb Scintillation Spectrometer. The results are shown in Fig. 8.

Each peak fraction from a Dowex-50W(H^+) column were taken to dryness, chromatographed on Whatman No. 1 paper with n-butanol:acetic acid:water as a solvent system and examined under ultraviolet light. The results are shown in Table III.

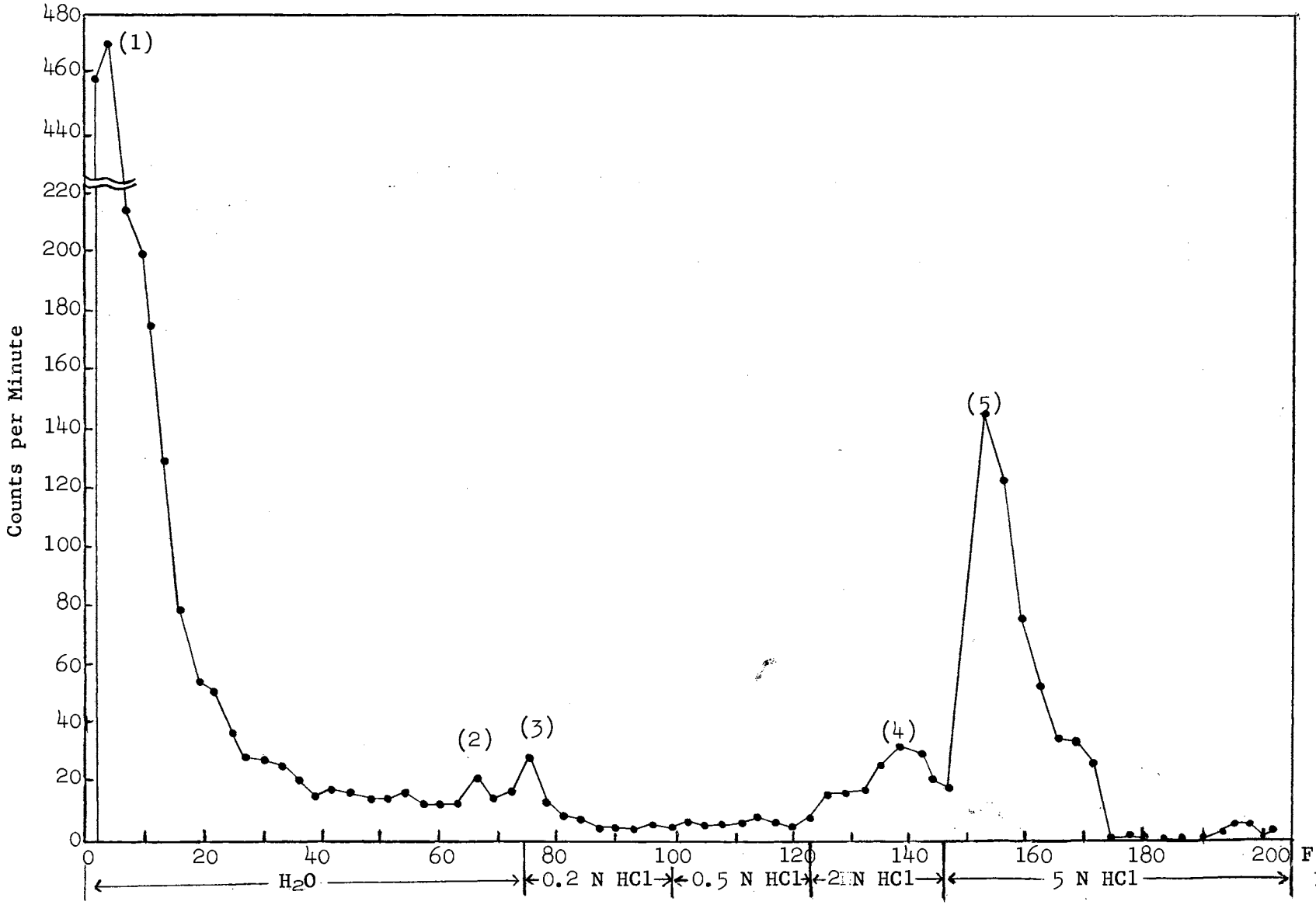


Fig. 8. Distribution of the Radioactivity in the Fraction from a Dowex-50W(H^+) Separation of the Photooxidation Products of DL-Tryptophan-7a-C^{14} (Exp. 7).

TABLE III

PAPER CHROMATOGRAPHY OF THE PEAK FRACTION OF EFFLUENT FROM A DOWEX-50W(H^+) COLUMN

Type of Column	Eluant	No. of Peak	No. of Fraction	R_F Value	Fluorescence
Dowex-50W(H^+)	H_2O	1	3 to 35	----	----
	H_2O	2	63 to 69	0.18	weak yellow
				0.40	yellow-white
	H_2O + 0.2 N HCl	3	72 to 78	0.29	yellow-white
	2 N HCl	4	132 to 144	0.88	dark green
	5 N HCl	5	148 to 173	0.175	yellow-white
				0.40	weak blue

The fractions (3-35) containing the first peak from a Dowex-50W(H^+) column were taken to dryness and chromatographed on a Dowex-2 (formate) column. The distribution of radioactivity in the fractions is shown in Fig. 9.

Each peak fraction from Dowex-2 (formate) was taken to dryness and the residue chromatographed on Whatman No. 1 paper. The results of this experiment are shown in Table IV.

Many experiments performed gave a large number of photooxidation products. However, photooxidation in Exp. 14, 15 and 16 produced a relatively small number of products and tryptophan had completely disappeared after 2 hours of illumination. In this experiment Hyamine X-10 was used to absorb $C^{14}O_2$. The rate $C^{14}O_2$ appearance from each specifically labeled tryptophan was observed. Duplicate experiments gave similar results. Fig. 10, 11 and 12 show the amount of radioactivity found in the photooxidation products from DL-tryptophan-α-C^{14}, DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14}. All of the spots which had high levels of radioactivity also fluoresced strongly when irradiated at 366 mμ or 253.7 mμ.

DL-Tryptophan-7a-C^{14} gave three major radioactive products, and DL-tryptophan-α-C^{14} and -5-C^{14} gave five products. In this series of experiments (Nos. 14, 15 and 16) a large amount of radioactivity was found at the origin when the paper chromatogram was developed with n-butanol:acetic acid:water as a solvent. A decrease in the radioactivity of kynurenine was accompanied by an increase in $C^{14}O_2$ evolution. After 1 hour approximately 25 per cent of the isotope from DL-tryptophan-α-C^{14}, -5-C^{14} and -7a-C^{14} was present in kynurenine. As shown in Table V disappearance of kynurenine was accompanied by a corresponding

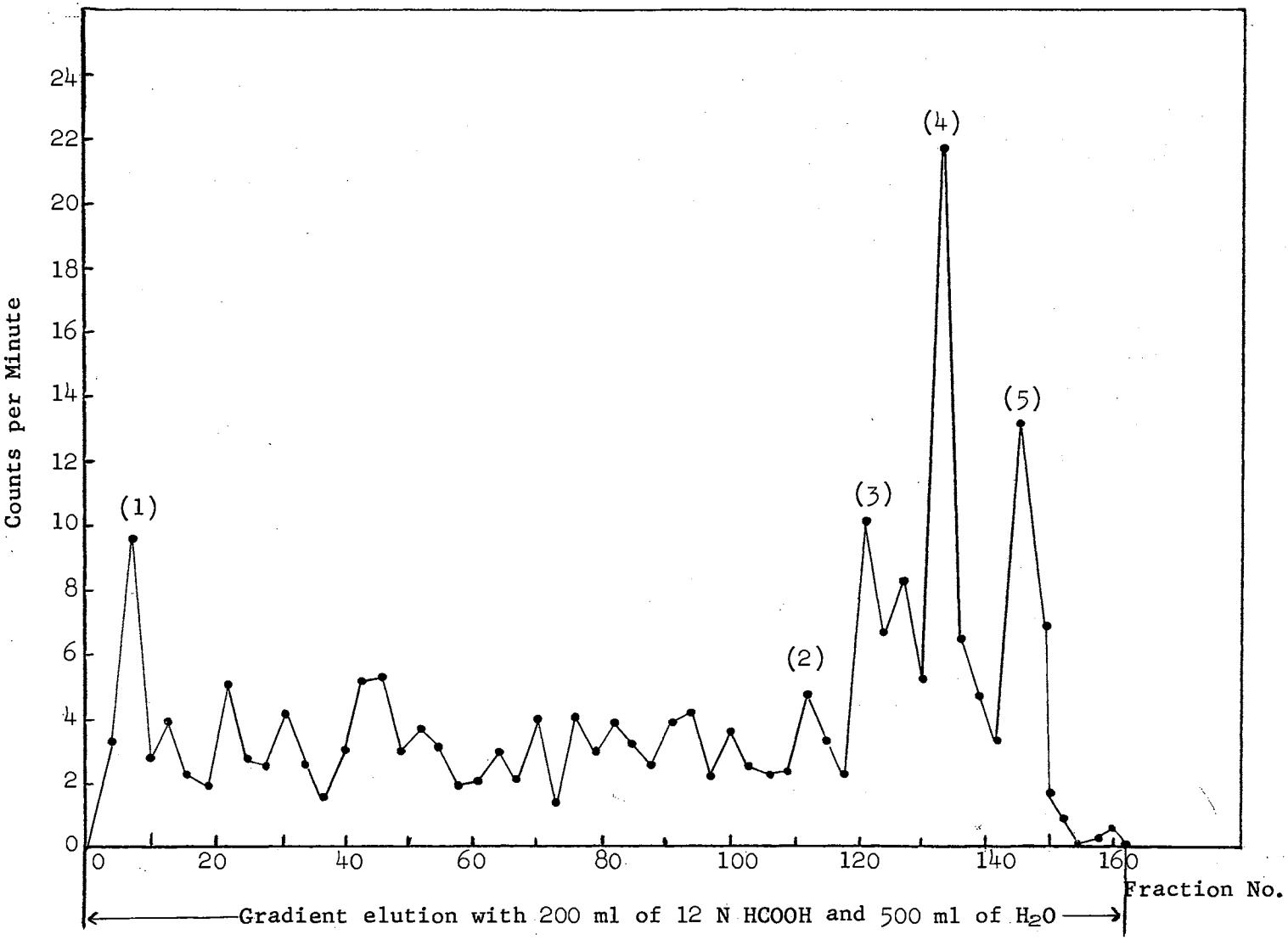


Fig. 9. Distribution of the Radioactivity of Eluate from Dowex-2(formate) Column by Gradient Elution with Concentrated Formic Acid and Water.

TABLE IV

PAPER CHROMATOGRAPHY OF THE PEAK FRACTIONS OF EFFLUENT FROM A DOWEX-2(FORMATE) COLUMN

Type of Column	Eluant	No. of Peak	No. of Fraction	R_F Value	Fluorescence
Dowex 2 (formate)	gradient elution	1	2 to 10	0.82	weak yellow
		2	119 to 123	0.867	violet
				0.90	pink
		3	125 to 129	0.92	pink
		4	131 to 138	0.068	weak yellow
				0.113	weak yellow
				0.165	weak yellow
		5	143 to 150	0.045	weak yellow

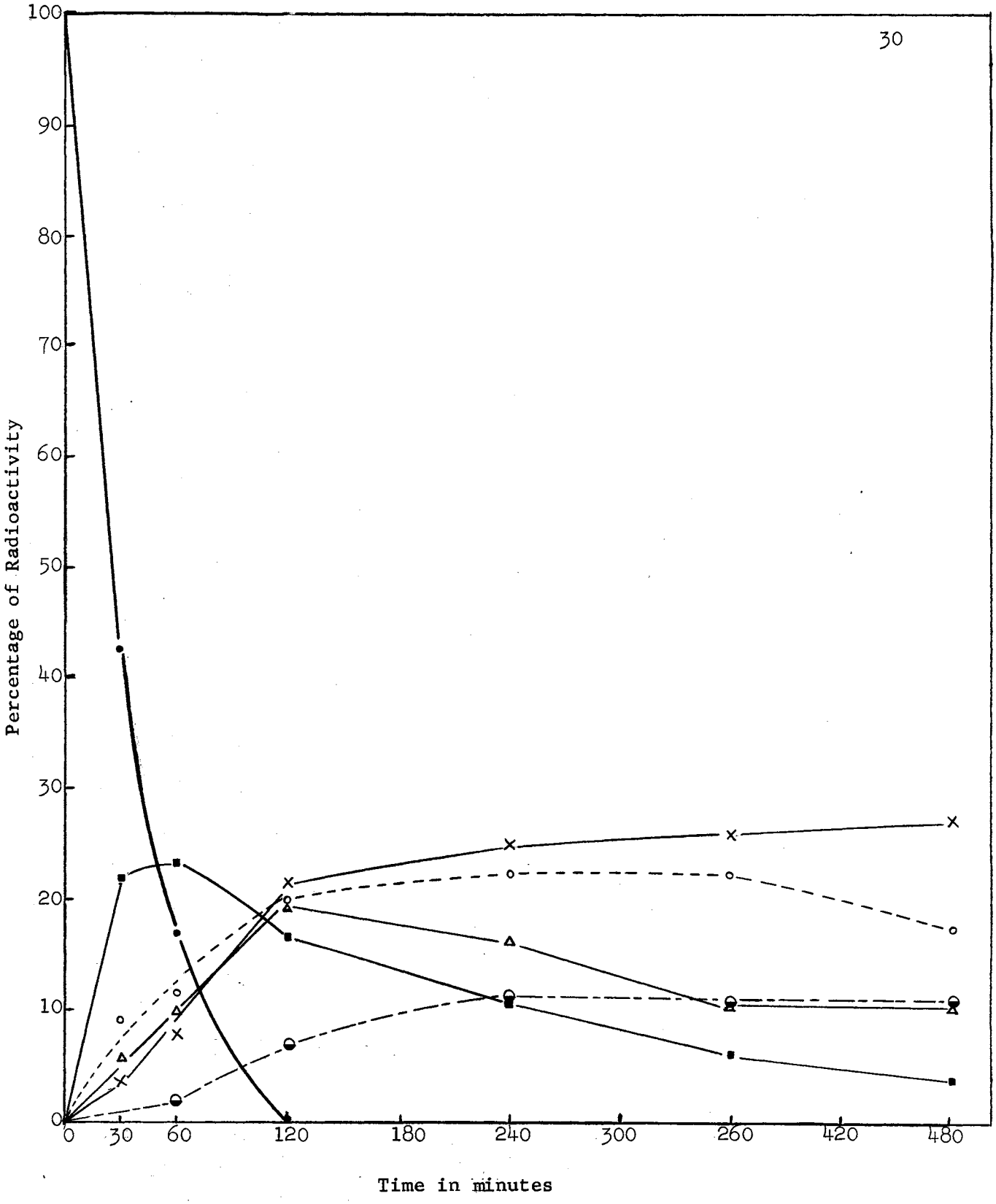


Fig. 10. Distribution of the Radioactivity from DL-Tryptophan-α-C^{14} in Products Separated by Paper Chromatography after Varying Periods of Photo-oxidation (Exp. 14). •——•, tryptophan; ▪——▪, kynurenine; x——x, R_F 0.0; △——△, R_F 0.10; o---o, R_F 0.17; ⊖---⊖, R_F 0.80.

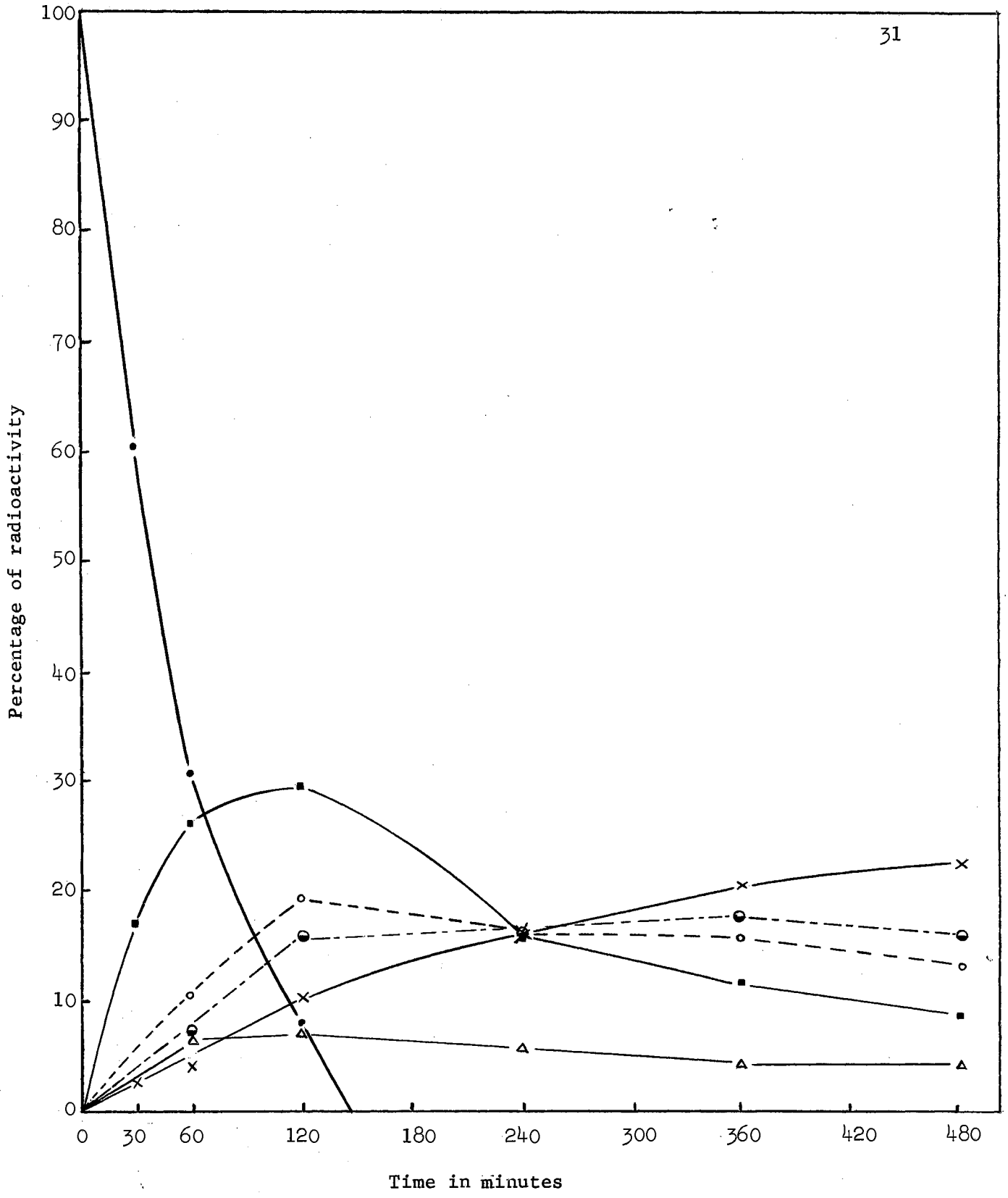


Fig. 11. Distribution of the Radioactivity from DL-Tryptophan-5-C^{14} in Products Separated by Paper Chromatography after Varying Periods of Photo-oxidation (Exp. 15). •——•, tryptophan; ▪——▪, kynurenine; x——x, R_F 0.0; o---o, R_F 0.17; ⊖---⊖, R_F 0.80.

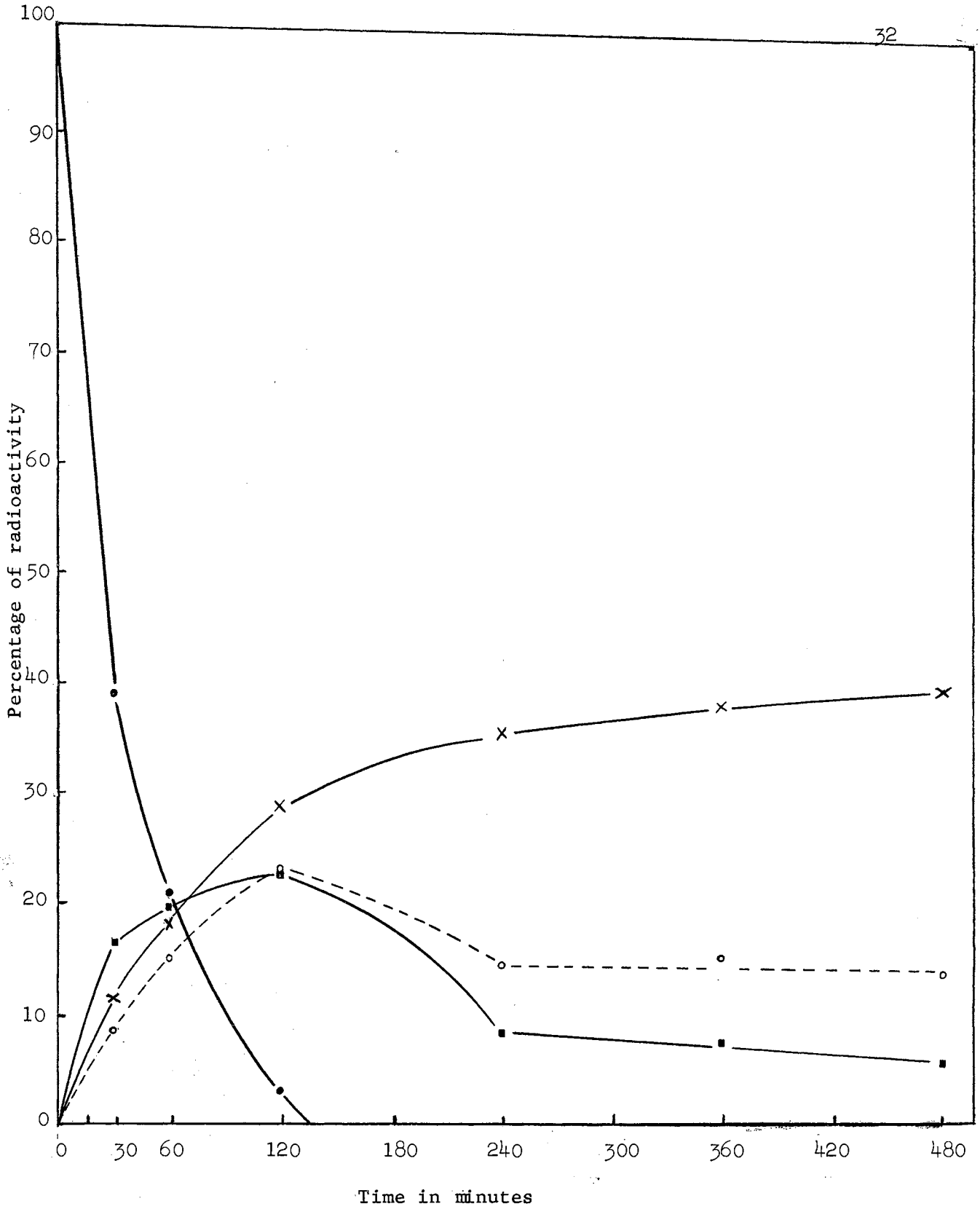


Fig. 12. Distribution of the Radioactivity from DL-Tryptophan-7a-C^{14} in Products Separated by Paper Chromatography after Varying Periods of Photo-oxidation (Exp. 16). •——•, tryptophan; ▪——▪, kynurenine; x——x, R_F 0.0; o---o, R_F 0.17.

TABLE V

THE RELATIONSHIP BETWEEN RADIOACTIVE KYNURENINE AND $C^{14}O_2$ AT DIFFERENT TIMES FROM DL-TRYPTOPHAN-α-C^{14} (Exp. 14)

Time (min)	Kynurenine (%)	$C^{14}O_2$ (%)	Total
60	23.5	10	33.9
240	10	24	34
360	6	26	32
480	4	27	31

appearance of the α-carbon of tryptophan as $C^{14}O_2$ indicating that the disappearance of kynurenine resulted from oxidation of the side chain, but as experiments with tryptophan-5 and -7a-C^{14} demonstrated $C^{14}O_2$ also arises from the benzene ring.

Results of column chromatography of the photooxidation products of Exp. 16 are shown in Fig. 13. The contents of the peak tubes from each fraction were pooled and lyophilized. The dried materials were dissolved in a small amount of methanol:n-butanol:benzene:water (4:2:2:2) mixture, spotted on Whatman No. 1 paper and developed with n-butanol: acetic acid:water as a solvent. Peak 1 located at the origin, and peaks 6 and 9, R_F values 0.17 and 0.30 respectively, were the major products. From peaks 2, 3, 4, 5, 7, 8 and 10 no C^{14} was detected on the paper chromatograms.

In an attempt to obtain purified material from each spot on the chromatograms, unlabeled DL-tryptophan (Exp. 17) was illuminated under the same conditions as was used in the case of labeled tryptophan. The products were separated by paper chromatography using n-butanol:acetic acid:water or 20 per cent potassium chloride solution as solvent. Fig. 14 shows a typical chromatogram and the R_F values using different solvent systems.

Each spot was cut out and eluted as described in Experimental Methods. These eluates were tested with many color reagents and compared with the products from labeled tryptophan. The results are summarized in Tables VI and VII. Table VI summarizes the properties of the products detected in the 8 hour oxidized samples and Table VII summarizes the rate of formation of each product.

The material remaining at the origin after development with n-butanol:

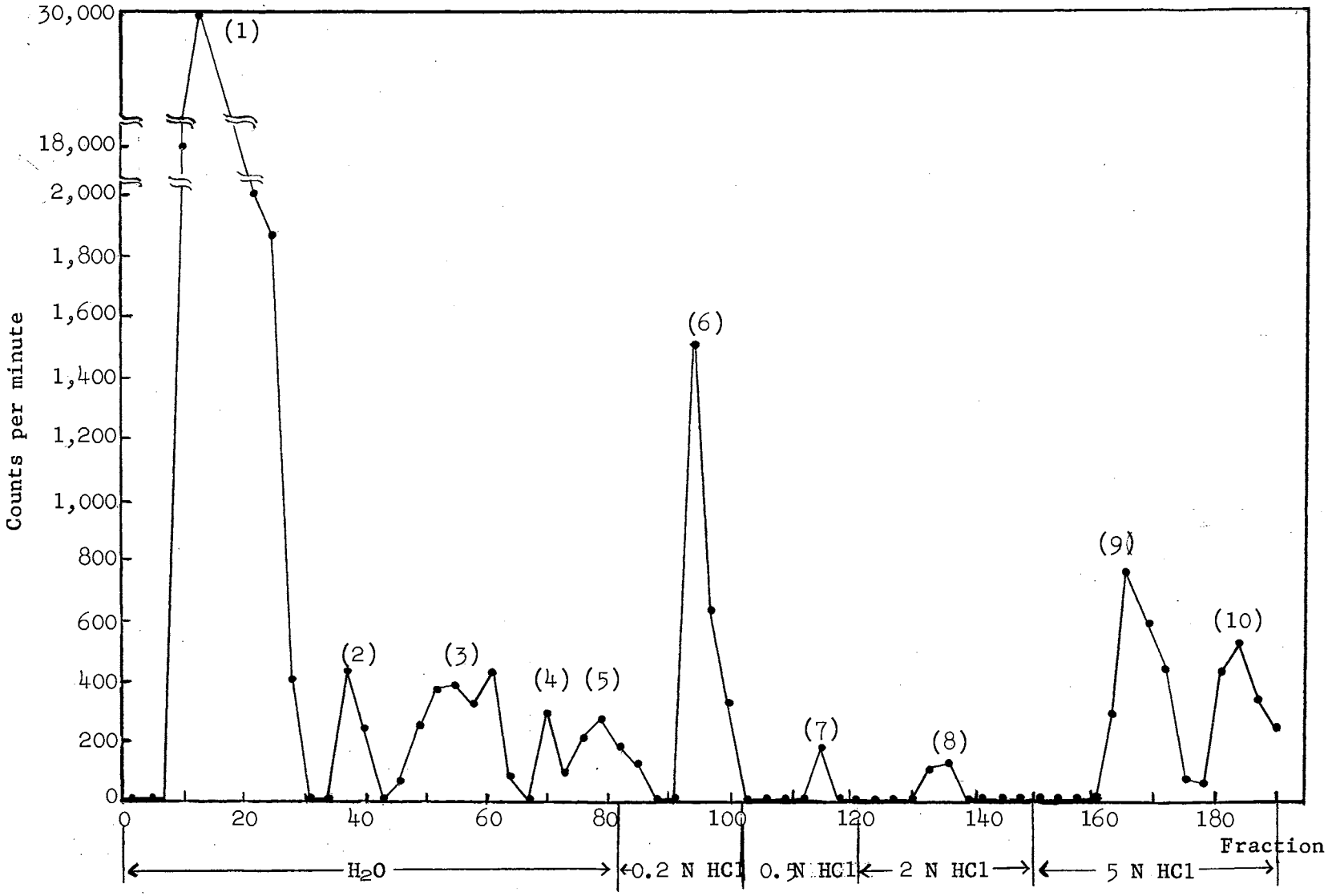
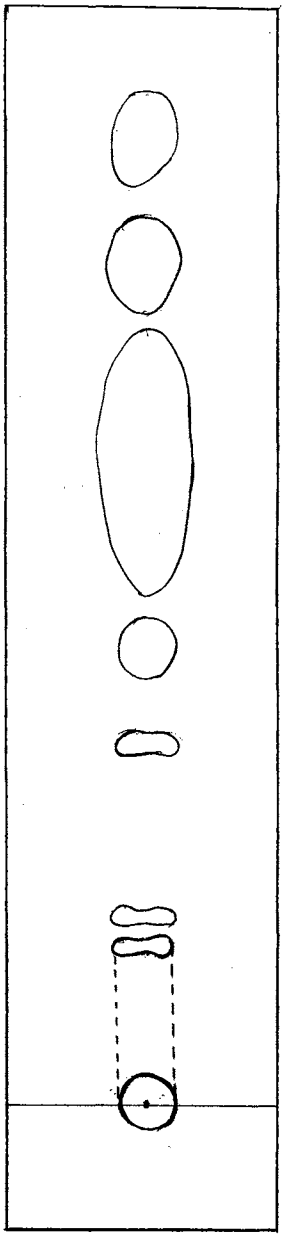


Fig. 13. Distribution of the Radioactivity in the Fraction from a Dowex-50W(H^+) Separation of the Photooxidation Products of DL-Tryptophan-7a-C^{14} (Exp. 16).



No. of spot	R_F value BuOH:HOAC:H_2O	20% KCl	U.V. (366.5 mμ)
7	0.81	0.734 0.014	Pink
6	0.72	spread	dark green
methylene blue			
5	0.31	0.63	violet
4	0.24	spread	golden yellow
3	0.17	spread	weak yellow
2	0.10	0.76	strong yellow
1	0	0	violet

Fig. 14. Location of Photooxidation Products (Exp. 17) of Unlabeled DL-Tryptophan on Paper Chromatogram.

TABLE VI

COMPARISON OF RADIOACTIVE PRODUCTS*1 FROM LABELED TRYPTOPHAN (EXP. 14, 15, and 16) AND THE PRODUCTS*2 FROM UNLABELED TRYPTOPHAN (EXP. 17).

No. of Compd.	Percentage of Radioactive Products from Three Specifically Labeled Tryptophan			Ninhydrin	Methyl red	P-diMe Amino Benz-aldehyde	Diazotized P-NO_2 aniline	Nessler Reagent after fixing NH_3
	7a	α	5					
1	39.4	31.5	20.4	-	+++	W. Y.	W. Y.	Golden Brown
2	0	13.1	11.1	+	+	Light Green	Y. Brown	W. Y.
3	14.3	15.5	13.8	-	-	Beige	Y.	-
4	0	0	0	+	+	Cream	Y.	-
5	6.1	3.6	5.6	+	++	Orange	Deep Orange	W. Y.
6	0	0	0	- (Purple)	+	Light Beige	W. Orange	M. Y.
7	0	14.8	24.6	-	++	Tan	Medium Y.	W. Y.

*1 *2 After 8 hours irradiation.

TABLE VII

DISTRIBUTION OF RADIOACTIVITY IN PHOTOOXIDATION PRODUCTS AFTER DIFFERENT PERIODS OF IRRADIATION

	7a-C^{14}				α-C^{14}				5-C^{14}			
No. of Compd.	1 hr %	4 hrs %	6 hrs %	8 hrs %	1 hr %	4 hrs %	6 hrs %	8 hrs %	1 hr %	4 hrs %	6 hrs %	8 hrs %
1	16.2	29.9	35.7	39.4	9.9	27.7	28.9	31.5	9.7	17.8	24.2	20.4
2					12.8	15.8	18.3	13.1	8.9	9.5	8.7	11.1
3	14.9	16.8	14.5	14.3	13.8	16.8	17.4	15.5	15 1	21.3	17.1	13.8
4												
5	19.3	9.3	7.1	6.1	23.5	10.0	6.0	4.0	35.1	10.3	7.6	5.6
6					Small							
7					5.9	11.0	13.3	14.8	21.3	21.3	27.7	24.6

acetic acid:water, was spotted on Whatman No. 1 paper and developed with various solvents. The spot remained at the origin using 20 per cent potassium chloride, methanol:n-butanol:benzene:water (4:2:2:2), 80 per cent phenol, and ethyl cellosolve:ammonia:water = 80:5:15. However, ethyl ether:88 per cent formic acid:water (5:2:1) separated a substance at R_F 0.79 which corresponded to the R_F value of succinic acid. However, when succinic acid was developed with n-butanol:acetic acid:water solvent system it had an R_F of 0.46 (elongated spot).

2. Larger and Very Large Scale Experiments

In larger scale experiments the reaction mixtures (containing 1.35 mg of DL-tryptophan-7a-C^{14} and 102 mg of unlabeled DL-tryptophan) were illuminated for 16 hours. At the end of 16 hours the reaction mixtures were almost dry. The residue was dissolved in n-butanol: acetic acid:water or methanol:n-butanol:benzene:water (4:2:2:2). Acetone, water and ether were unsatisfactory solvents. The products were dissolved in 30 ml of methanol:n-butanol:benzene:water mixture and placed on a Dowex-50W(H^+) column which was developed with water then with hydrochloric acid. The conditions of development and the distribution of radioactivity are shown in Fig. 15.

The contents of the peak tubes from each fraction were pooled and lyophilized. The dried materials were extracted first with water, and then with methanol:n-butanol:benzene:water mixture, and spotted on Whatman No. 1 paper. The paper was developed using n-butanol:acetic acid:water as a solvent, and the spots were detected using ultraviolet light (Fig. 16 and 17).

A photooxidation product showing blue fluorescence, an R_F 0.90, was insoluble in water, but the other products dissolved readily

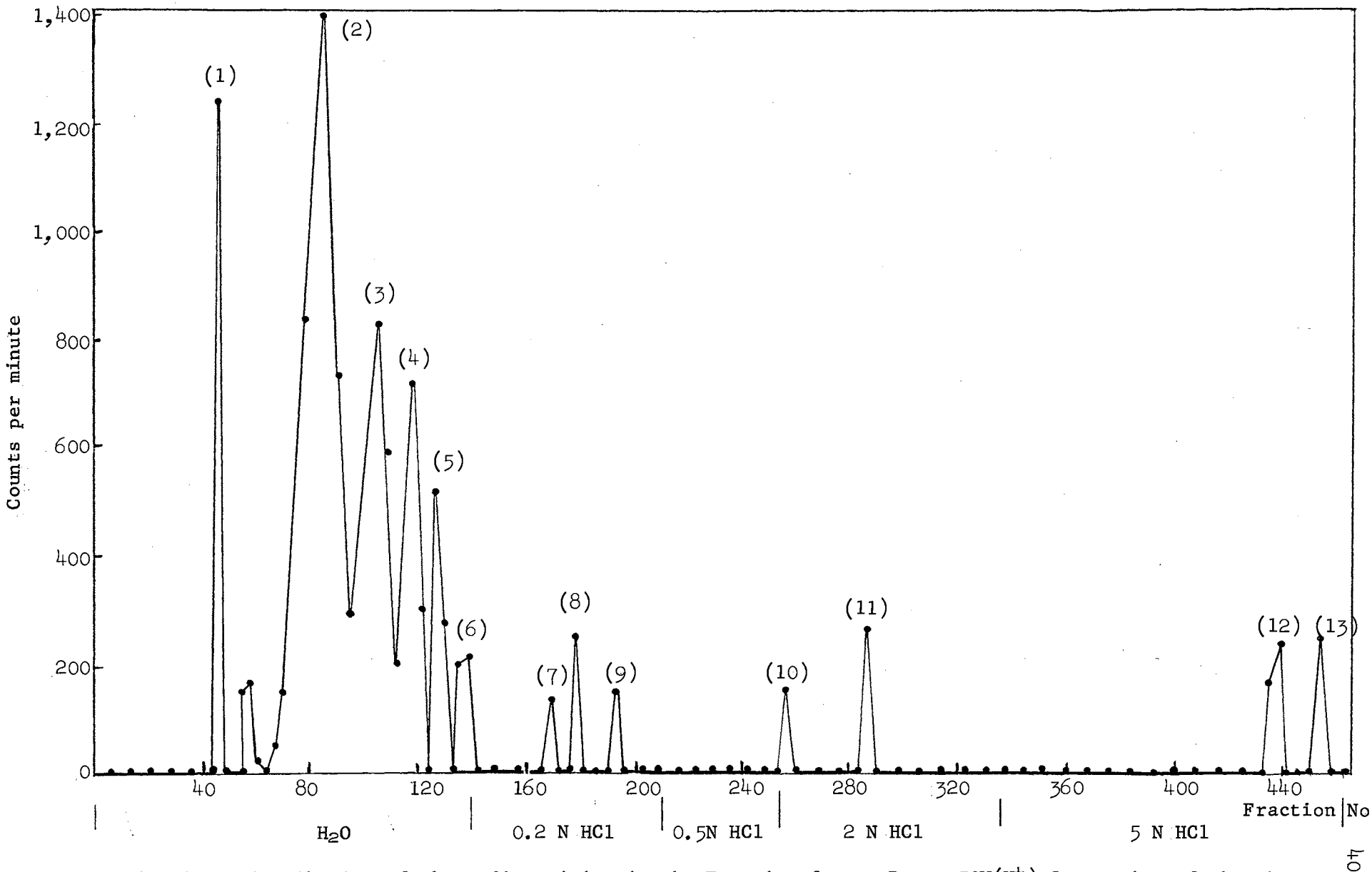


Fig. 15. Distribution of the Radioactivity in the Fraction from a Dowex-50W(H^+) Separation of the Photo-oxidation Products of DL-Tryptophan-7a-C^{14} and Unlabeled DL-Tryptophan Mixture.

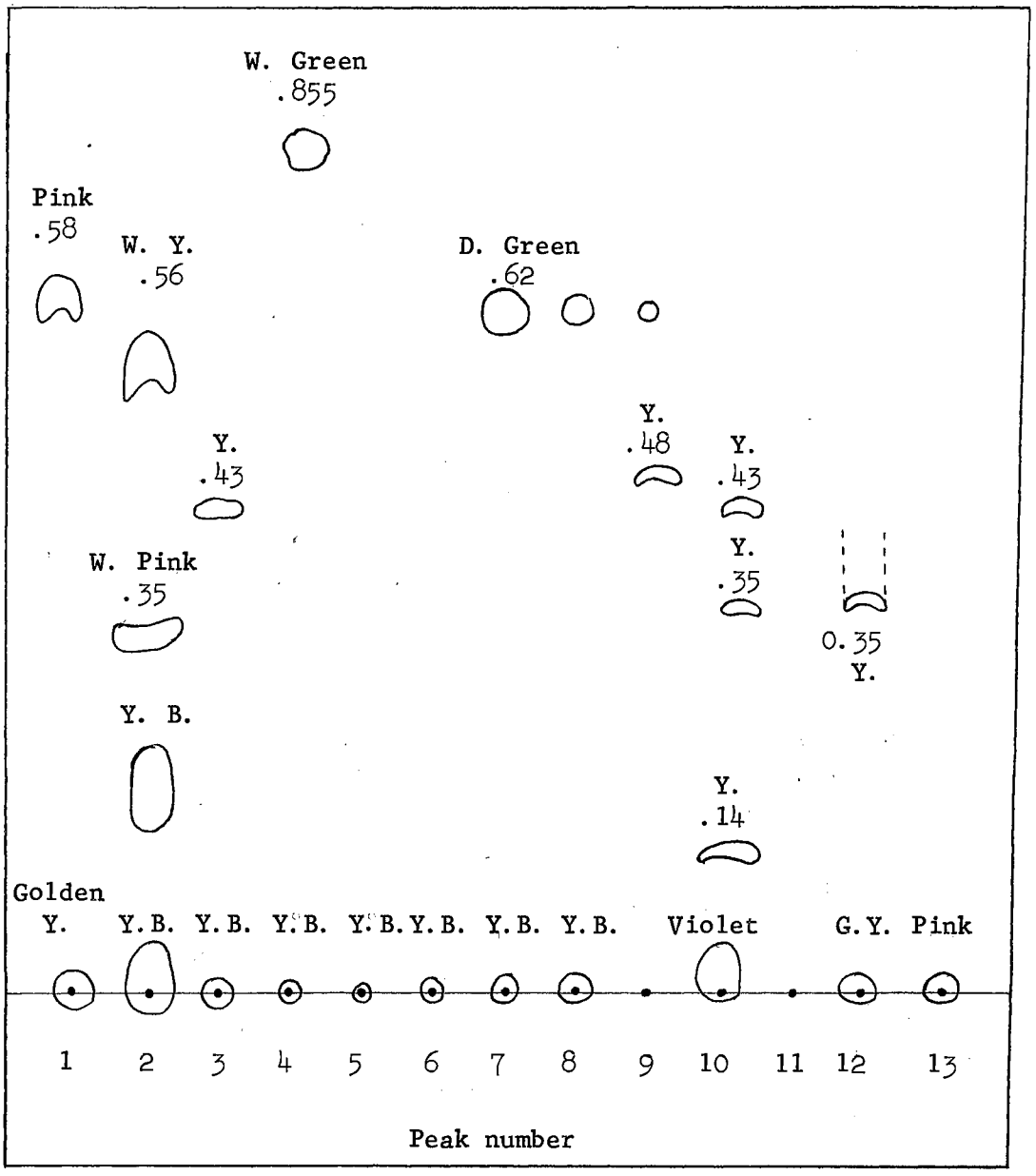


Fig. 16. R_F Value and Fluorescent Spots on Paper Chromatogram of the Water Soluble Extract of the Dried Materials of the Peak Fractions from a Dowex-50W(H^+) Separation.

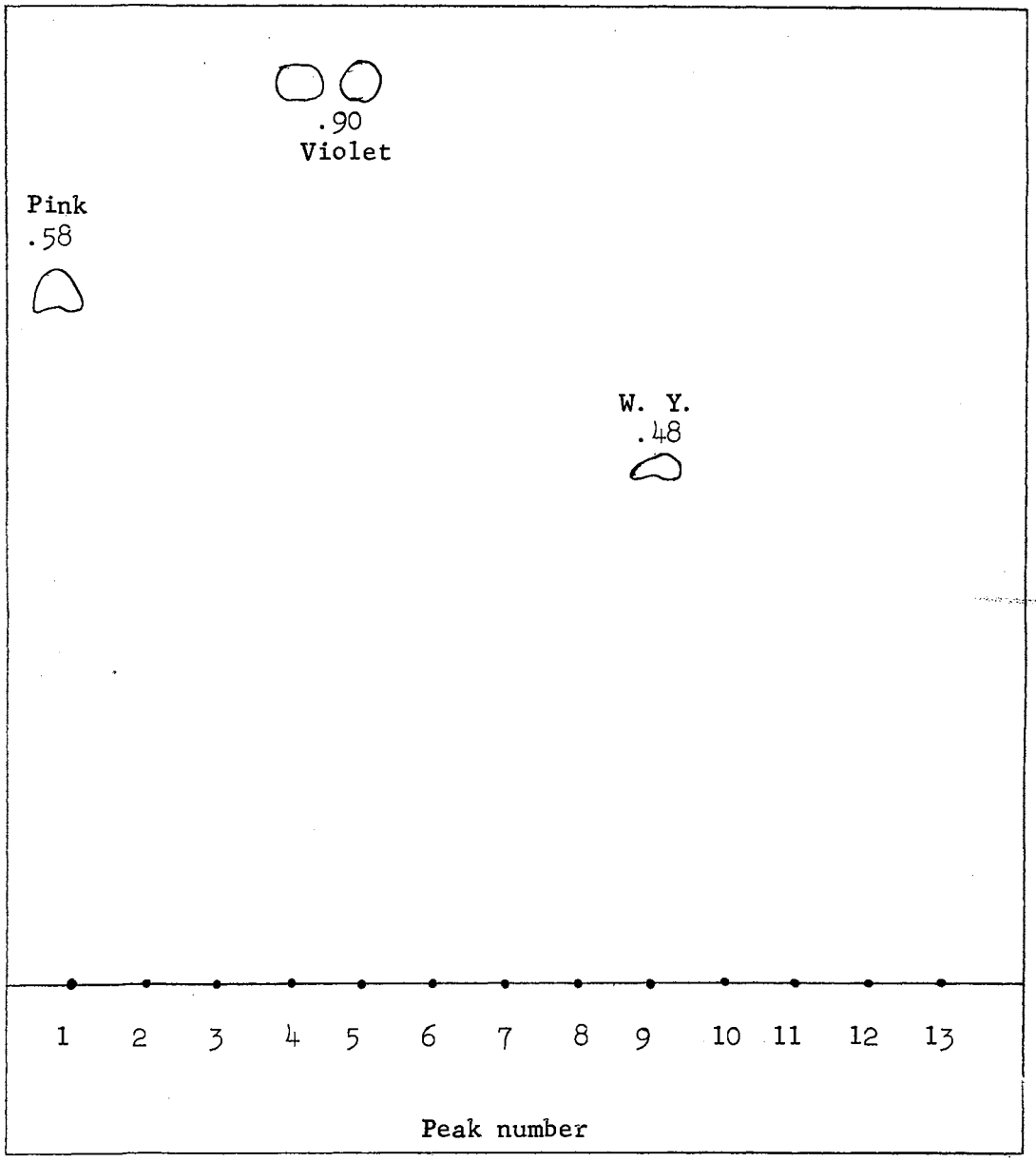


Fig. 17. R_F Value and Fluorescent Spots on Paper Chromatogram of the Water Insoluble Material which was Soluble in Methanol: n-Butanol: Benzene: Water Mixture.

in water. The overall recovery of radioactivity was 6 per cent in this experiment. Presumably most of the C^{14} was evolved as $C^{14}O_2$.

In a similar experiment, the products from 71.5 mg of DL-tryptophan in 200 ml of solution illuminated for 8 hours were mixed with 0.4 ml of photooxidation products of DL-tryptophan-α-C^{14} (Exp. 14). This mixture was passed through a Dowex-2 (formate) column and the column was washed with about 3 liter of deionized water to remove methylene blue. The radioactivity of the effluent was negligible. The column was then developed by gradient elution using formic acid and water as shown in Fig. 18. Recovery of C^{14} was about 35 per cent from the Dowex-2 column.

Each peak fraction from Dowex-2 (formate) column was taken to dryness and the residue chromatographed on Whatman No. 1 paper. The radioactive spots from paper chromatograms are shown in Table VIII.

In a very large scale experiment, the photooxidation products from 400 mg of tryptophan were mixed with 0.4 ml of products of DL-tryptophan-α-C^{14} (Exp. 14). This mixture was placed on a Dowex-50W(H^+) column, eluted with water and next with hydrochloric acid as shown in Fig. 19. Recovery of the C^{14} was about 62 per cent from a Dowex-50W(H^+) column.

Each peak fraction from the Dowex-50W(H^+) column was taken to dryness and the residue chromatographed on Whatman No. 1 paper. The radioactive spots from paper chromatograms are shown in Table IX.

Recovery of radioactivity from Dowex-50W(H^+) column was higher than from Dowex-2 (formate) column. However, the material remaining at the origin could not be found in the effluent from the Dowex-50W(H^+) column when the paper chromatograms were developed using n-butanol: acetic acid:water.

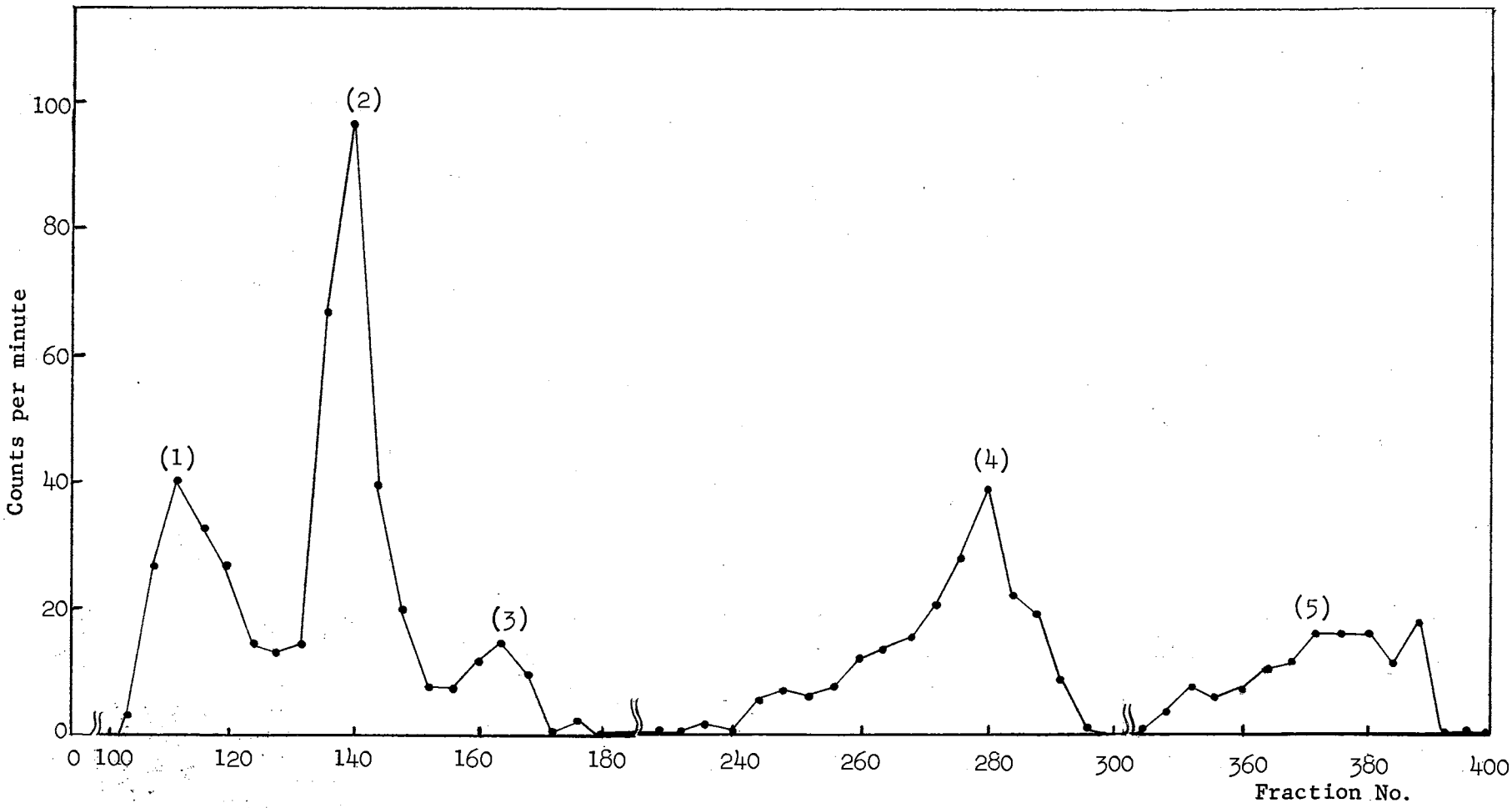


Fig. 18. Distribution of the Radioactivity in the Fraction from a Dowex-2(formate) Separation using Gradient Elution with Concentrated Formic Acid and Water.

TABLE VIII

R_F VALUES OF THE RADIOACTIVE MATERIALS ON PAPER CHROMATOGRAMS OF FRACTIONS FROM THE DOWEX-2 (FORMATE) COLUMNS (FIG. 18)

No. of Fraction	R_F Value
104 to 128	0.07; 0.80
129 to 152	0.18; 0.75; 0.86
156 to 180	0.07; 0.17
228 to 288	0.07; 0.21
348 to 390	Origin

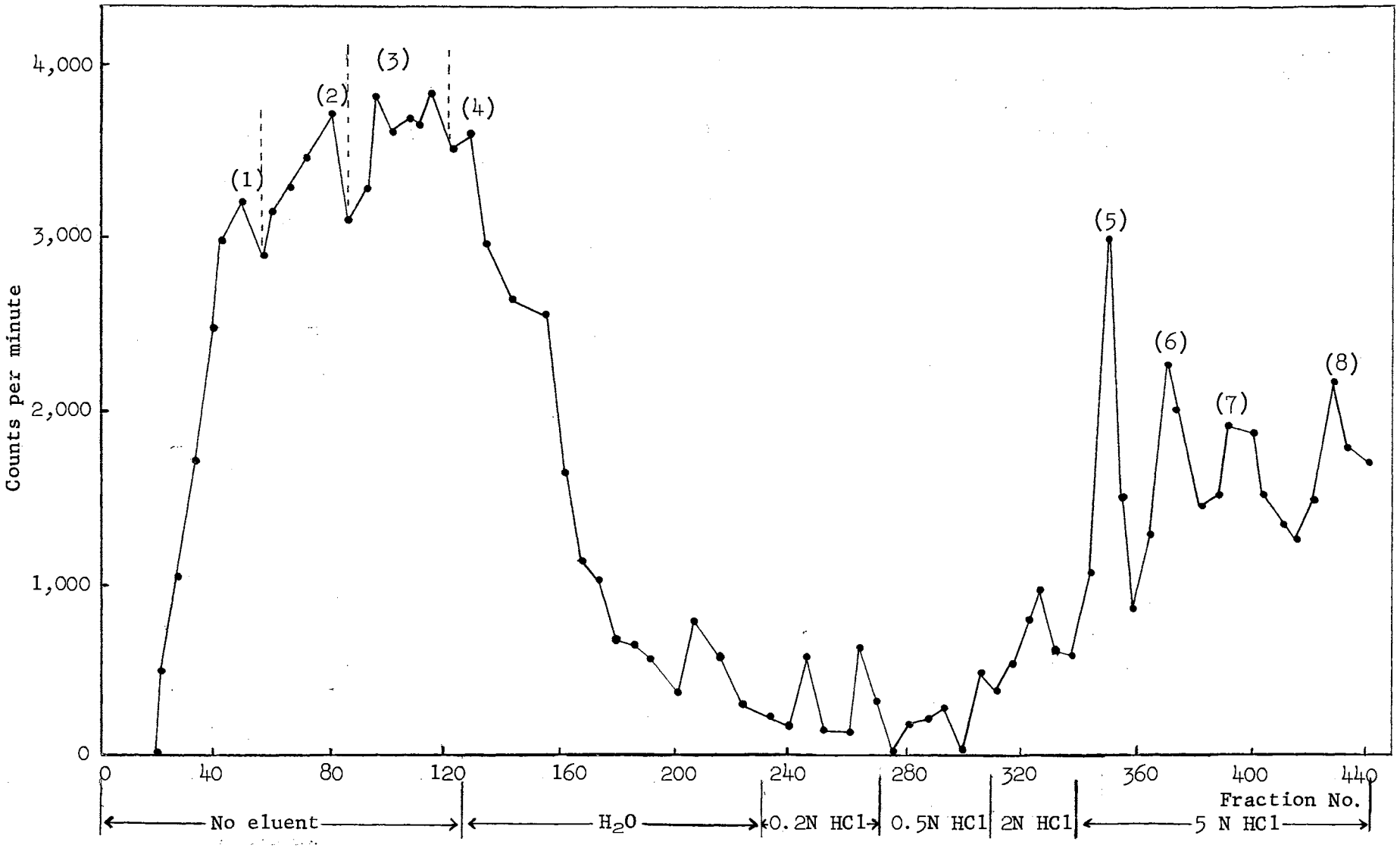


Fig. 19. Distribution of the Radioactivity in the Fraction from a Dowex-50W(H^+) Column.

TABLE IX

R_F VALUES OF THE RADIOACTIVE MATERIALS ON PAPER CHROMATOGRAMS OF FRACTIONS FROM DOWEX-50W(H^+) COLUMN (FIG. 19)

No. of Fraction	R_F Value
18 to 57	no spots
58 to 87	no spots
88 to 125	0.84
126 to 180	0.43
385 to 418	0.45
422 to 442	0.17; 0.41

DISCUSSION

Weil (19, 20) observed that tryptophan was the amino acid most susceptible to photooxidation when it was illuminated with visible light in the presence of methylene blue and that the pyrrole ring was degraded. He found, however, that the benzene nucleus was resistant to photooxidation even with 4 moles of oxygen uptake per mole of tryptophan. His observation that the benzene ring does not participate in photooxidation under these conditions is incorrect.

To date, none of the photooxidation products of tryptophan have been identified. The experiments described here were designed to provide information in this area. In these experiments the benzene nucleus was found to be initially resistant to photooxidation since $C^{14}O_2$ was released more slowly from DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14} then from DL-tryptophan-α-C^{14}. However, after seven hours illumination, DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14} released a greater percentage of the C^{14} as $C^{14}O_2$ than did DL-tryptophan-α-C^{14} as shown in Fig. 2. This evidence clearly shows that the benzene nucleus participates in the later stages of photooxidation. The rates of $C^{14}O_2$ release from DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14} was very similar.

When high concentrations of tryptophan (Exp. 5-13) were irradiated, a major product with an R_F value of 0.90 (weak pink fluorescence) using n-butanol:acetic:water solvent was obtained. On the other hand, in the experiment using low concentrations of tryptophan (Exp. 14, 15, and 16)

most of the radioactivity remained at the origin when the same solvent system was used. In this latter case, DL-tryptophan-7a-C^{14} produced this major product from the start of the reaction and in larger amounts. In contrast DL-tryptophan-5-C^{14} and DL-tryptophan-α-C^{14} in the early stages of illumination gave only small amounts of this product.

The pyrrole ring of tryptophan was broken down in the early stages of photooxidation and kynurenine was formed. However, it is possible that other products are also produced directly from tryptophan as well as kynurenine, since the material which had R_F value of 0.17 (Fig. 12) was produced together with kynurenine at the start of the reaction, but did not appear to be derivative from kynurenine.

The concentrations of tryptophan and light intensity were the most important factors in the photooxidation. Changes in these variables not only altered the quantity of products but also changed the nature of the products.

Although other experiments were performed to determine if tryptophan metabolites other than kynurenine were formed during photooxidation, no evidence was found to indicate that the photooxidative process was similar to any of the known biological pathways of tryptophan degradation. Kynurenic acid, 3-hydroxykynurenine, 3-hydroxyanthranilic acid, quinolinic acid or nicotinic acid could not be found in the photooxidized mixture.

DL-tryptophan-5-C^{14} and 7a-C^{14} released $C^{14}O_2$ during photooxidation at about the same rates. The radioactive products formed, differed significantly, however. Tryptophan-5- and α-C^{14} gave labeled products with R_F values of 0.10 (no fluorescence, ninhydrin positive) and 0.17 (yellow fluoresence under ultraviolet light, ninhydrin negative) while

tryptophan-7a-C^{14} did not. The latter compound gave rise to a labeled product whose R_F value is 0.27-0.30. None of this product was labeled when the tryptophan had C^{14} in the 5 or α- position.

These findings suggest that the equal rate of release of $C^{14}O_2$ from positions 5 and 7a- is a fortuitous circumstance and that a major fissiou takes place leaving carbon atoms α and 5, but not 7a in the same compound. The substance with an R_F of 0.10 contains an amino group (amino acid or amine) while the substance at R_F 0.17 does not have an amino group.

It is not clear whether the major product (R_F = 0) in the low concentration tryptophan experiments is a single substance or a mixture.

When the mixture of photooxidation products from tryptophan-α-C^{14} and from unlabeled tryptophan oxidized on a large scale was fractionated on a Dowex-50W(H^+) column, several closely associated peaks were collected in fractions 20 to 165 (Fig. 19). When each of these fractions were lyophylized and chromatographed on paper, no distinct labeled components were detected. This loss of C^{14} labeled compounds may have resulted from the extended exposure to the acid used in developing the column. In contrast to these results where the same reaction mixtures were fractionated over a Dowex-2(formate) column, several distinct radioactive fractions were widely separated. Paper chromatography of these separated fractions yielded radioactive zones usually with at least two such spots from each peak from the Dowex-2 column. (Table VIII).

SUMMARY

1. The photooxidation of tryptophan was studied using DL-tryptophan-α-C^{14}, DL-tryptophan-5-C^{14} and DL-tryptophan-7a-C^{14} in the presence of methylene blue and visible light.

2. Oxygen uptake and carbon dioxide released were measured by manometric techniques.

3. The rate of $C^{14}O_2$ evolution during photooxidation was measured for each specifically labeled tryptophan. The results showed that the side chain of tryptophan was readily oxidized CO_2. Contrary to reports in the literature, the benzene ring of tryptophan was found to be degraded during photooxidation to the extent of 25 per cent after a 3 hour lag period.

4. Photooxidation products were separated by paper chromatography. Between 3 and 9 radioactive products were obtained depending upon the condition employed for the photooxidation.

5. Kynurenine was identified as the major product in the early stages of photooxidation of tryptophan.

6. Attempt to isolate and identify the photooxidation products using Dowex-2 (formate) and Dowex-50W(H^+) columns is described.

BIBLIOGRAPHY

1. Oster, G., and McLaren, A. D., J. Gen. Physiol., 33, 215 (1950).

2. Raab, O., Biol., 39, 524 (1900).

3. Beck, L. V., and Nichols, A. C., J. Cellular Comp. Physiol., 10, 123 (1937).

4. Tung, T., and Zia, S. H., Proc. Soc. Expt. Biol. Med., 36, 326 (1937).

5. Tung, T., Proc. Soc. Expt. Biol. Med., 38, 29 (1938).

6. Chin, T. L., Proc. Soc. Expt. Biol. Med., 38, 697 (1938).

7. Gomez-Vega, P., Arch. Dermatol. u Syphilis., 33, 49 (1935); Chem. Abs., 30, 5721 (1936).

8. Perdrau, J. R., and Todd, C., Proc. Roy. Soc., (London) 112B, 277 (1933); Chem. Abs., 27, 2469 (1933).

9. Perdrau, J. R., and Todd, C., Proc. Roy. Soc., (London) 112B, 288 (1933); Chem. Abs., 27, 2706 (1933).

10. Clifton, C. E., Proc. Soc. Exptl. Biol. Med., 28, 745 (1931).

11. Lippert, K. M., J. Immunol., 28, 193 (1935); Chem. Abs., 29, 80482 (1935).

12. Jodlbauer, A., and Tappeiner, H., Deut. Arch. klini. Med., 72, 520 (1905).

13. Jodlbauer, A., and Tappeiner, H., Deut. Arch. klini. Med., 75, 386 (1905).

14. Blum, H. F., Pace, N., and Garrett, R. L., J. Cellular Comp. Physiol., 9, 217 (1937).

15. Hausmann, W., Naturwissenschaften 11, 945 (1923).

16. Sheard, C., Caylor, H. D., and Schlotthauer, C., J. Expt. Med., 47, 1013 (1928).

17. Blum, H. F., Physiol. Rev., 12, 23 (1932).

18. Schmidt, C. L. A., and Norman, G. F., J. Gen. Physiol., 4, 681 (1922).

19. Weil, L., and Buchart, A. R., Arch. Biochem. Biophys., 34, 1 (1951).

20. Weil, L., and Maher, J., Federation Proc., 9, 244 (1950).

21. Weil, L., Buchert, A. R., and Maher, J., Arch. Biochem. Biophys., 40, 245 (1952).

22. Weil, L., Buchert, A. R., Arch. Biochem. Biophys., 46, 266 (1953).

23. Shugar, David, Bull. acad. polon. sci., 1, 39 (1954); Chem. Abs., 48, 11518h (1954).

24. Weil, L., and Seibles, T. S., Arch. Biochem. Biophys., 54, 368 (1955).

25. Lieben, F., Biochem. Z., 184, 453 (1927).

26. Carter, C. W., Biochem. J., 22, 575 (1928).

27. Weil, L., Gordon, W. G., and Buchert, A. R., Arch. Biochem. Biophys., 33, 90 (1951).

28. Errera, M., Progress in Biophysics, 3, Academic Press Inc., New York, 1953, P. 96.

29. Yoshida, Z., and Kato, M., J. Chem. Soc. Japan, Pure Chem. Sect., 75, 112 (1954); Chem. Abs., 49, 12437f (1955).

30. Henderson, L. M., Rao, D. R., and Nystrom, F. R., Biochem. Prep., 6, John Wiley and Sons, Inc., New York, 1958, P. 90.

31. Mathur, G. P., Dissertation for the Degree of Ph.D., Oklahoma State University, May, 1961.

32. Dalgliesh, C. E., Biochem. J., 52, 3 (1952).

33. Dalgliesh, C. E., Biochem. J., 61, 334 (1955).

34. Mason, M., and Berg, C. P., J. Biol. Chem., 188, 783 (1951).

35. Palmer, J. K., Conn. Agri. Exp. Sta. Bull., 589, 12 (1955).

36. Cheftel, R. I., Munier, R., and Macheboeuf, M., Bull. soc. chim. biol., 34, 380 (1952).

37. Cheftel, R. I., Munier, R., and Macheboeuf, M., Bull. soc. chim. biol., 35, 1085, 1095 (1953).

38. Jepson, J. B., and Smith, I., Nature, 172, 772 (1953).

39. Jepson, J. B., and Stevens, B. J., Nature, 172, 1100 (1953).

40. Dalgliesh, C. E., Biochem. J., 52, 3 (1952).

41. Fewster, M. E., and Hall, D. A., Nature, 168, 78 (1951).

42. Bray, H. G., Thorpe, W. V., and White, K., Biochem. J., 46, 271 (1951).

43. Rodnight, R., Biochem. J., 64, 621 (1956).

44. Asquith, R. S., Nature, 168, 738 (1951).

45. Palevsky, H., Swank, R. K., and Greenchick, R., Rev. Sci. Instr., 18, 298 (1947).

46. Roy, J. K., and Price, J. M., J. Biol. Chem., 234, 2759 (1959).

47. Lindenblad, G. E., Kaihara, M., and Price, J. M., J. Biol. Chem., 219, 893 (1956).

48. Dent, C. E., Biochem. J., 41, 240 (1947).

49. Matsuda, G., Nagasaki Igakkai Zassi, 28, 811 (1953).

VITA

Je Hyun Kim

Candidate for the Degree

Master of Science

Thesis: PHOTOOXIDATION OF TRYPTOPHAN BY VISIBLE LIGHT IN THE PRESENCE OF METHYLENE BLUE

Major Field: Chemistry (Biochemistry)

Biographical:

Personal Data: Born at Seoul, Korea, July 13, 1927, the son of Sang Lim Kim.

Education: Graduated from the Preparatory School of Liberal Arts and Sciences College, Seoul National University in 1948; received the Bachelor of Science degree from Seoul National University, with a major in Chemistry, in September, 1953; completed requirements for the Master of Science degree in August, 1962.

Professional experience: Chief of organic division of the Technical Research Laboratory of Ministry of Transportation, Republic of Korea, from October, 1953 to June, 1955. Assistant professor, Department of Chemistry, Kyungpook National University, Taegu, Korea. Graduate research assistant, Department of Biochemistry, Oklahoma State University from 1960 to 1962.