Radiation Effects on the Metabolism of Phospholipids in the Central Nervous System of Albino Rats

National Aeronautics and Space Administration
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Progress Report V

Effects of X-Rays on Sphingomyelin Biosynthesis in Brain and Spine Mitochondria of Albino Rats.

Starting Date of Grant

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July 1, 1967 through August 31, 1968

Submitted by

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Abstract

The effect of x-rays on the biosynthesis of the phospholipid, sphingomyelin, in brain and spinal cord mitochondria has been studied. Mitochondria were isolated from male rats, irradiated and incubated with precursors of sphingomyelin. Sphingomyelin was isolated from the reaction mixture as alkali stable phospholipid. Identification of sphingomyelin was accomplished by infrared spectra, thin layer chromatography, and phosphorus determinations. Spinal cord mitochondria appeared to be the best source of ceramide transferase for sphingomyelin synthesis. X-irradiation caused an increase in sphingomyelin from both spinal cord and brain mitochondria. Moderate doses of x-rays caused a destruction of the carbonyl function of myristaldehyde.

Mechanism of Action of X-Rays on Phospholipids and Precursors

Introduction

As set forth in the proposal "Radiation Effects on the Metabolism of Phospholipids in the Central Nervous System of Albino Rats," three levels of organization were to be included in long term studies relative to the effects of radiation on phospholipid metabolism, (1) the molecular level, (2) the subcellular level, and (3) the organism level. Studies on the molecular level were to include the following:

a. The effects of x-rays on chemical structure of phospholipids and phospholipid precursors.

b. The effects of x-rays on the chemical and biochemical synthesis of phospholipids, especially sphingomyelin.

c. The isolation, purification, and characterization (with respects to radiation effects) of the enzyme, phosphorylcholine-ceramide transferase and phosphorylcholine-glyceride transferase.

The molecular level of organization has been investigated, and only certain aspects of (a) and (b) have been studied to any great extent. Experiments on the biosynthesis of sphingomyelin from irradiated rat brain and spine mitochondria are now in progress.

Spectroscopic and chromatographic evidence for the alteration of the molecular structure of phospholipids and related compounds was reported in Progress Report I (July 1, 1965-January 31, 1966) and Progress Report II (February 1, 1966 to August 31, 1966).

Analyses of infrared and ultraviolet spectra showed the intensification of carbonyl and amino group frequencies in x-irradiated phospholipids. At least two components were present in thin layer chromatograms of N-acylsphingosine (ceramide), choline chloride, lecithin, sphingomyelin, and cephalin. In the absence of oxygen and aqueous solvents, structural

changes were apparent in irradiated compounds. Powers and co-workers (1961) described oxygen-independent, temperature dependent irradiation induced changes as Class I type damage. Pasynskii and co-workers (1964) believed that Class I damage was due to direct radiation effects on a very small number of molecules. From our observations, this damage must follow a somewhat random course.

A kinetic model for radiation damage based upon the work done by Lindblom (1961) with choline chloride was proposed for ceramide in Progress Report III (September 1, 1966 to December 31, 1966). Progress Report IV was concerned with the characterization of breakdown products, resulting from the irradiation of phosphatidylserine, sphingomyelin, sphingosine, N-acylsphingosine, lysolecithin, lecithin, cytidine diphosphate choline, choline chloride, and other phospholipid precursors on thin-layer chromatogram sheets in the presence of oxygen.

This report (Progress Report V) presents preliminary findings on studies of the biosynthesis of sphingomyelin from an x-irradiated particulate enzyme from rat brain and spine mitochondria. Additional data on functional groups affected by x-rays are presented also.

Experimental Procedures

A colony of albino rats (Wistar strain) was developed from weanling rats originally obtained from Charles River Breeding Farms.

Rat brains and spines were obtained from male and female animals lightly anesthetized with chloroform. Only the cerebrum and spinal cords were used as a source of enzymes for these studies. The medulla oblongota and cerebellum were discarded.

A particulate enzyme fraction was prepared from the fresh brain or spine by the procedure of Sribney and Kennedy (1958). The protein content

of the enzyme fraction as determined by the method of Waddell (1956) was 0.5 to 1.2 mg of protein per ml of Tris (0.02 m) - Versene (0.001 m) buffer, pH 8.0. These preparations contained both microsomes and mitochondria. Particulate enzyme fractions were either stored at -20°C or subjected to x-irradiation from a Norelco MG 300 X-ray machine. Dosages of 100r were obtained from the 150 KV machine with physical conditions of 2 ma, 8 inch focal distance for 4 minutes. Dosages of 1000r were obtained on the same machine with 10 ma, 8 inch focal distance for two minutes.

Chromatographically pure compounds were used in all experiments. Sphingosine (DL Erythro form, Miles Laboratory), cytidinediphosphate choline (Boehringer - Mannheim Corporation), N-acylsphingosine (mixed ceramides, Applied Sciences Laboratories) were chromatographically pure. Sodium tetraethylenediamine tetra-acetate (Versine, Fisher Scientific) and Tris (hydroxymethyl) aminoethane (THAM, Fisher Scientific) were of the highest purity. All commercial solvents were redistilled prior to use. Tetradecyl aldehyde (Myristaldehyde, Aldrich Chemical Company) and 2,4-dinitrophenylhydrazine (Eastman Organic Chemicals) were routinely recrystallized from 95 per cent ethyl alcohol prior to use.

The protocol for a typical experiment is shown in Table I. All solutions were made up in 0.07 m Tris buffer. The enzymatic experiments were run in a volume of 3.2 ml on a Warburg Respiration Apparatus at 37°C for two hours. Absolute methanol (9.6 ml) was used to stop the enzymatic reaction. Extraction procedures were a combination of the procedures of Sribney and Kennedy (1958) and Rapport and Lerner (1957). The incubation mixture was successively extracted for labile lipids with 9.6 ml portions of methanol at 55°C for 5 minutes. Combined extracts were adjusted to pH 12.6 (0.4 N) with methanolic KOH. This mixture was incubated at 37°C for

2 hours, hydrolyzing the alkali unstable phospholipids (lecithin, cephalin, etc.). All vessels were adjusted to pH 7.0 with acid following incubation. Extraction was continued following the addition of 2 M KCl and chloroform. The chloroform phase was evaporated on a biodryer and re-extracted three times with KCl solution. After washing with water, the chloroform phase which contained the sphingomyelin and closely related compounds was chromatographed on thin layer chromatograms (Eastman, TLC or Gelman I TLC plates, types SG and A, respectively). Phosphorus determinations (Bartlett, 1959) were run on individual components eluted from chromatograms with 3 ml of hot water. The eluate was evaporated and infrared spectra were run on the dry materials in $CHCl_3$ using matched KRS-5 cells 0.93 mm path length.

Table I. Protocol for a Typical Experiment [a,b]

Additions	Amounts	Tube Number 1	2	3	4	5	6	7
1. Cysteine, µmoles	64	+	+	+	+	+	+	+
2. Enzyme		-	+	+ boiled		+ boiled		+ boiled
3. Protein, ug								
Male Brain	922							
Male Spine	393							
4. Mixed Ceramide, µmoles	12.8	+	-	-	+	+	-	-
5. Sphingosine, µmoles	12.8	+	-	-	-	-	+	+
6. CDP-Choline, µmoles	2.56	+	-	-	+	+	+	+
7. Tris Buffer, µmoles	168	+	+	+	+	+	+	+
8. Tween-20, Mg	3.2	+	+	+	+	+	+	+
9. $MnCl_2$, µmoles	32	+	+	+	+	+	+	+
10. Water, ml	0.8	+	-	-	-	-	-	-
TotalVolume, ml	3.2	+	+	+	+	+	+	+

a. Tubes numbers 2-7 contained enzyme particles obtained from either brain or spine tissues.

b. The same experiments were run with x-irradiated particles replacing the non-irradiated particles for both brain and spine enzyme particles.

Activity was represented as micromoles of sphingomyelin formed per microgram of protein per hour.

Aldehydes were determined quantitatively as the basic 2,4-dinitrophenylhydrazines by a combination of the methods of Wittenberg, Korey and Swenson (1956); Dhont and DeRooy (1961); and Sibley and Lehninger (1949).

Results

Effect of X-Rays on Sphingomyelin Biosynthesis

Female brain mitochondria appeared to be the best source of enzymes for sphingomyelin synthesis as is indicated in Table II. The next best source of these enzymes is male spine. Activities of 7.0 and 4.9 x 10^{-3} µmoles sphingomyelin/µg protein/hour were obtained for female brain and male spine enzymes, respectively. The sphingomyelin formed was homogeneous by thin layer chromatography using two solvents, diisobutyl-ketone-Acetic acid- Water (40:20:3) and N-heptane-diisobutyl-ketone (96:6)-and a modification of the procedure of Marinetti and Stotz (1960).

Table II. Sphingomyelin Formation by Brain and Spine Mitochondria of Adult Male and Female Albino Rats

	Conditions	Protein used µg	Sphingomyelin found in µmoles	Activity µmoles sphingomyelin/µg protein/hour x 10^{-4}	Identification by TLC
	Control	-	-	-	-
Brain	+Complete				
Male	"	922	8.6	1.6	+
Female	"	416	17.6	7.1	+
Spine					
Male	"	393	11.6	4.9	+
Female	"	870	6.6	1.3	+

+ The complete experiment contained substituents as indicated in item 4, Table I. + indicates sphingomyelin.

Components were visualized by iodine vapors, Rhodamine 6-G and 0.005 per cent solution or Munier and Macheboeuf modification of the Dragendorff Reagent (for choline phosphatides such as lecithin, lysolecithin and sphingomyelin). (See chromatographic separation patterns 1 and 2 in appendix).

Because of the availability of adult male rats, all of the remaining data will be given for males only.

When irradiated rat brain mitochondria was incubated with sphingosine (12.8 µmoles) and CDP-choline (2.56) µmoles, an alkali stable phosphorus containing substance was formed (sphingomyelin concentration was calculated from phosphorus content); however, the as yet unidentified substance was not sphingomyelin as seen by the negative results obtained by TLC shown in Table III. It should be noted that X-rays seemed to enhance the formation of this substance in the irradiated enzyme. Addition of the natural precursor of sphingomyelin, N-acylsphingosine, was necessary before sphingomyelin could be identified from the chromatograms, although there was not a net change in activity (Table IV). (See chromatograms 3-5 in appendix).

Table III. Effect of X-rays on Sphingomyelin Synthesis from Sphingosine by Brain Mitochondria [a]

Conditions	Sphingomyelin µmoles	Activity x 10^{-4}	Identification by TLC
1. Blank	0	0	
2. Enzyme	2.6	0.5	Sphingomyelin + sphingosine
3. Boiled Enzyme	0	0	-
4. Irradiated Enzyme	7.3	1.3	Sphingomyelin + sphingosine
5. Boiled-Irradiated Enzyme	0	0	No sphingomyelin, sphingosine
6. Enzyme + Sphingosine	15.1	2.7	No sphingomyelin, sphingosine
7. Boiled Enzyme + Sphingosine	0	0	Sphingosine
8. Irradiated Enzyme + Sphingosine	13.2	2.4	Sphingosine Major component

[a] Dose 1000r. All experiments with sphingosine also contained 2.56 µmoles of cytidine diphosphate choline. Activity expressed as micromoles of sphingomyelin formed per microgram protein per hour. Conditions outlined in Table I.

Table IV. Sphingomyelin Synthesis from N-Acylsphingosine by Brain Mitochondria [a]

Conditions	Sphingomyelin μmoles	Activity x 10^{-4}	Identification by TLC
1. Blank	0	0	0
2. Enzyme	2.6	0.5	Sphingomyelin, sphingosine
3. Boiled Enzyme	0	0	-
4. Irradiated Enzyme	7.30	1.3	Sphingosine Major component
5. Enzyme + Mixed N-Acyl-sphingosine (complete)	11.9	2.2	Sphingomyelin Major component
6. Complete + Boiled Enzyme	0	0	0
7. Complete + Irradiated Enzyme	13.2	2.4	Sphingomyelin
8. Complete + Boiled-Irradiated Enzyme	0	0	Some sphingosine

[a] Dose 1000r. All experiments with N-Acylsphingosine also contained 2.56 μmoles of cytidine diphosphate-choline. Other conditions outlined in Table I.

Again it should be noted in these experiments that X-rays had an enhancing effect.

In Table V, it can be seen that sphingomyelin synthesis was present to a much larger extent in spine mitochondria than in brain mitochondria. Irradiated spine mitochondria had greater activity for sphingomyelin synthesis without co-factors. In fact, co-factors normally used for sphingomyelin synthesis appeared inhibitory. Sphingosine was inhibitory (Table VI). N-acylsphingosine enhanced sphingomyelin synthesis while irradiation plus ceramide caused a decline in synthesis. In experiments not shown, it was found that a ten-fold reduction in the irradiation dose was without effect. (Chromatograms 6-9 in appendix show identification patterns for these experiments).

Table V. Effect of X-Rays on Sphingomyelin Synthesis from Sphingosine by Spine Mitochondria [a]

Conditions	Sphingomyelin μmoles	Activity x 10^{-4}	Identification by TLC
1. Blank	0	0	0
2. Enzyme	16.1	6.8	+ Sphingomyelin + other components
3. Boiled Enzyme	0	0	- Large amount of unidentified component
4. Irradiated Enzyme	90.5	34.8	+ Sphingomyelin and other components
5. Boiled-Irradiated Enzyme	0	-	Some unidentified components
6. Enzyme + Sphingosine	11.1	4.7	- No sphingomyelin
7. Boiled Enzyme + sphingosine	5.0	2.1	- No sphingomyelin
8. Irradiated Enzyme + sphingosine	26.3	11.2	+ Sphingomyelin present

[a]Dose 1000r. Other conditions same as in Table III and Table I. (See chromatograms 10-15a in appendix).

Table VI. Sphingomyelin Synthesis from N-Acyl-Sphingosine by Spine Mitochondria [a]

Conditions	Sphingomyelin μmoles	Activity x 10^{-4}	Identification by TLC
1. Blank	0	0	-
2. Enzyme	16.1	6.8	+
3. Enzyme + N-Acyl-sphingosine	55.9	23.7	+ also sphingosine
4. Boiled Enzyme N-Acylsphingosine	0	0	+
5. Irradiated Enzyme + N-Acylsphingosine	36.3	15.4	+
6. Boiled-Irradiated Enzyme + N-Acylsphingosine	0	0	-
7. Boiled Enzyme	0	0	-
8. Irradiated Enzyme	90.5	34.8	+ (TLC plates 11, 11a)

[a] Dose 1000r. (See chromatograms 10-15a in appendix).

Effect of X-Rays on Aldehydes

When myristaldehyde (C_{14}) and propionaldehyde (C_3) were exposed to a dose of 10,000r X-rays, mixed results were obtained. Myristaldehyde lost 43 per cent of its carbonyl function as measured by infrared spectra and from 64-28 per cent when activity was measured by its 2,4-dinitrophenyhydrazone (Table VII). Oppositely, the three carbon aldehyde, propionaldehyde, showed no change in its 5.8 carbonyl function by IR; however, there were changes in the amount of 2,4-dinitrophenylhydrazone at concentrations above 0.2 micromoles. Careful analysis of the infrared spectra of both short and long chain aldehydes showed changes in number of carbon-carbon double bonds in myristaldehyde, carbon-hydrogen bonding in both propionaldehyde and myristaldehyde, and possibly the formation of alcoholic OH groups in both compounds.

Table VII. Effect of X-Rays on Carbonyl Function of Myristaldehyde and Propionaldehyde [a]

	Concentration in μmoles			Infrared Spectral Changes at 5.8μ	
	Before	After	Per cent Change	Per cent	Other IR Changes
Myristaldehyde					
1.	2.00	0.72	64	43	
2.	1.00	0.44	56	"	increased OH, C-C,
3.	0.50	0.36	28	"	and C-H splitting
4.	0.10	0.22	22	"	
Propionaldehyde					
1.	0.50	0.55	10	No Change	increased OH, and
2.	0.20	0.14	30		C-H splitting
3.	0.10	0.10	0		
4.	0.01	0.01	0		

[a] Dose 10,000r. Other conditions described in text.

Discussion

Animal mitochondria is generally considered to be particularly radiosensitive. Phosphorylation by rat spleen and liver mitochondria was suppressed by 800r whole-body (Benjamin and Yost, 1960). Data presented in this report showed that exposure of isolated brain and spinal cord mitochondria to 100 and 1000r of x-rays enhanced the formation of sphingomyelin and related compounds. This finding supports the reports of Soviet investigators who reported an increase in lipid synthesis by microorganisms exposed to irradiation (discussed by Hoptman, 1962).

An apparent increase of sphingomyelin in irradiated cerebellum and spine mitochondria could also be associated with the "patchy demyelination sometimes prominent throughout the white matter of the cerebrum, cerebellum, pons, and spinal cord" reported by VanCleave (1963) in work done on x-irradiated monkey brain. VanCleave also mentioned demyelinated areas of human spinal cord which were densely packed with fat-containing cells. An excessive amount of fat was reported to have been found in many of the ventral horn cells. That a large amount of this fat was due to sphingomyelin is supported by the findings by Sheltway and Dawson (1966) who reported that between 20 and 30 per cent of the lipid phosphorus of myelinated nerve fibers was due to sphingomyelin. The amount of sphingomyelin in the gray matter is higher than it is in the white matter of young humans, whereas; the reverse is true for adults (O'Brien, 1955).

A phosphatidylinositide like compound could possibly be one of the unidentified phosphorus compounds identified by infrared spectra. Possmayor and Strickland (1967, 1967a) and Paulus and Kennedy (1960) found that cytidinodiphosphate (CDP) choline, CDP-glycerol, CDP-ethanolamine, and cytidinotriphosphate stimulated phosphomonoinositide formation in rat brain

preparations. The reaction presumably was by way of the phosphatidic acid to CDP-diglyceride route.

Perusal of the infrared spectra obtained from isolated alkali stable brain and spine phospholipids showed a sphingomyelin like compound which contained trans double bonds, covalent phosphates, trimethylammonium groups and very prominent carbonyl groups. The spectrum of commercially available sphingomyelin contained no carbonyl functions. The differences in the two spectra may be due to a keto enol rearrangement within the sphingomyelin molecule either during synthesis or during the alkaline conditions present during isolation. A quantitative determination of the total carbonyl content of sphingomyelin was not undertaken.

In view of the above findings, it is not unreasonable to assume that the increased accumulation of sphingomyelin, post-radiation was due, not to synthesis, but to demyelination in the spinal cord mitochondria. This idea is supported by the finding that precursors (sphingosine and N-acyl-sphingosine) of sphingomyelin, which should have enhanced synthesis, really were inhibitory. The effect of irradiation on phospholipid synthesis in the brain and central nervous system is very complex, and definitive answers to the question of what molecular reactions are involved in the response of isolated mitochondria to x-rays await additional study. Likewise, the series of reactions which take place when pure phospholipids and related compounds are irradiated in the absence of oxygen and external heat require further study.

Summary

It has been found that x-irradiation (1) increases the amount of sphingomyelin and related compounds released in cerebellum and spinal cord mitochondria, (2) may render sphingosine and N-acylsphingosine (ceramide)

inhibitory to ceramide transferase for sphingomyelin synthesis, and (3) causes an aberration in carbonyl functional groups, especially of aldehydes.

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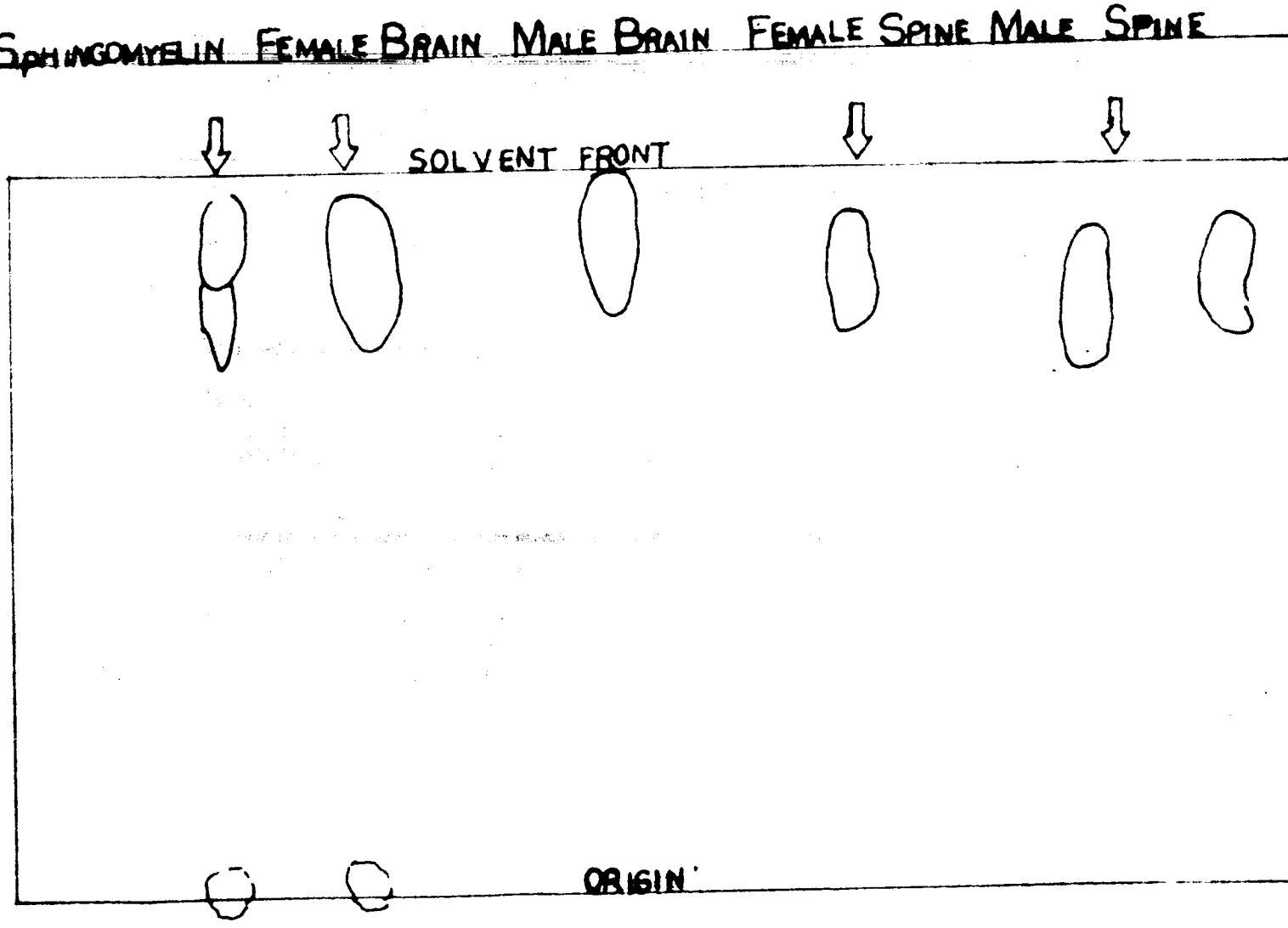
Appendix

Major Expenditures to Date (July 1, 1967 to August 31, 1968)

I.	Personnel (Salaries and Wages)	
	A. Principal Investigator, Charlotte O. Lee, Ph.D. (One-twelfth of annual salary per month).	$ 2,430.00
	B. Technician, Mrs. Yvonne Allen, B.S. (Fulltime). . . .	4,010.00
	C. Undergraduate Assistants	
	1. Mr. Jimmie Echols, Junior (Chemistry, Major). . .	457.20
	2. Miss Sandral Hulett Senior (Biology, Major, July and August, 1967).	361.88
	3. Miss Committer Booker, Senior (Chemistry Major) .	319.77
	4. Mr. Ben Echols (Chemistry Major (Animal Care) . .	40.00
	5. Miss Shirley Williams (Chemistry Major, Animal Care)	32.50
	6. Mr. L. C. Holloway (Animal Care).	10.00
	D. Fringe Benefits (Institutions's share of F.I.C.A.). .	172.95
	E. Total Personnel	$ 7,834.30
II.	Major Items of Equipment.	$ 233.92
III.	Consumable Supplies	$ 1,157.92
IV.	Travel:	
	Redstone Arsenal, Technician's car.	$ 13.62
	Federation Meetings, April, 1968.	254.10
V.	Total Direct Expenses to Date (August 31, 1968)	$ 9,493.86
VI.	Overhead (indirect costs) - 20%	$ 2,848.16
VII.	Total Funds Available (July 1, 1967).	$16,564.34
VIII.	Total Funds Expended (July 1, 1967 - August 31, 1968) . .	$12,342.02
X.	Balance in Grant Funds.	$ 4,222.32

CHROMATOGRAM 1. SYNTHESIS OF SPHINGOMYELIN BY MALE AND FEMALE RAT BRAIN AND SPINE MITOCHONDRIA.

GELMAN PAPER DIISOBUTYL KETONE: ACETIC ACID: H_2O, SOLVENT B



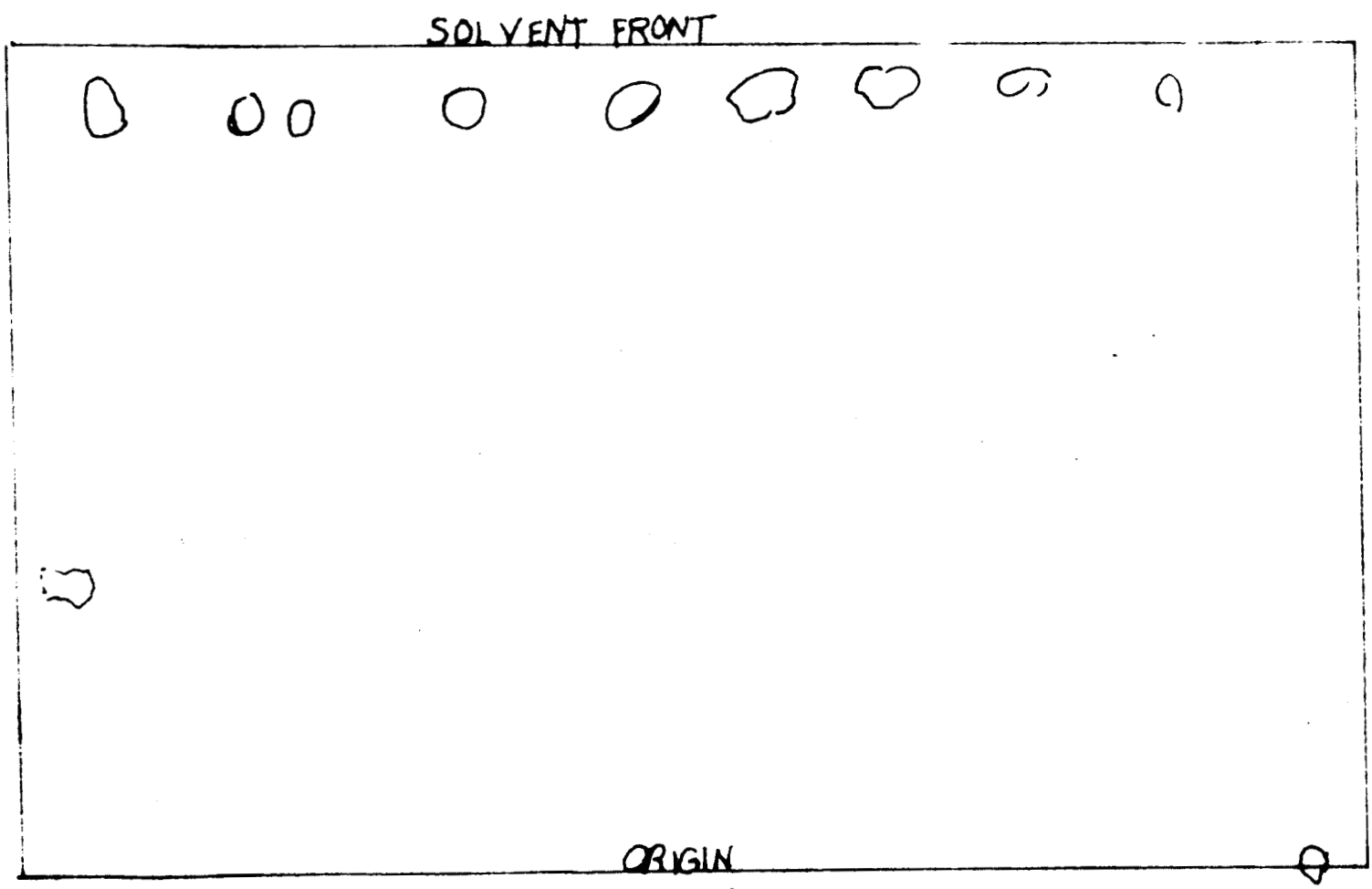
CHROMATOGRAM II. SYNTHESIS OF SPHINGOMYELIN BY MALE AND FEMALE RAT BRAIN AND SPINE MITOCHONDRIA.

EASTMAN PAPER $CHCl_3 : CH_3OH : CH_3COOH : H_2O$,

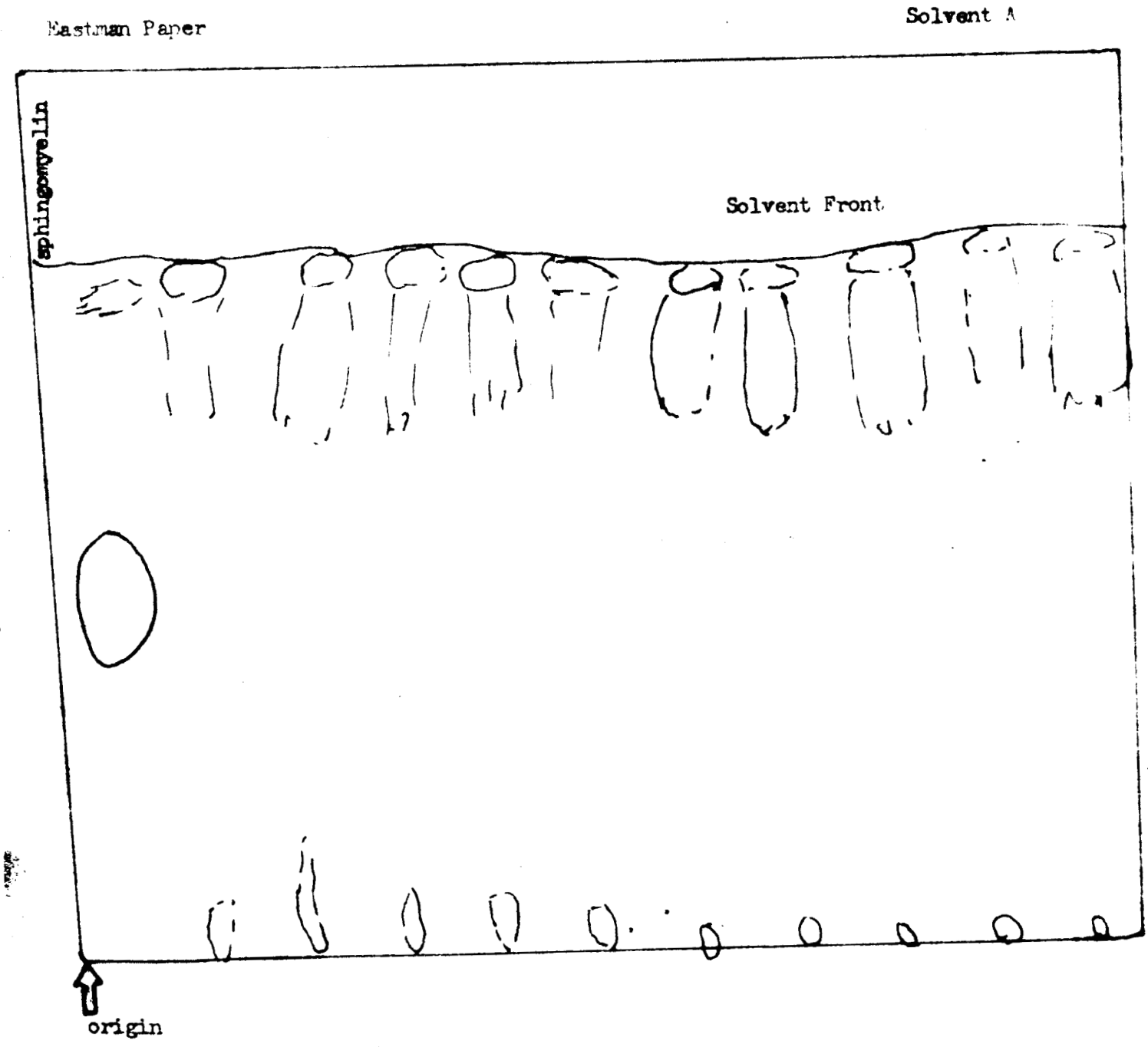
SOLVENT A

MALE

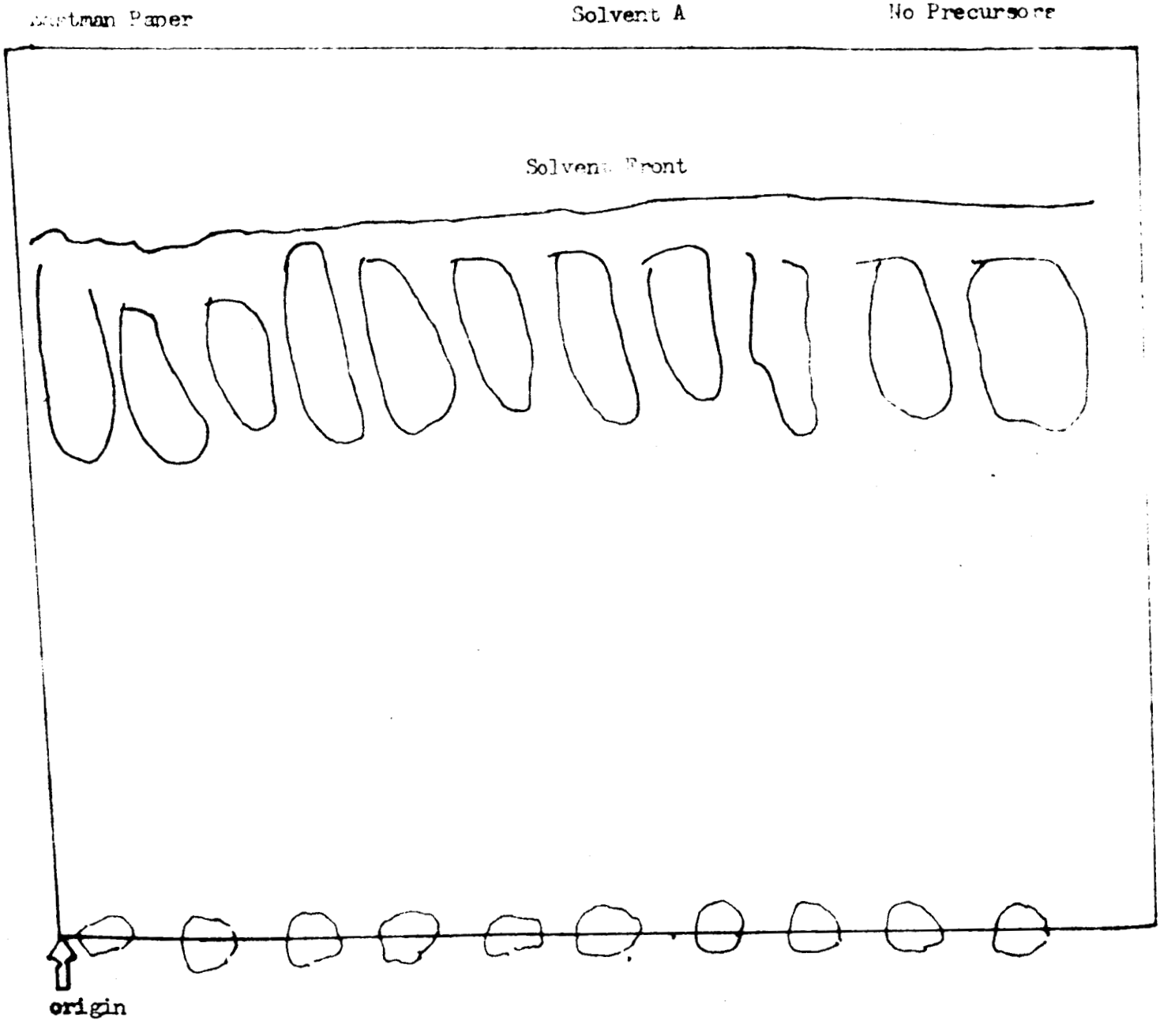
SPHINGOMYELIN, FEMALE BRAIN, BRAIN, FEMALE SPINE, MALE SPINE, BLANK



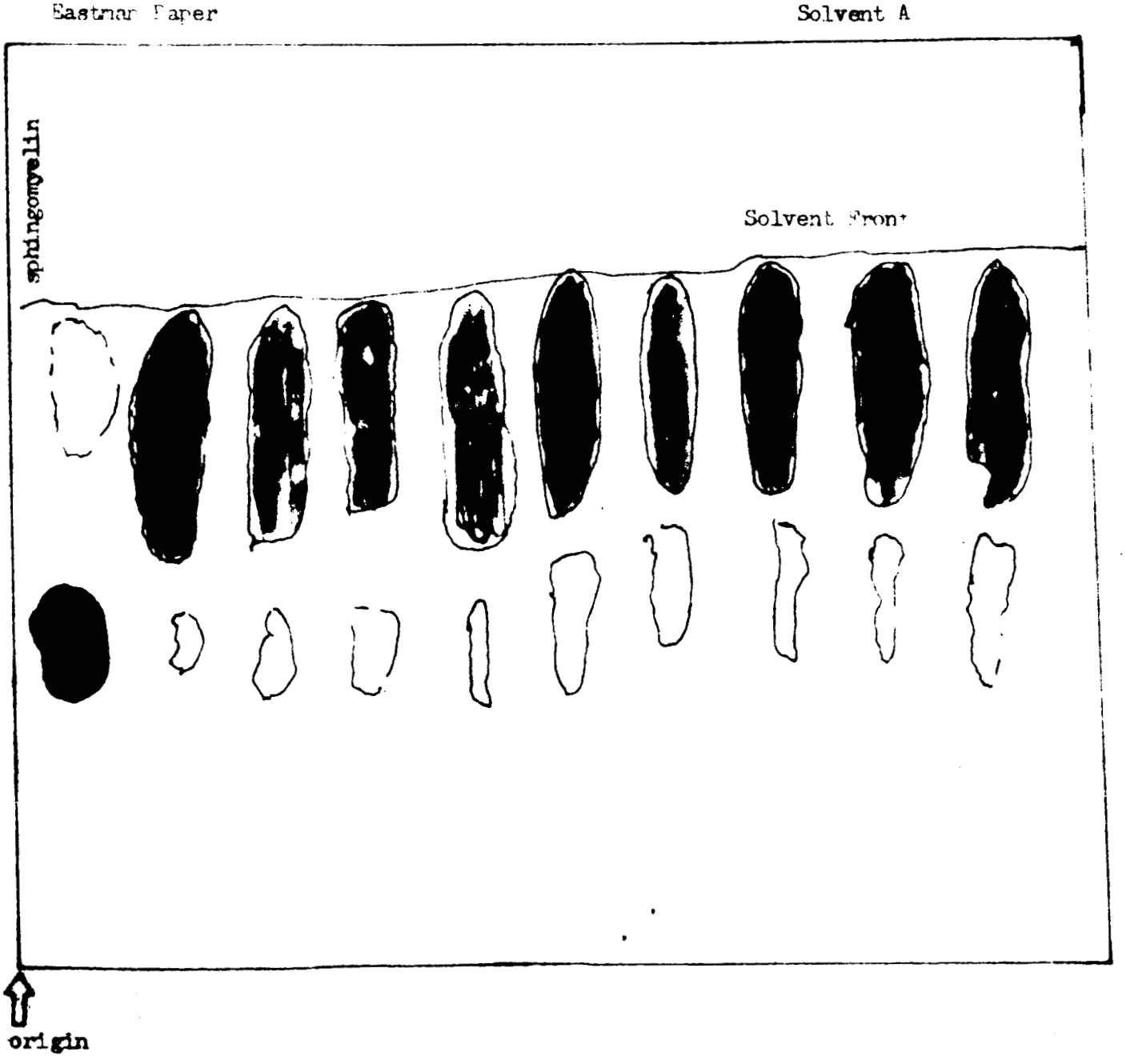
CHROMATOGRAM IV. Sphingomyelin by Rat Brain Mitochondria in Presence of Sphingosine



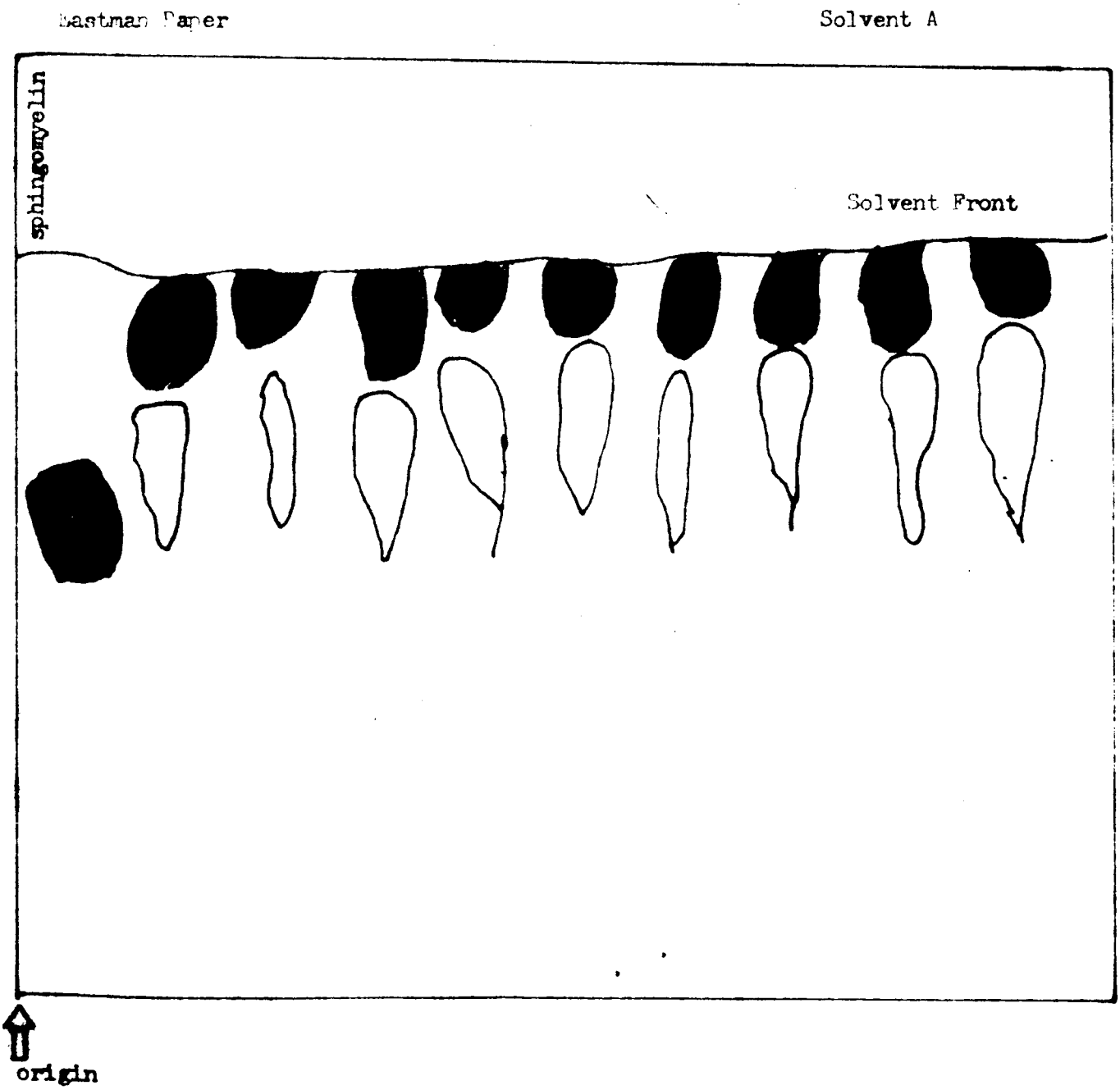
CHROMATOGRAM III. Sphingomyelin Formation by Rat Brain Mitochondria



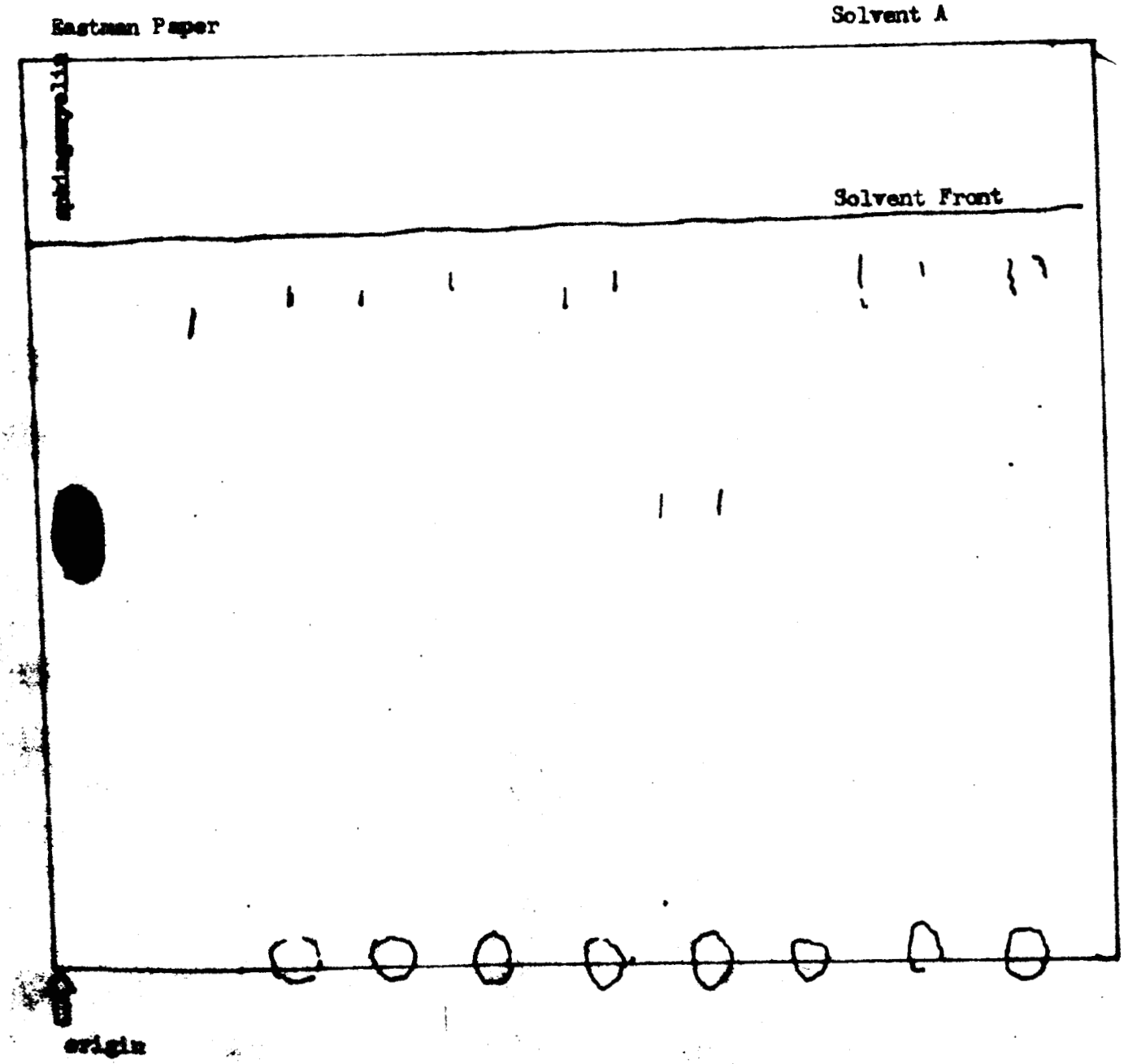
CHROMATOGRAM V. Effect of X-Rays on Sphingomyelin Formation by Brain Mitochondria in Presence of N-Acylsphingosine



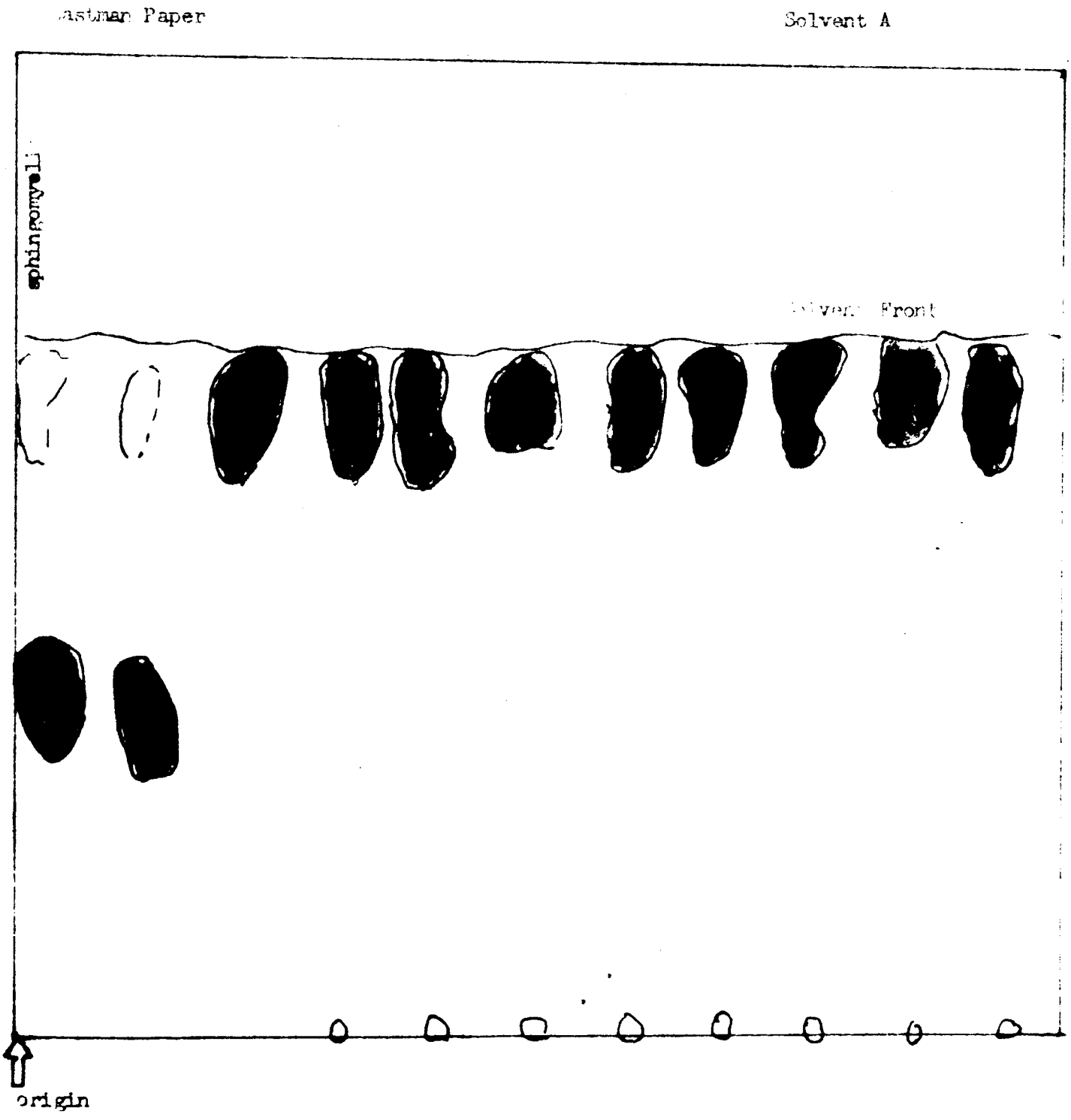
CHROMATOGRAM VI. Sphingomyelin Formation by Brain Mitochondria in Presence of N-Acylsphingosine



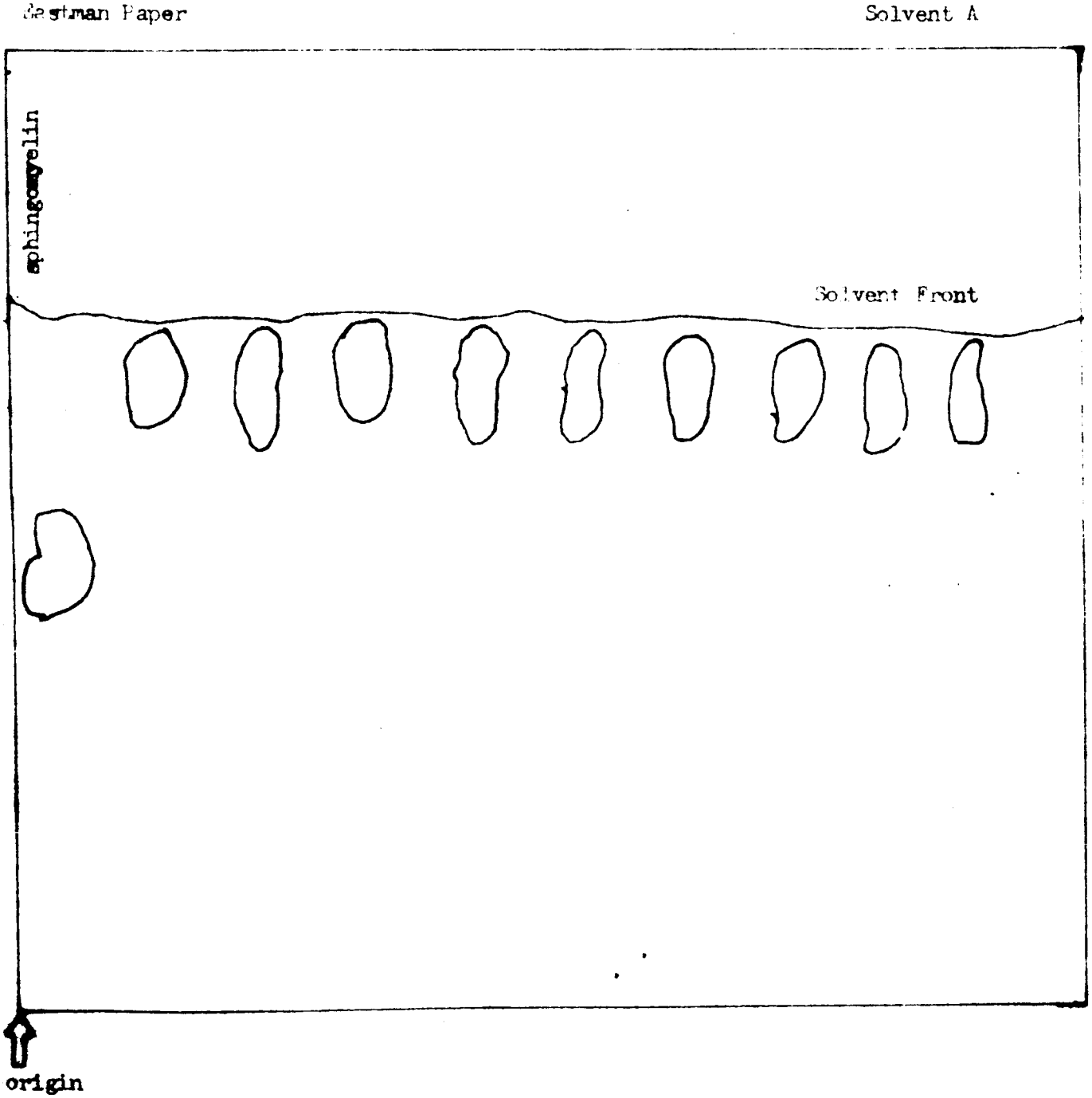
CHROMATOGRAM VII. Effect of Heat on Sphingomyelin Formation by Brain Mitochondria in Presence of N-Acylsphingosine



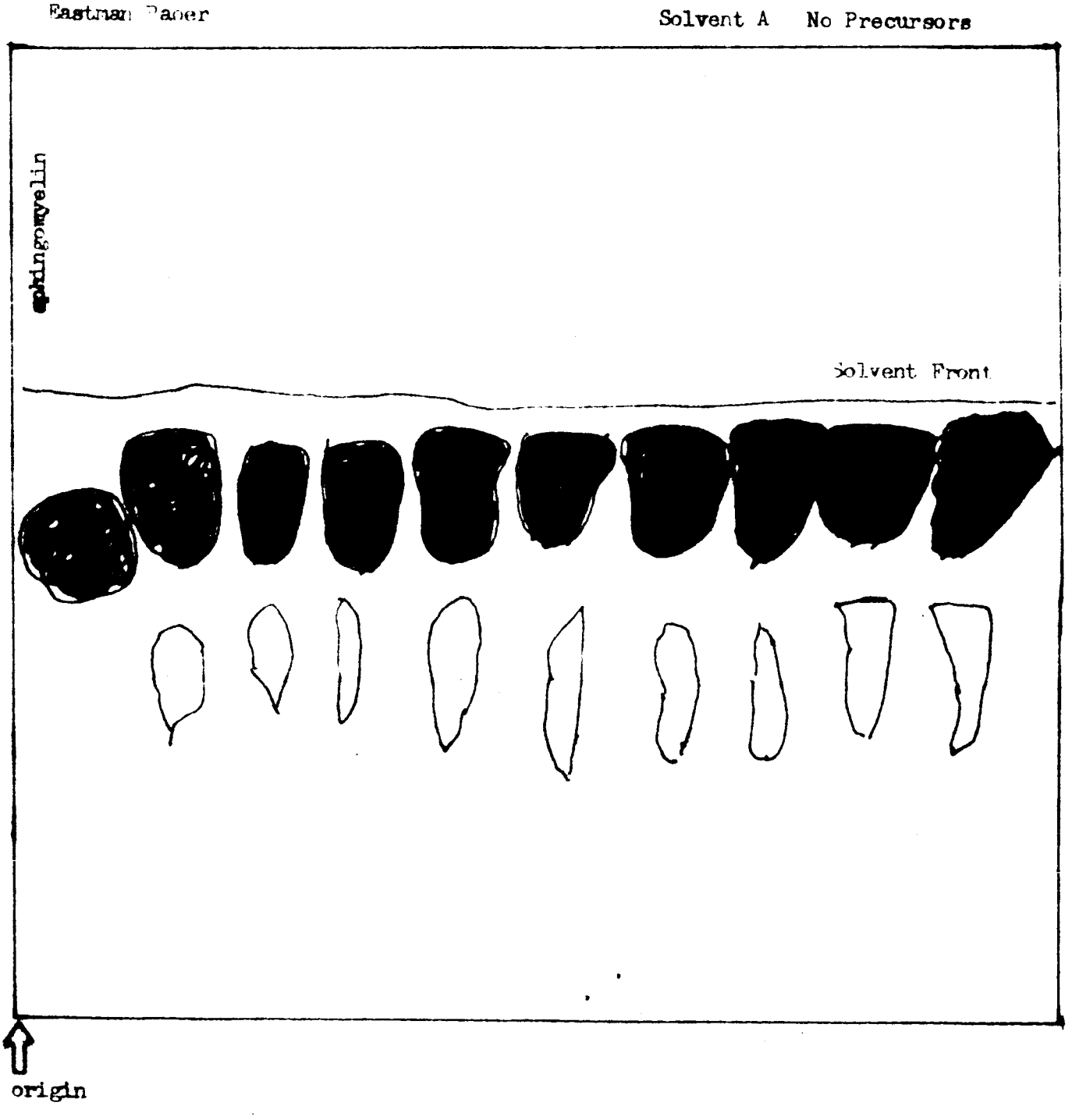
CHROMATOGRAM VIII. Effect of X-Rays on Sphingomyelin Formation by Boiled Brain Mitochondria in Presence of N-Acylsphingosine



CHROMATOGRAM IX. Effect of X-Rays on Sphingomyelin Formation from Sphingosine by Brain Mitochondria



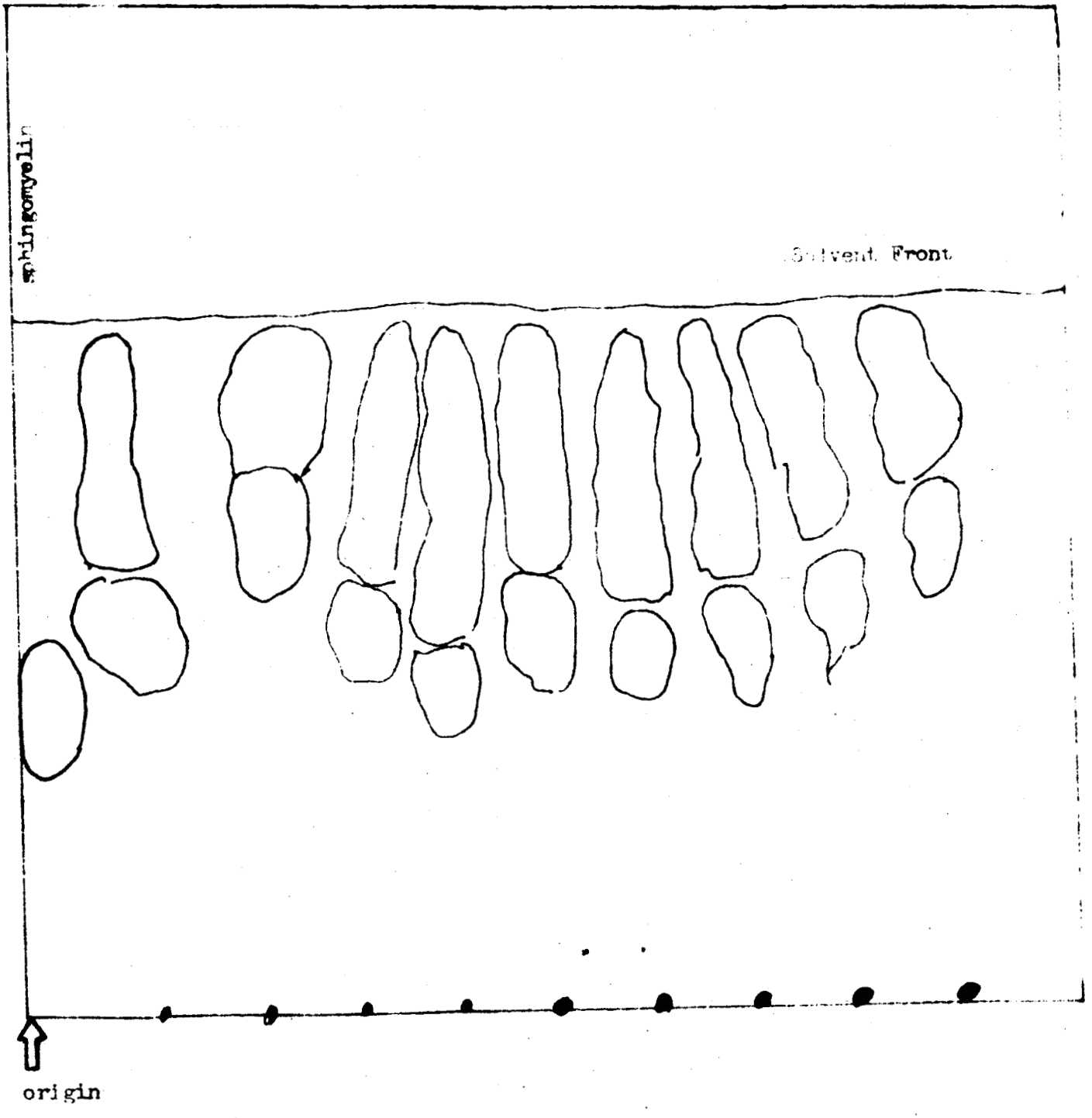
CHROMATOGRAM X. Sphingomyelin Formation by Spine Mitochondria



CHROMATOGRAM XI. Sphingomyelin by Irradiated Spinal Cord Mitochondria

Whatman Paper

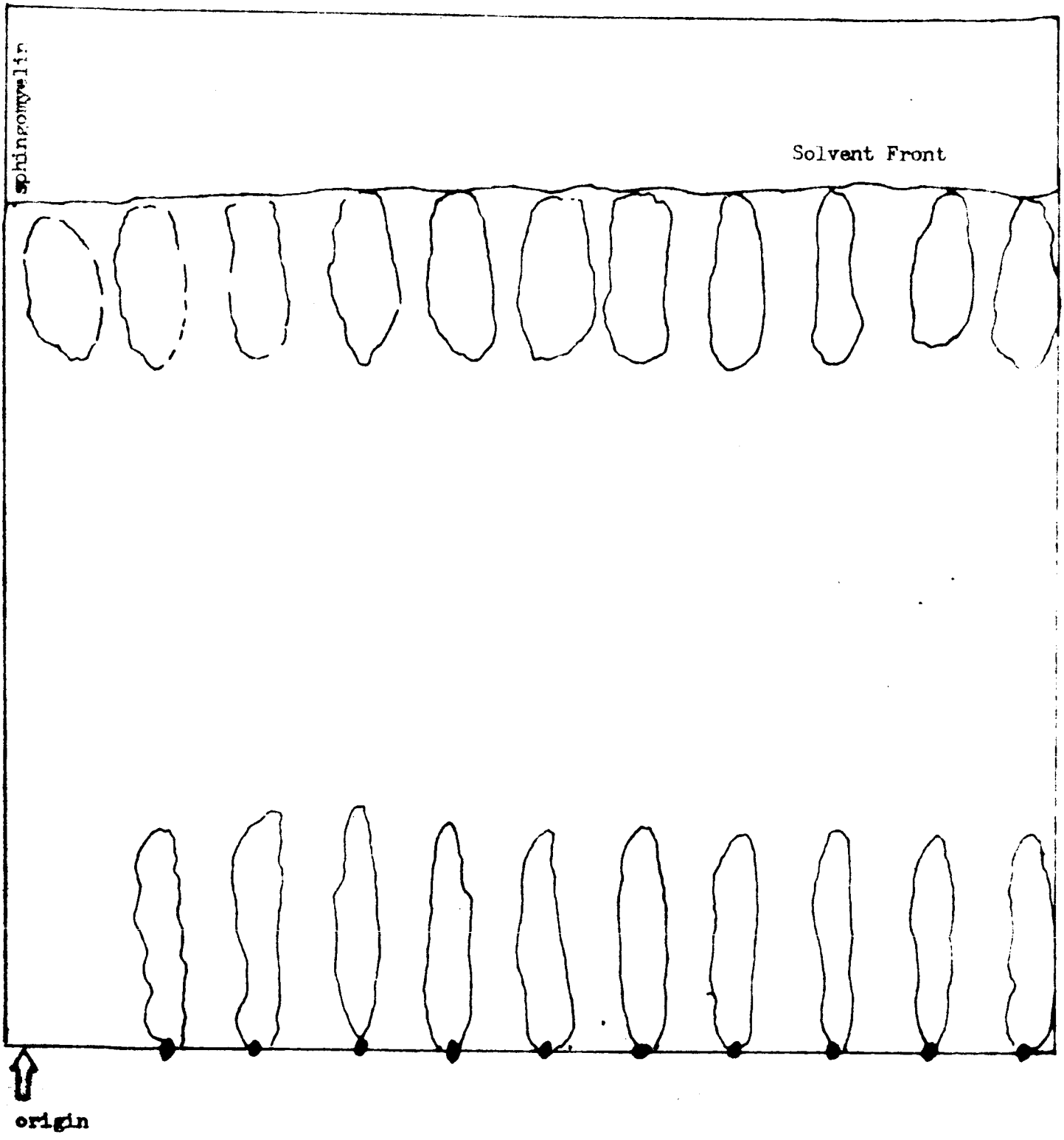
Solvent A No Precursors



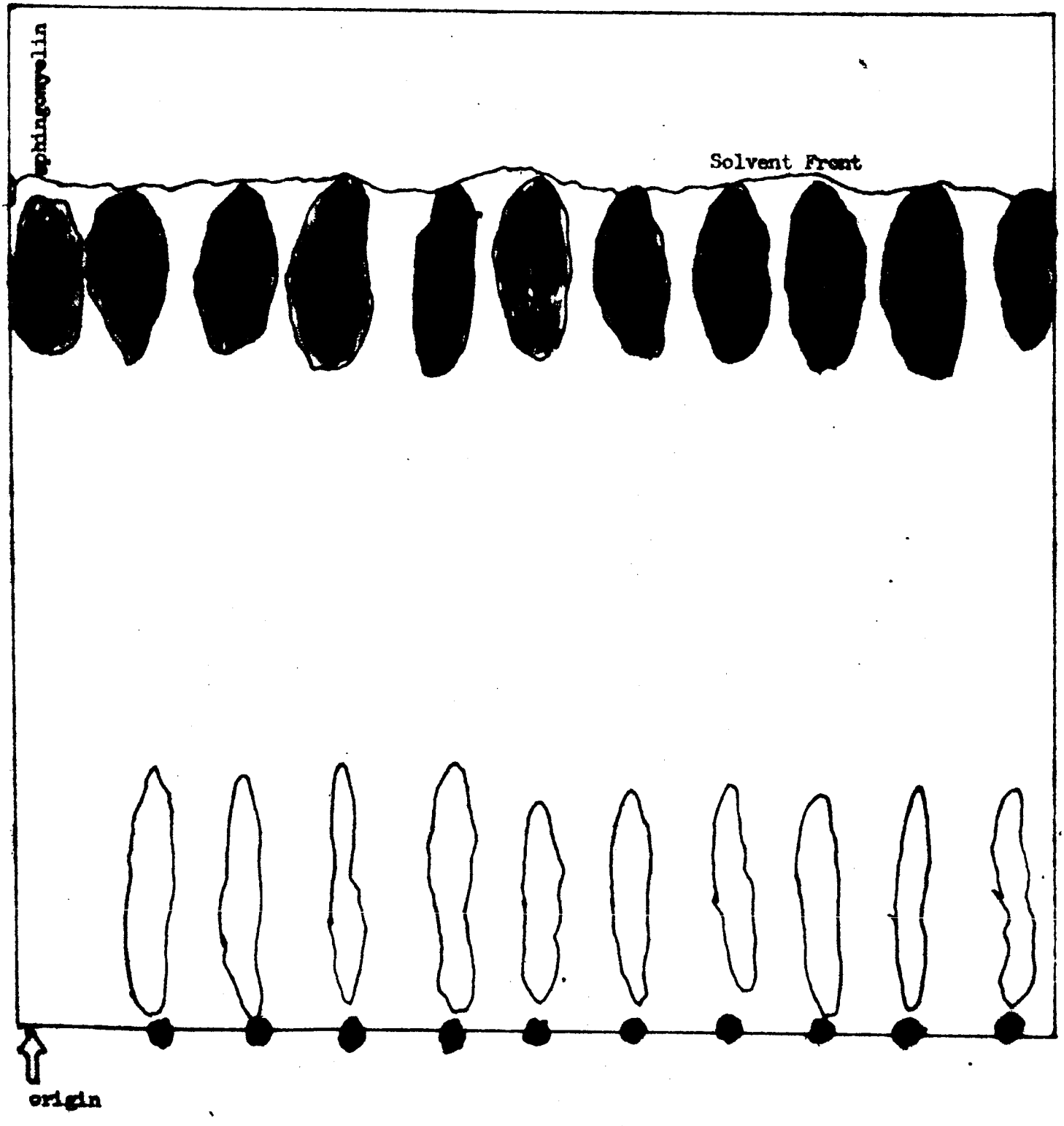
CHROMATOGRAM XI-A.

Gelman Paper

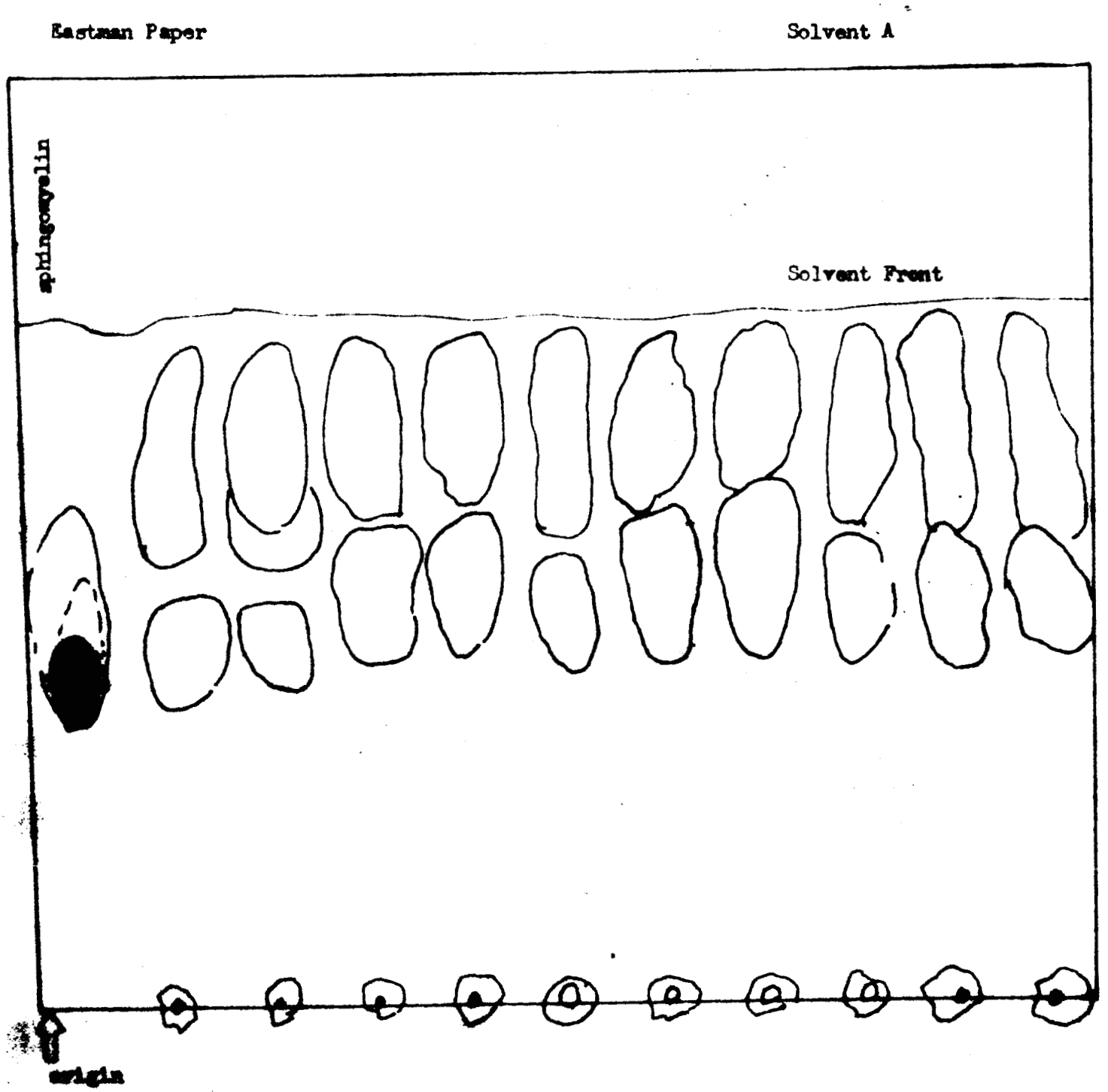
Solvent B No Precursors



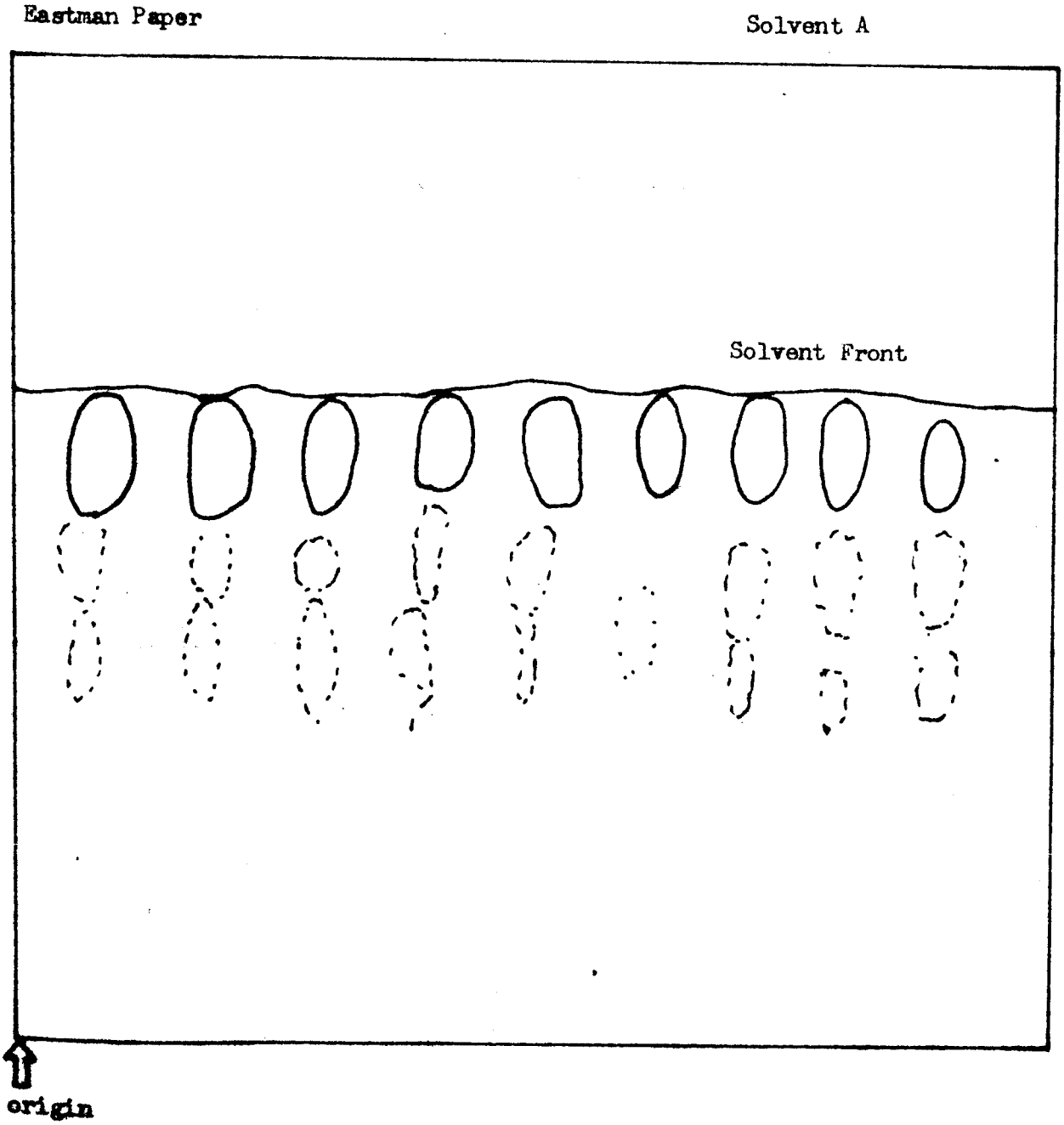
CHROMATOGRAM XII. Effect of X-Rays on Sphingomyelin Formation by Spine Mitochondria in the Presence of Sphingosine



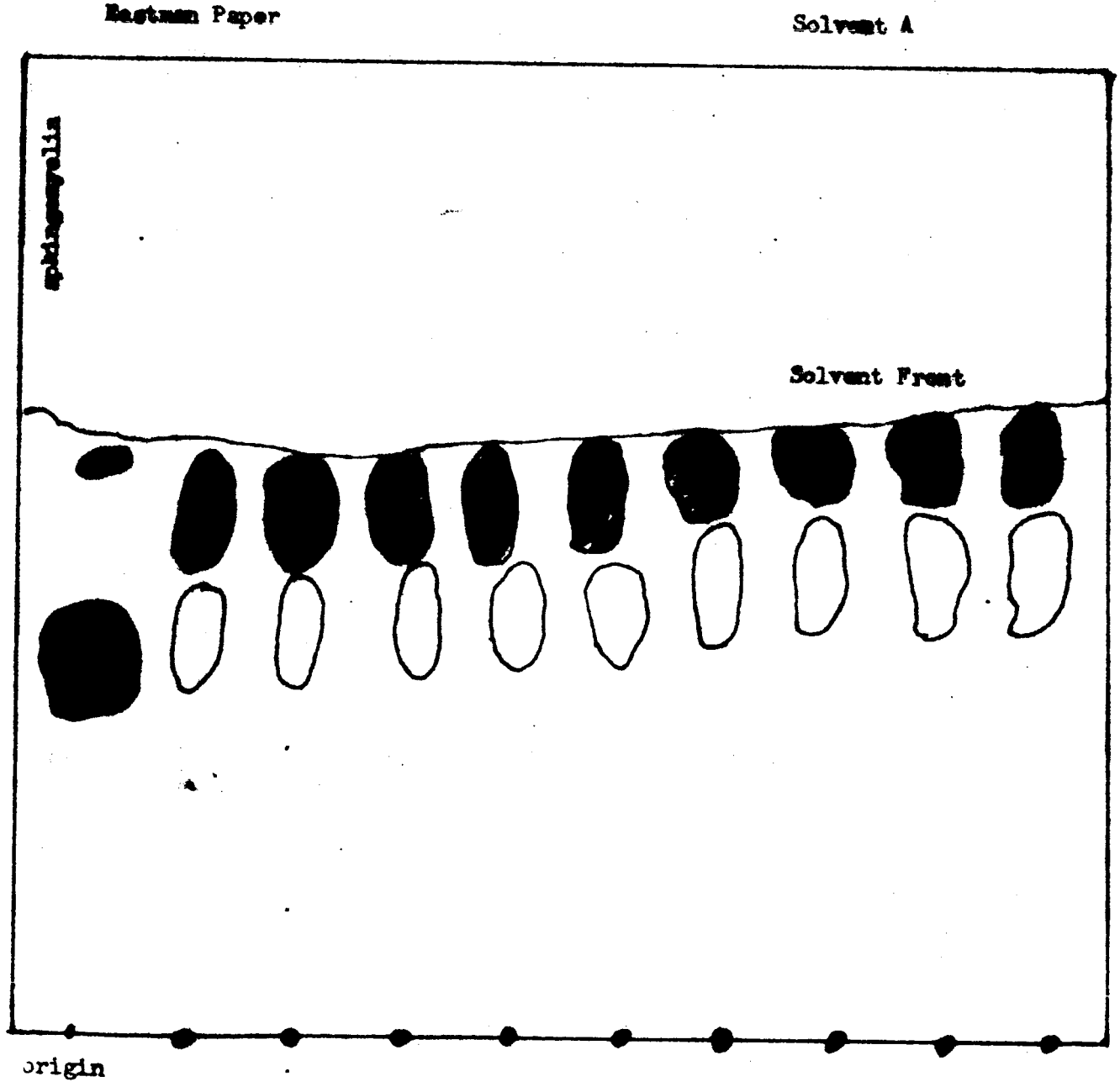
CHROMATOGRAM XIII. Sphingomyelin Formation by Spine Mitochondria in Presence of N-Acylsphingosine



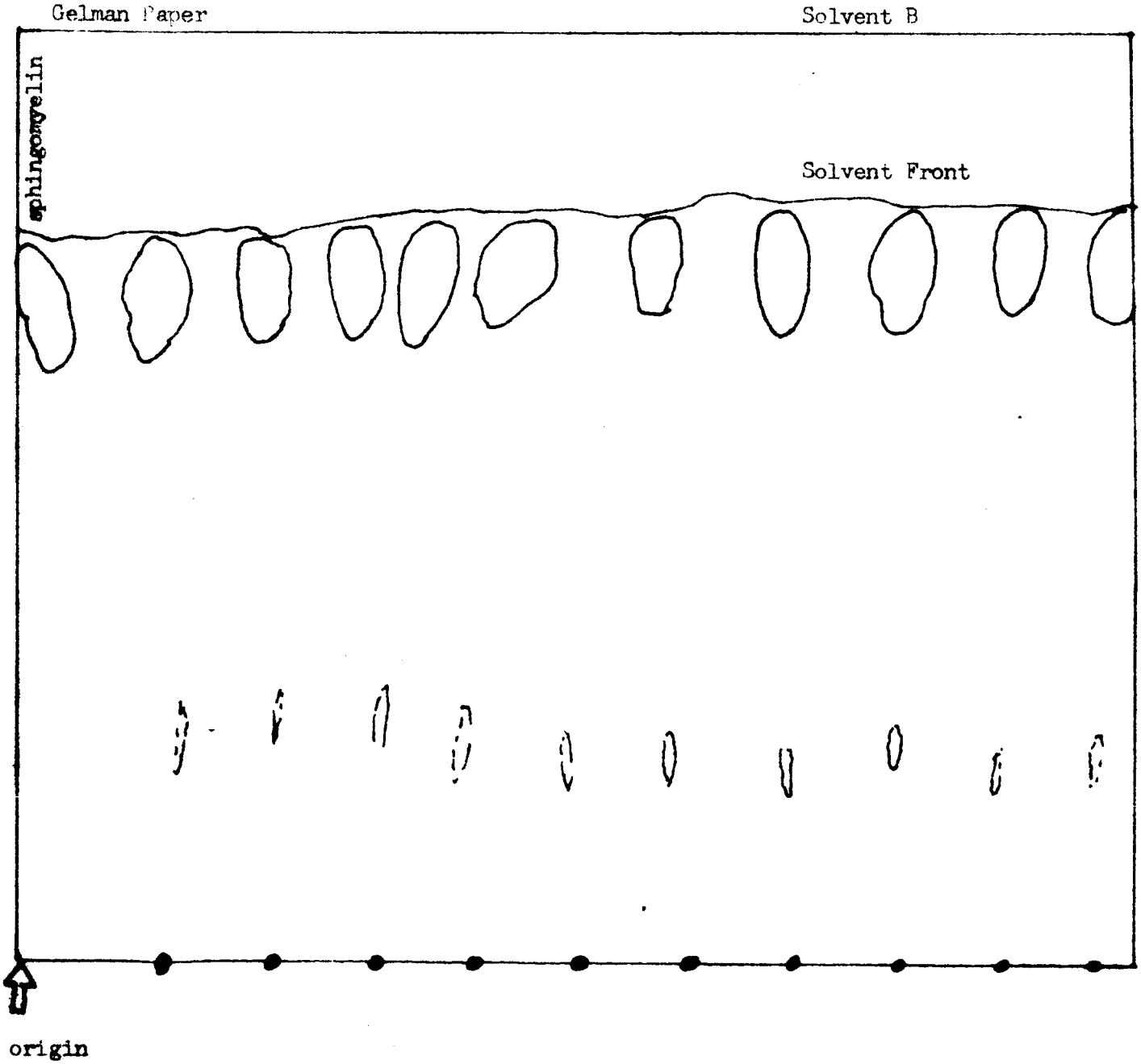
CHROMATOGRAM XIV. The Effect of Heat on Sphingomyelin Formation by Spine Mitochondria in the Presence of N-Acylsphingosine

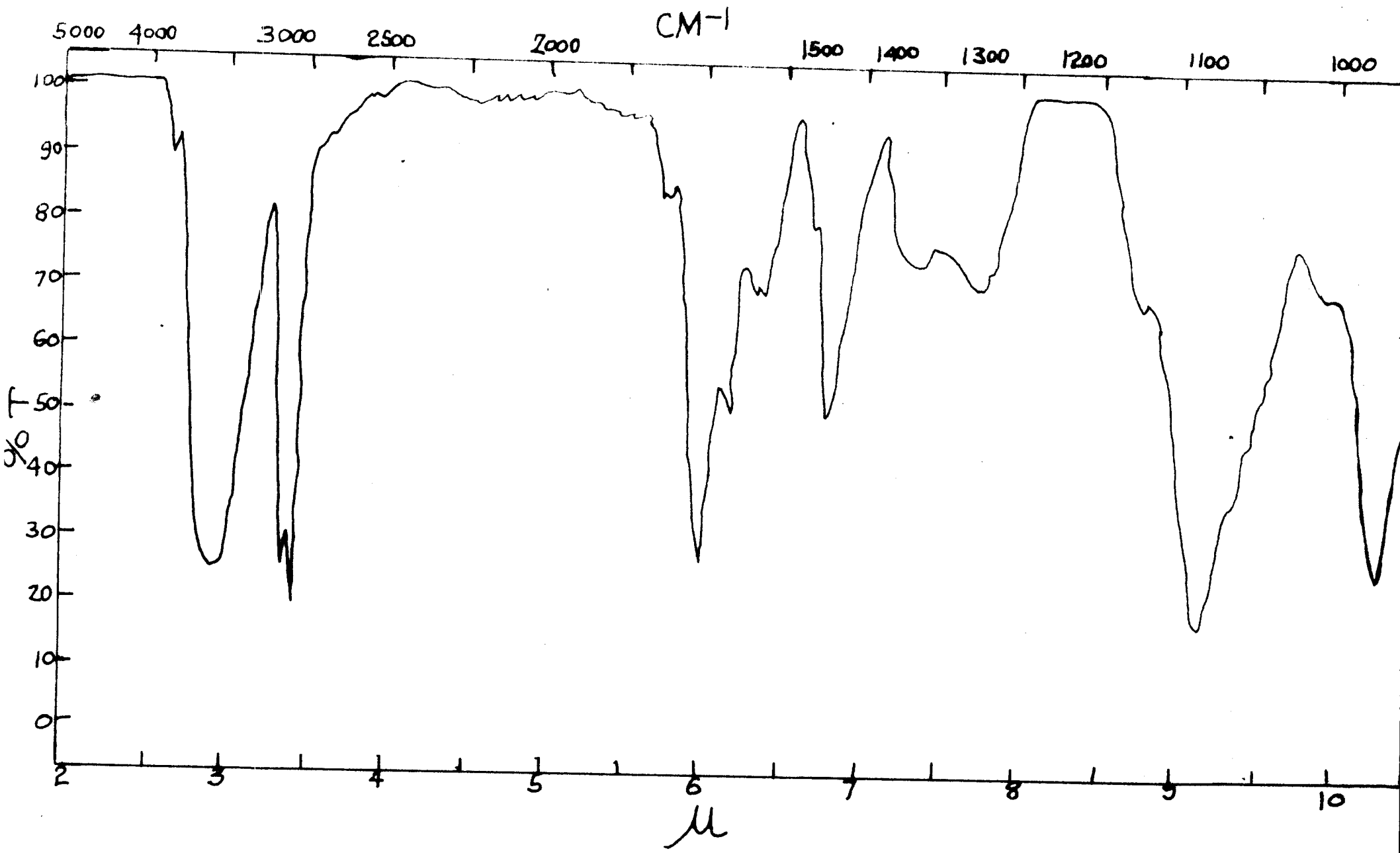


CHROMATOGRAM XV. Effect of X-Rays on Sphingomyelin Formation by Spine Mitochondria in Presence of N-Acylsphingosine

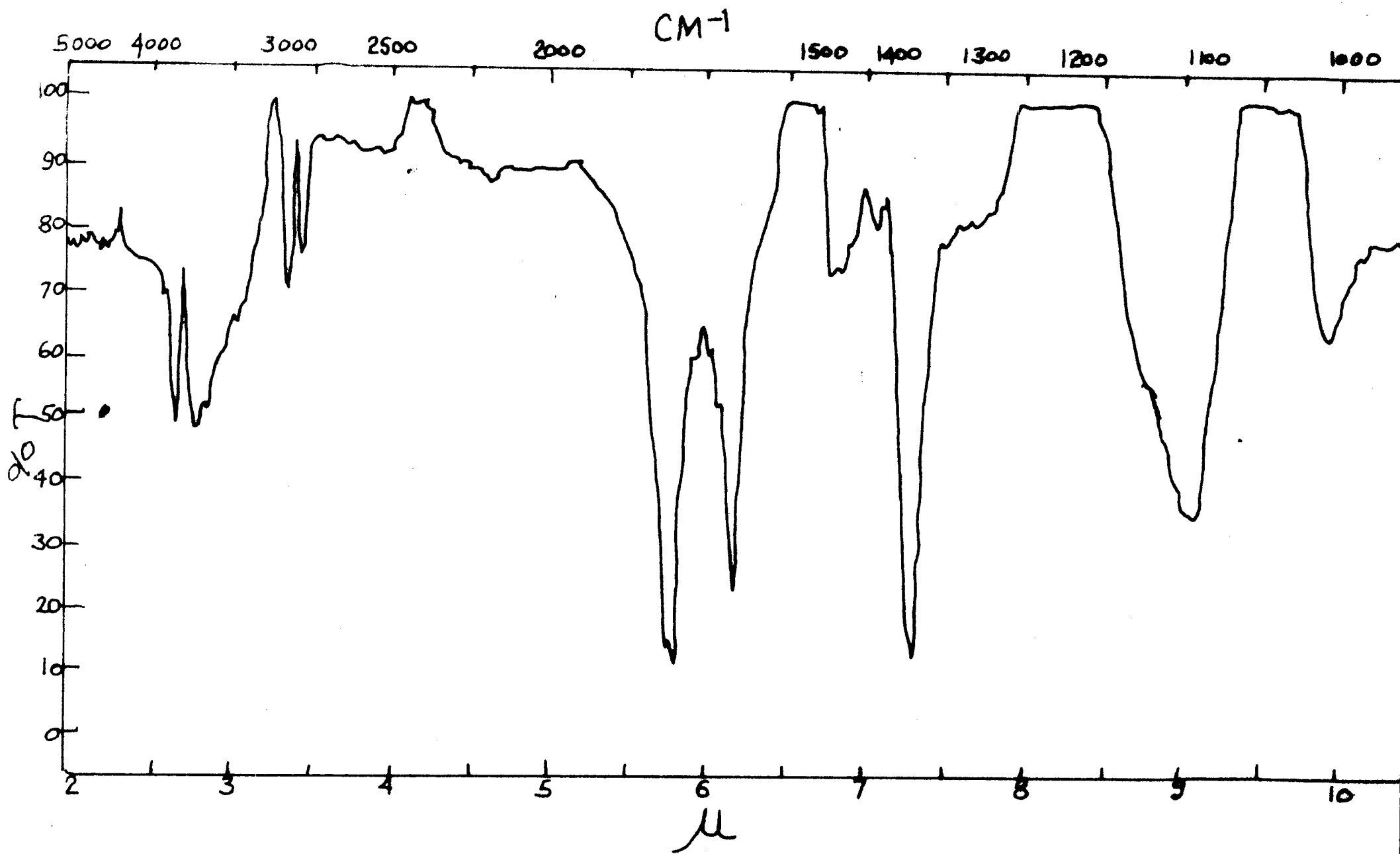


CHROMATOGRAM XV-A.

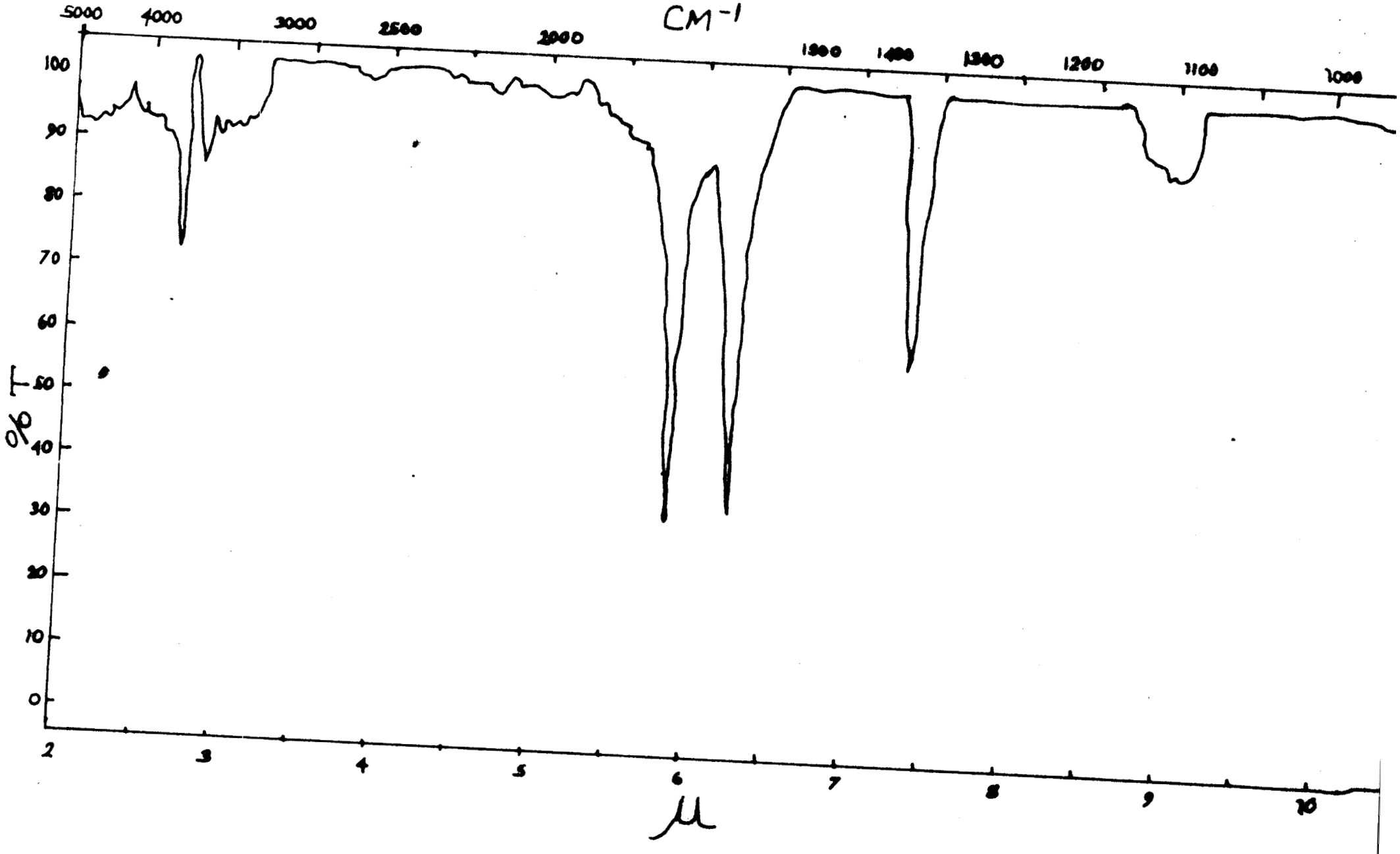




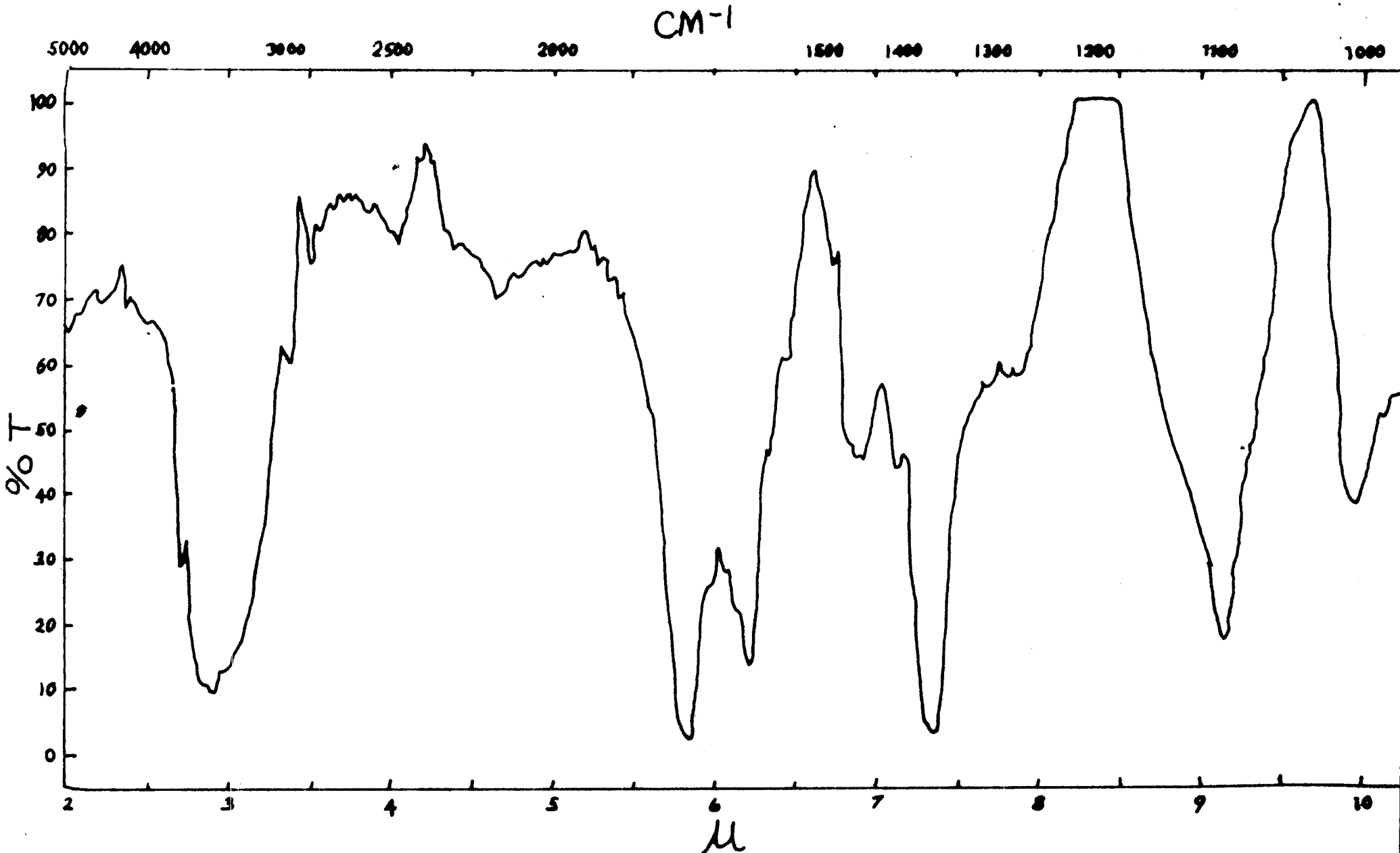
Infrared Spectrum No. 1. Sphingomyelin. Path 0.093 mm
KRS-5 Matched Cells Solvent $CHCl_3$ Concentration 3.26 mg/ml
Instrument Beckman IR-5A



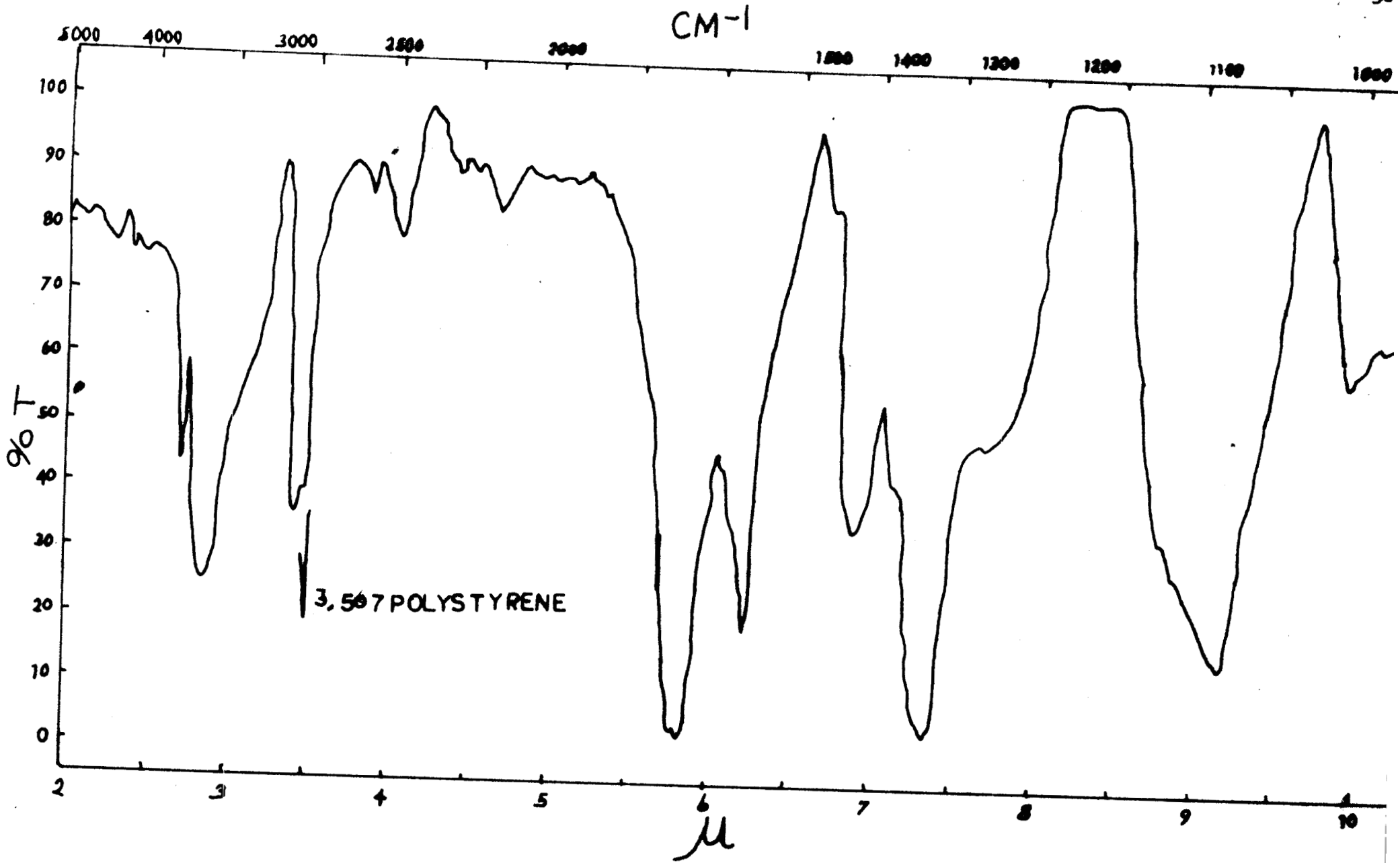
Infrared Spectrum No. 2. Sphingomyelin Formation from Rat Brain Mitochondria in Presence of N-Acylsphingosine. Alkali Extract. For Other Details See Spectrum 1.



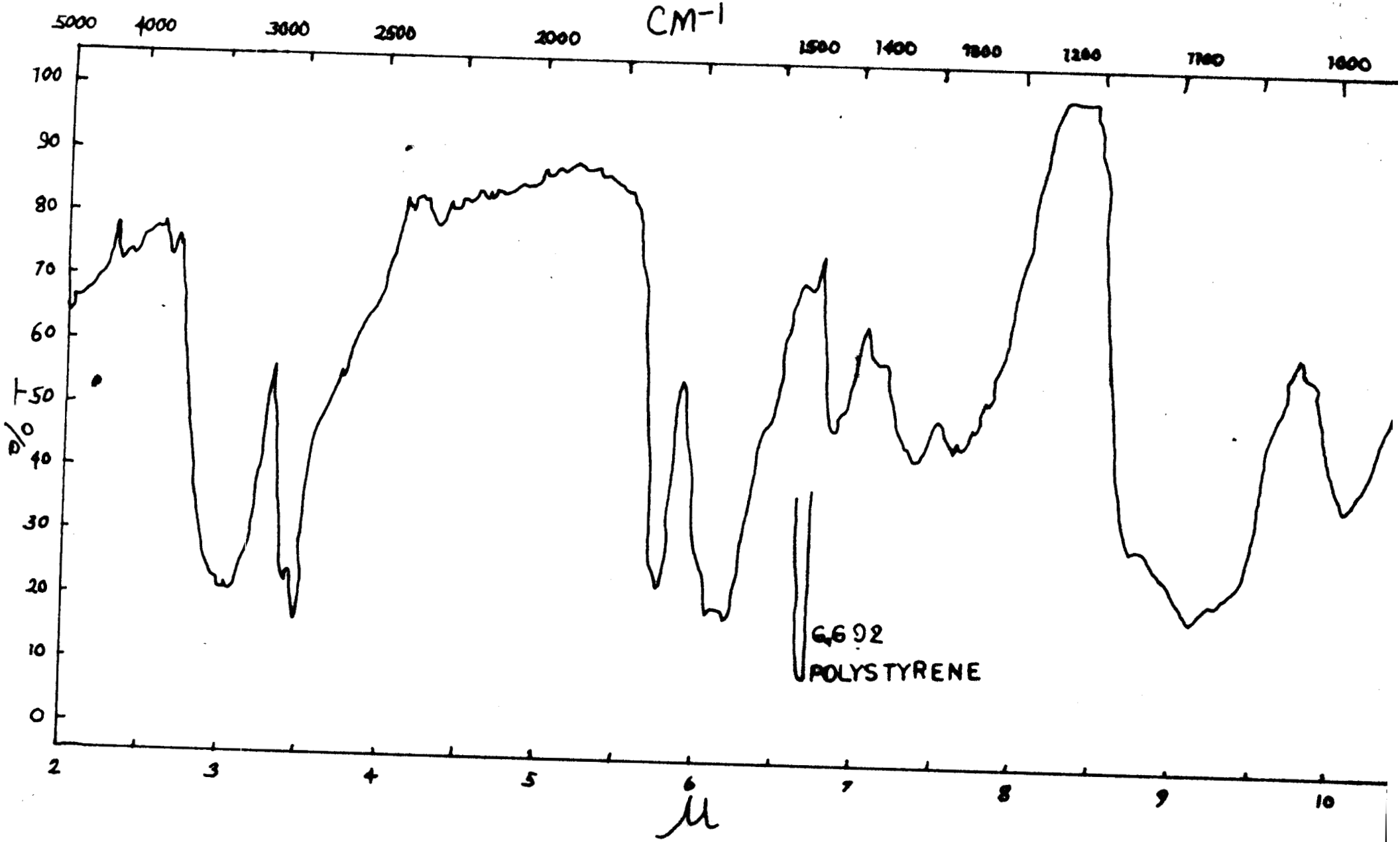
Infrared Spectrum No. 3. Effect of Heat on Sphingomyelin Formation from Rat Brain Mitochondria in Presence of N-Acylsphingosine. Alkaline Extract. For Other Details See Spectrum 1.



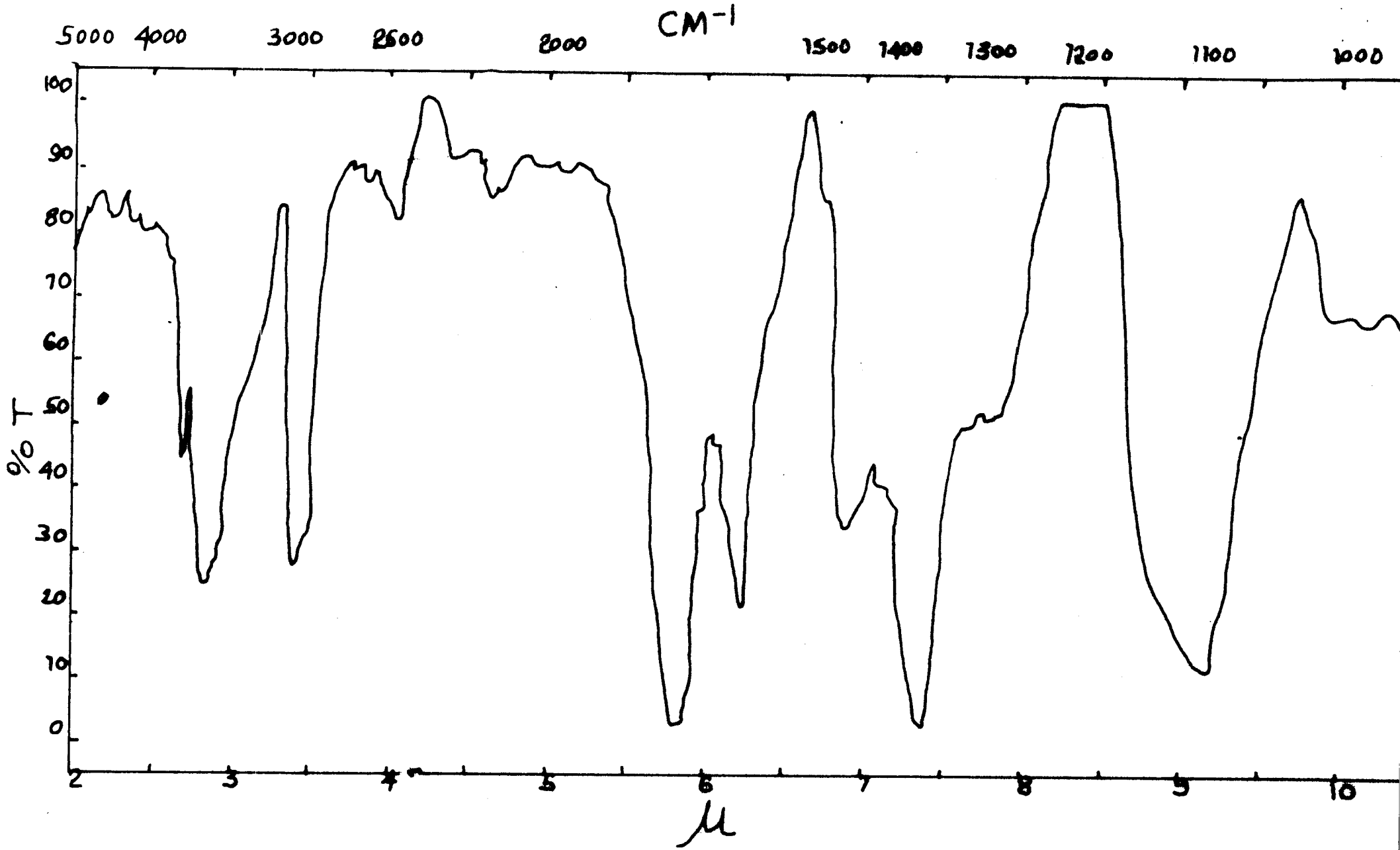
Infrared Spectrum No. 4. Effect of X-Rays on Sphingomyelin Formation by Brain Mitochondria in the Presence of N-Acylsphingosine (Complete System).



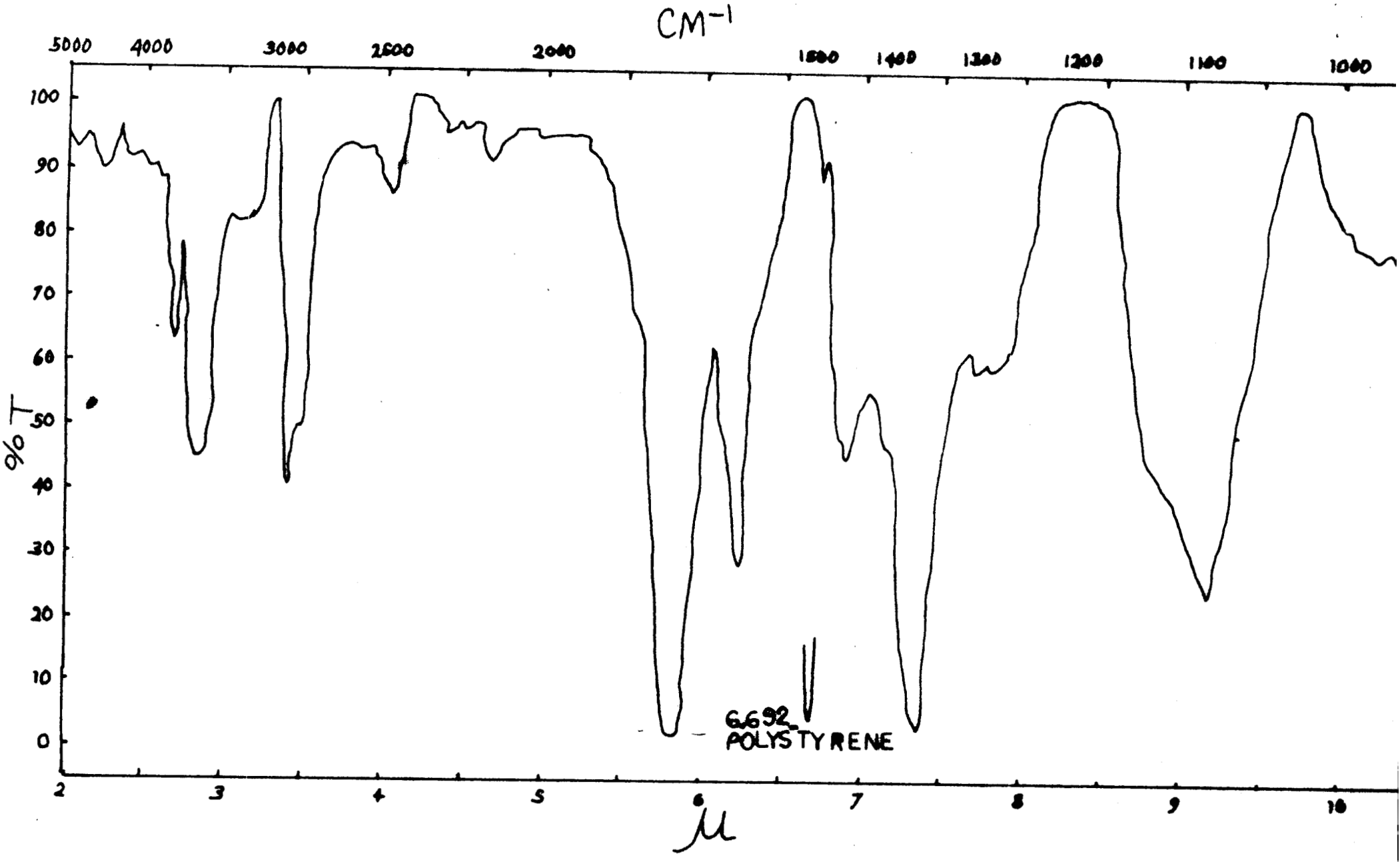
Infrared Spectrum No. 5. Sphingomyelin Formation by Spine Mitochondria.



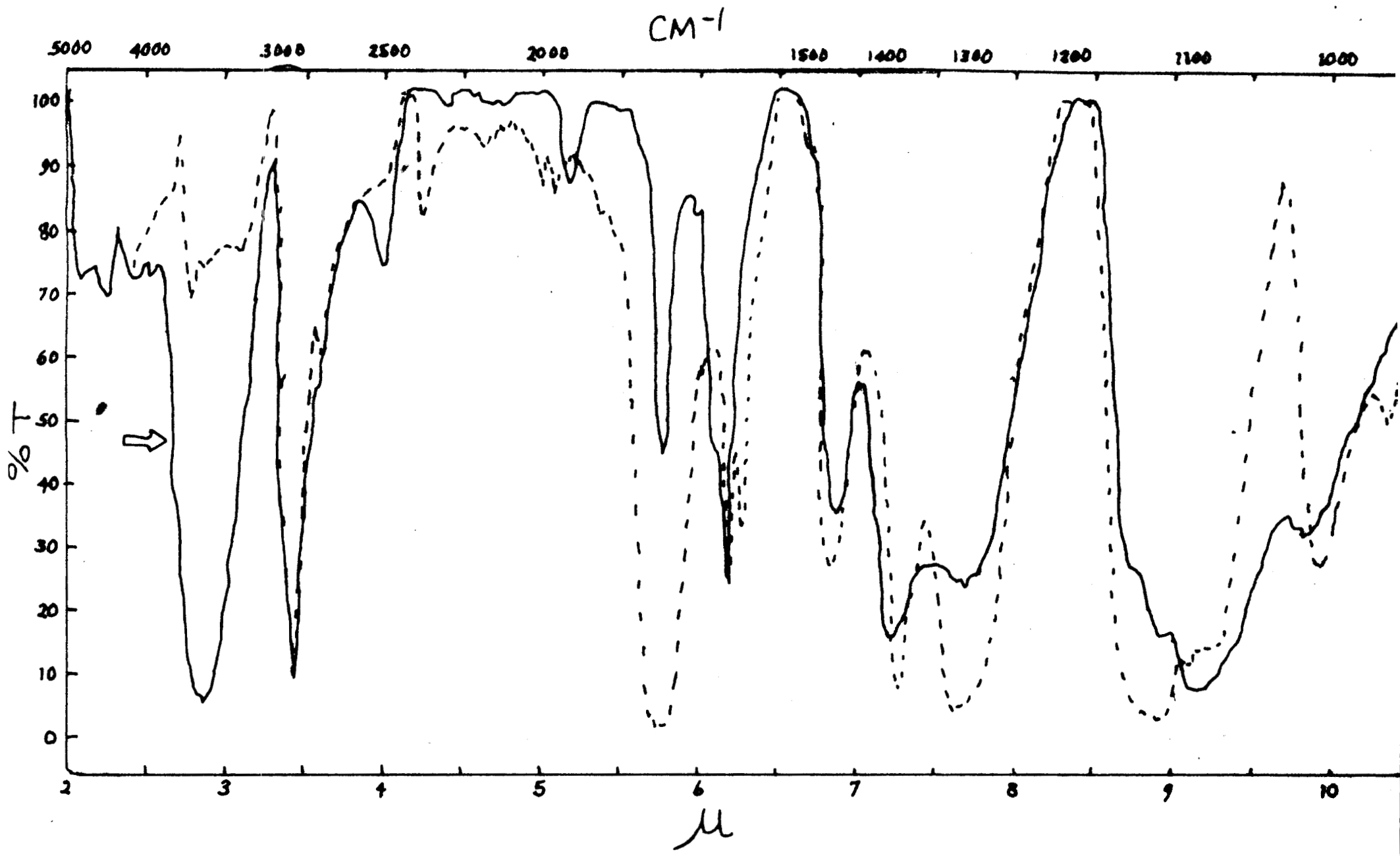
Infrared Spectrum No. 6. Phosphatidylinositol.



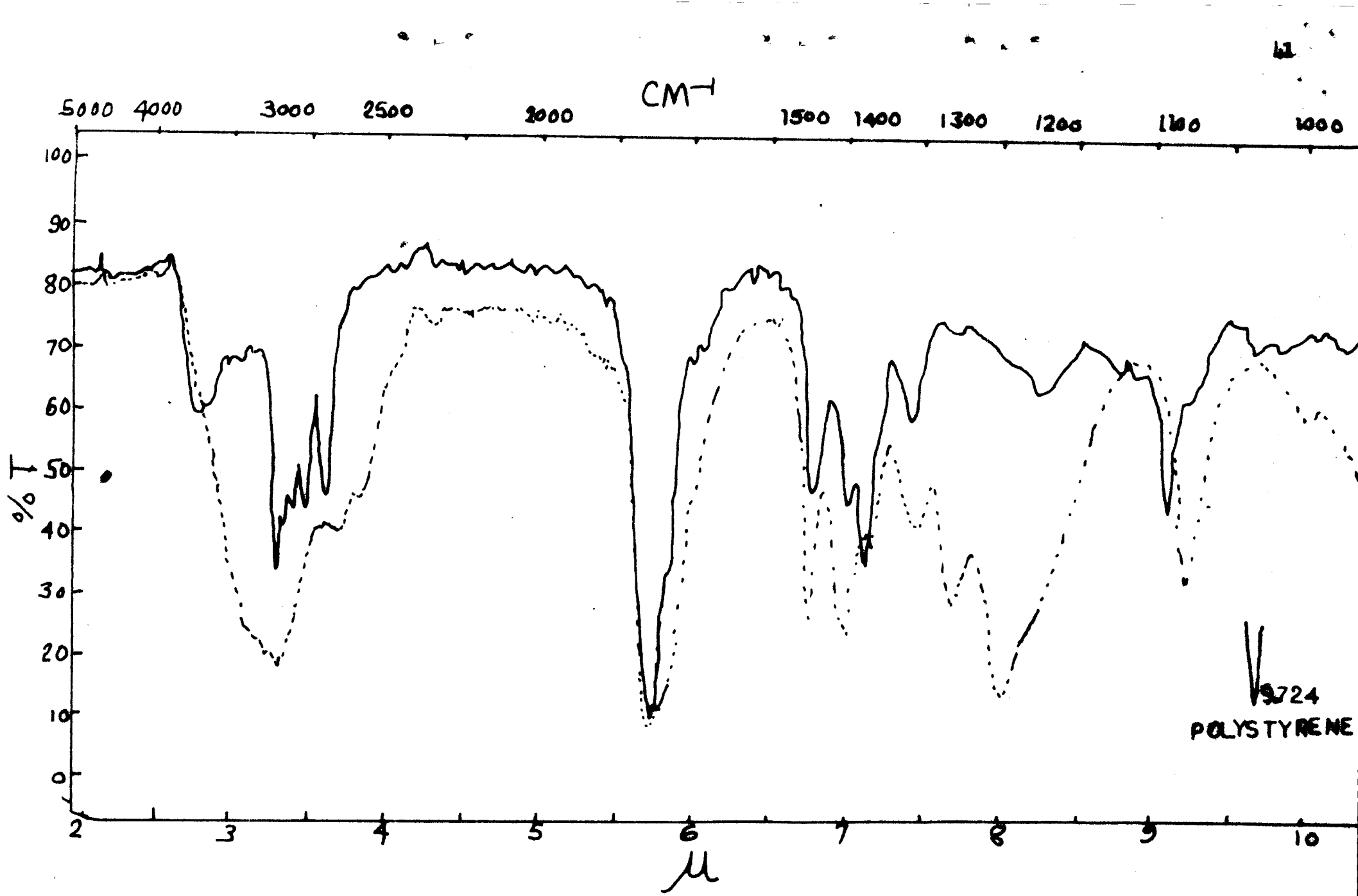
Infrared Spectrum No. 7. Phospholipid Formation by Spine Mitochondria.



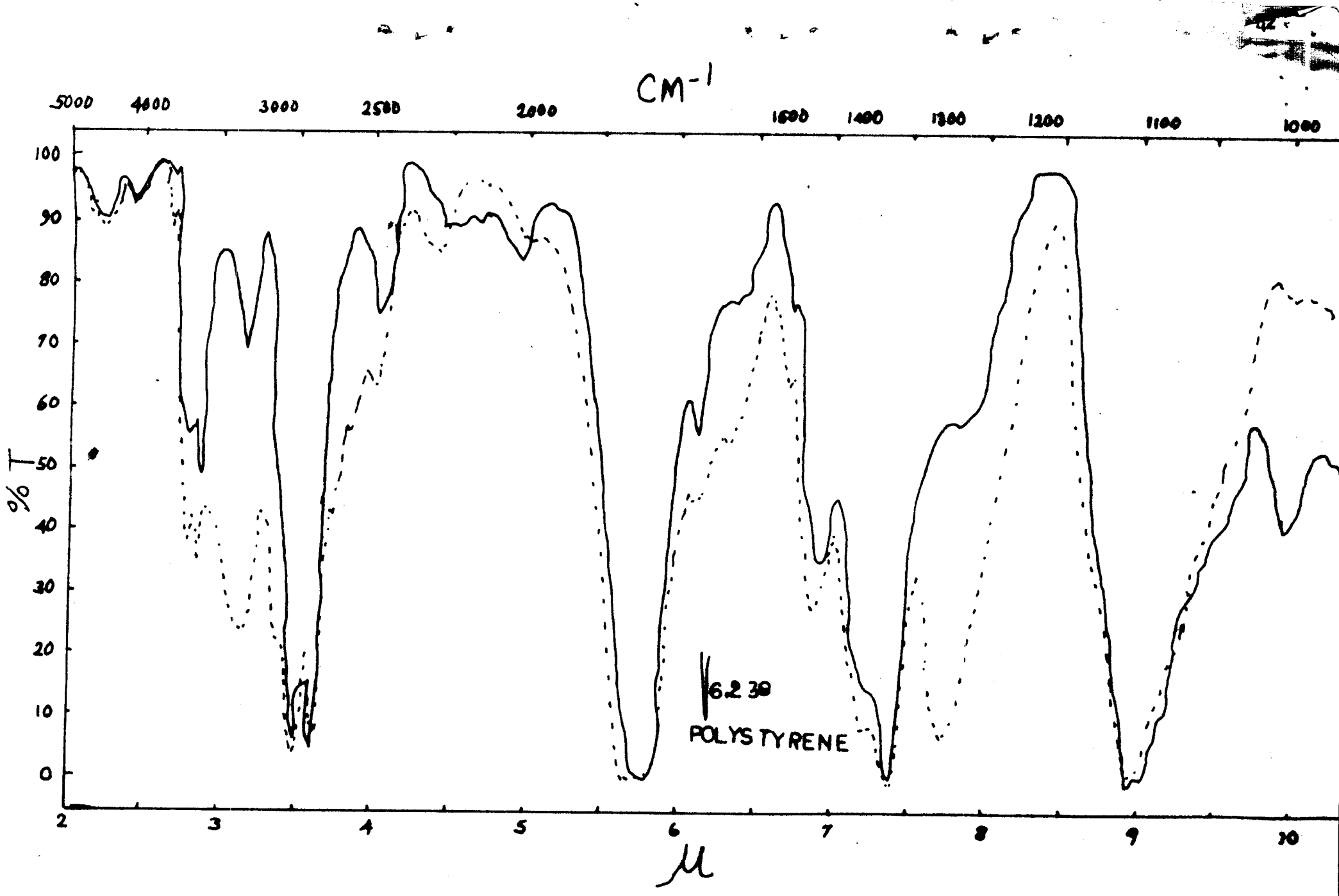
Infrared Spectrum No. 8. Phospholipid Formation by Irradiated Spine Mitochondria.



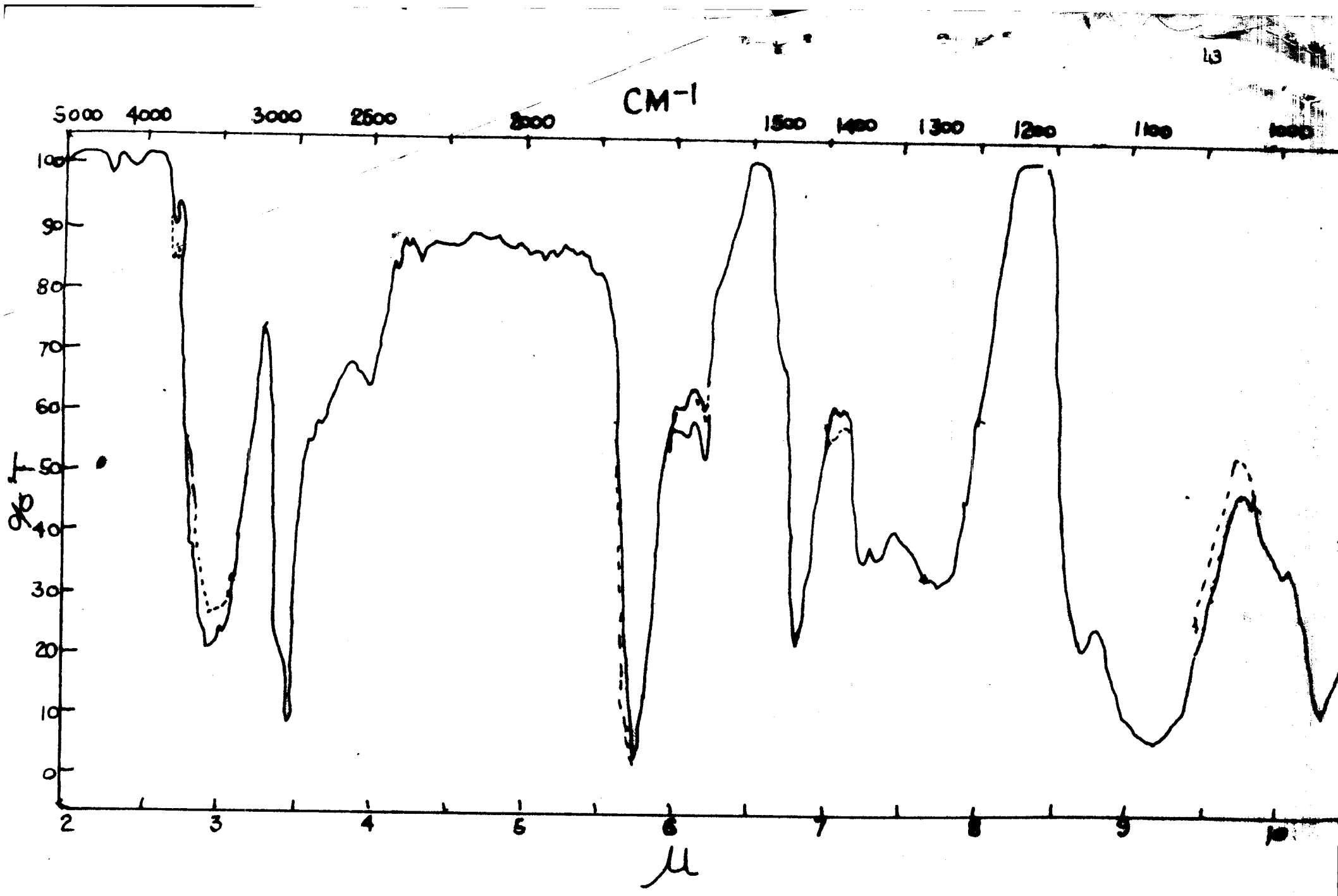
Infrared Spectrum No. 9. Effect of X-Rays on Myristaldehyde. 0.05 M in $CHCl_3$; 0.093 mm path - - - - Spectrum of Nonirradiated Pure Myristaldehyde, _____ Spectrum of Irradiated Pure Compound.



Infrared Spectrum No. 10. Effect of X-Rays on Propionaldehyde.
- - - - Spectrum of Non-irradiated Pure Propionaldehyde,
_____ Spectrum of Irradiated Pure Propionaldehyde.
Pure Substances Run on Rock Salt Plates.



Infrared Spectrum No. 11. Effect of X-Rays on Acetaldehyde.
- - - - Pure Acetaldehyde, _____ Irradiated Pure Acetaldehyde. 0.05 M in $CHCl_3$.



Infrared Spectrum No. 12. Effect of X-Rays on DL-α-DipalmitoylLecithin.
9.24 mg in $CHCl_3$.
- - - - Pure DL-α-DipalmitoylLecithin;
_____ Irradiated DL-α-DipalmitoylLecithin.