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Characterization of a soluble form of structural protein from Neurospora crassa

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CHARACTERIZATION OF A SOLUBLE FORM OF STRUCTURAL PROTEIN FROM NEUROSPORA CRASSA

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April 29 1971
Date

Typed by Charyl Warner

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INTRODUCTION

The assembly process of a membrane is not known. The presence of a class of proteins called structural proteins which are ubiquitous to membranes of eucaryotes implies a significant role in membrane assembly. Membranes have been fractionated into their components but as yet no native precursors to membrane protein have been studied. This thesis reports the study of a soluble form of membrane structural protein which is in the cytosol of Neurospora crassa. The purpose of this work is to characterize the soluble form of structural protein and to learn more of membrane assembly. An important aspect of this work is the development of techniques for characterizing an insoluble protein such as structural protein and the components associated with it. Elucidation of the steps involved in membrane assembly awaited the development of these techniques.

REVIEW OF THE LITERATURE

Structural protein was first isolated by Green and co-workers (1961) from beef heart mitochondria. Woodward and Munkres (1966) prepared structural protein from the mitochondrial membranes of Neurospora crassa. They later reported that this protein could be isolated from nuclear membranes, plasma membranes, the microsomal fraction and from the soluble fraction of N. crassa (Woodward and Munkres, 1967). The soluble form of the structural protein was obtained as a quasi-crystalline material by Kuehn and co-workers (1969). Shannon et al. (1971) have shown that the soluble form of the structural protein appears in high concentration in the cytosol during periods of rapid membrane assembly (early logarithmic growth) and disappears from the cytosol during periods of slow membrane assembly (stationary phase). They have also shown that the soluble form of the structural protein is associated with phospholipid, ribose, and phosphate.

Some workers have contested the concept that membranes are assembled from a single structural protein (Kiehn and Holland, 1970). Others have reported the presence of a major protein species in the membranes of various organisms (Yu and Masoro, 1970; Yang and Criddle, 1970). To date, all attempts to study membrane assembly have involved the solubilization of biological membranes followed by reaggregation of the membranes with the removal of the detergents (Rottem et al., 1968; Razin et al., 1969).

Evidence for a pool of protein precursor to membranes was found by Ray et al. (1968) in rat liver cells using radioactive tracer techniques. However, no protein was isolated. The soluble form of the structural protein from N. crassa is the first proposed precursor to membrane structural protein which has been isolated.

METHODS AND PROCEDURES

Neurospora crassa, St. Lawrence strain 74 A, was grown with vigorous aeration in Vogel's medium (Vogel, 1964) to logarithmic phase growth and harvested by filtration through cheesecloth. The mycelial pad was washed with distilled water and pressed dry. The pad was weighed and ground in liquid nitrogen in a pre-cooled mortar. Each gram of powdered, frozen N. crassa was extracted with 2 ml of 0.1 M Tris-sulfate buffer containing 1.0 M monosodium carbonate and 20 mM beta-mercaptoethanol (pH 7.4, 25°). The extract was centrifuged at 105,000 g for 1 hour at 4° and the supernatant, containing the cytosol, was carefully decanted.

Crystallization of the soluble form of the structural protein occurred during dialysis of the supernatant against a 1:200 dilution of the extraction buffer containing 0.01 M magnesium chloride. The quasi-crystalline material was collected after 24 hours of dialysis by centrifugation and was washed in ten volumes of glass distilled water five times by resuspension and recentrifugation. The quasi-crystalline material was lyophilized and stored in a dessicator over calcium chloride.

Delipidization of the quasi-crystalline material was accomplished by sequential extraction with acetone, ethanol, chloroform and 2-chloroethanol. One gm of the lyophilized material was suspended in 10 ml of the organic solvent and then centrifuged to pellet the quasi-crystalline material. The same technique was used with each solvent and the sequence was repeated three times. The organic solvent washes were combined and evaporated under reduced pressure to prepare the

extracted lipids for thin-layer chromatography.

Thin-layer chromatography of the lipids was conducted on silica gel G plates in two solvents. The non-polar solvent system used for identification of the neutral lipids was hexane, diethyl ether, and acetic acid (90:10:1). The polar solvent system used for identification of the phospholipids was chloroform, methanol, acetic acid and water (50:25:8:4). The plates were prepared with a 1 mm layer of silica gel G and were activated for 30 min at 100° immediately before use. Samples were dissolved in 2-choloroethanol and applied to the plates with a microsyringe. The standards used in the thin-layer chromatography of the neutral lipids were: 1, 2-diolein, cholesterol, oleic acid, 1, 3-diolein, triolein, and cholesterol oleate. Phosphatidyl choline, phosphatidyl serine and phosphatidyl ethanolamine were used as standards for the polar solvent system. Lipid standards were obtained from Applied Science Laboratories, State College, Pennsylvania. Visualization of the lipids following chromatography was by oxidation of the lipids at 200° with 50% sulfuric acid containing 5% sodium dichromate. Lipids extracted from the quasi-crystalline material were identified by comparing the relative mobility of each component with the relative mobilities of the standards.

The fatty acids of the lipids in the quasi-crystalline material were prepared for gas-liquid chromatography by transmethylation in methanolic boron trifluoride (Supelco, Inc., Bellafonte, Pennsylvania). One gm of the lyophilized quasi-crystalline material was refluxed for 6 hr in 30 ml of anhydrous, methanolic boron trifluoride under a nitrogen atmosphere. The reaction mixture was quenched with 30 ml of water and the methyl esters of the fatty acids were extracted with 30 ml of chloroform.

The chloroform extraction was repeated three times. The combined extractions were evaporated to dryness under reduced pressure. The methyl esters of the fatty acids were solubilized in chloroform and injected into a Perkin-Elmer gas chromatograph. The chromatograph utilized a 90 cm column with a 6 mm outer diameter packed with 10% diethylene glycol succinate on gas-chrom Q (80-100 mesh). Diethylene glycol succinate and gas-chrom Q were obtained from Supelco, Inc., Bellafonte, Pennsylvania. The individual methyl esters of the fatty acids were identified by their respective retention times on the column when compared to the retention times of standards. The relative amount of each methyl ester was determined by triangulation of the peak on the recorder paper after the solvent blank had been subtracted.

Solvent systems used to solubilize the quasi-crystalline material were: 2% dodecyl sodium sulfate, 6 M guanidine hydrochloride, 99% formic acid, or 8 M urea in 25% formic acid. Two-chloroethanol, freshly distilled and adjusted to pH 8 with ammonium hydroxide, was used on specific occasions to solubilize the structural protein from the quasi-crystalline material. Eight molar urea kept the structural protein soluble after previous solubilization using one of the above solvent systems. Structural protein was also solubilized in 1 M sodium hydroxide.

Solubilized quasi-crystalline material was gel filtered on G-50 sephadex equilibrated with dodecyl sodium sulfate or with 8 M urea in 25% formic acid. Gel filtration was also conducted on LH-20 sephadex equilibrated with freshly distilled 2-chloroethanol which was adjusted to pH 8 with ammonium hydroxide. The sephadex columns contained 50 ml with a height-diameter ratio of 10:1 and a flow rate of 0.5 ml per min.

Elution of samples was monitored with an ISCO 270 UV flow monitor. Protein was quantitated by triangulation of the peaks on the ISCO recorder paper followed by comparison with peaks of known standards.

Three alkaline conditions of hydrolysis were used to treat the quasi-crystalline material: (1) aqueous sodium hydroxide, 1.0 N, at 25° for 12 hr; (2) anhydrous, methanolic potassium hydroxide, 0.1 M, at 25° for 6 hr; (3) anhydrous, methanolic potassium hydroxide, 0.1 M, refluxed for 1 hr.

Ultraviolet absorption spectra were obtained with a Cary 14 double beam, recording spectrophotometer. The solvents used in the spectral analysis were dodecyl sodium sulfate, guanidine hydrochloride and 2-chloroethanol.

Electrophoresis was conducted in 7% polyacrylamide gels (Davis, 1964) containing 8 M urea. Samples were applied to the gels either after solubilization in guanidine hydrochloride or after dialysis against 8 M urea. The acrylamide was polymerized in 7 cm glass tubes with outer diameters of 0.7 cm. The electrophoresis was conducted for 2 hr with a current of 2 mAmp per tube. Following the electrophoresis the gels were removed from the tubes and either fixed with 7% trichloroacetic acid or stained with aniline black before scanning on a Gilford 2400 recording spectrophotometer with a 2410 linear transport.

Electrofocusing was conducted in 7% polyacrylamide gels with 1% LKB ampholine, pH 4 to pH 6, and 8 M urea. The LKB ampholine was obtained from LKB-Produkter AB, Stockholm, Sweden. Samples were applied to the gels in formic acid, in guanidine hydrochloride, or after dialysis against 8 M urea. Tube size was the same as in electrophoresis. Electrofocusing was conducted for one hour at 150 volts between baths of 5%

phosphoric acid and 5% ethylene diamine. Following the electrofocusing the gels were removed from the tubes, fixed in 7% trichloroacetic acid, and scanned at 260 nm or 280 nm on a Gilford 2400 recording spectrophotometer with a 2410 linear transport. A sample gel from each electrofocusing run was sliced into 0.5 cm pieces. The ampholyte from each piece was eluted into 1 ml of distilled water and the pH of the resulting solution determined to estimate the pH gradient within the gel.

Protein was anlyzed on the Beckman Spinco 120 C automatic amino acid analyzer after 24 hr hydrolysis at 110° in a sealed vial of constant boiling hydrochloric acid. Amino acids were identified by their retention volume on the amino acid analyzer when compared to the retention volume of known amino acids. The relative amount of each amino acid was determined by triangulation of the peaks on the recorder paper and using color coefficients to correct for differential reactions of the various amino acids with ninhydrin.

Protein was determined by the Lowry method (Lowry et al., 1951) and compared to bovine serum albumin standards. Protein samples and the bovine serum albumin standards were dissolved in dodecyl sodium sulfate.

Total phosphorus was determined by a modification of the Fiske-Subbarow assay for inorganic phosphate (Fiske and Subbarow, 1925). One ml of 10% ethanolic magnesium nitrate (six hydrate) was added to 1 mg of the quasi-crystalline material in a pyrex test tube. The ethanol was evaporated at 100° and the mixture was heated in the flame of a Bunsen burner until brown fumes were no longer visible. The sample was then heated in 0.3 ml of 1 N hydrochloric acid for 30 min at 100° to hydrolyze any pyrophosphate present. The Fiske-Subbarow assay was performed following dilution to 5 ml.

Ribose was assayed using the orcinol colorimetric determination described by Ashwell (1957). Ethanolamine was determined by a modification of the method developed by Dittmer and co-workers (1958). The ammonia released from ethanolamine by periodic acid treatment was measured with the use of Nessler's reagent.

Mitochondrial membrane structural protein from N. crassa was a gift from Dr. D. O. Woodward of Stanford University.

RESULTS

The soluble form of the membrane structural protein was obtained as a quasi-crystalline material (Figure 1). Amino acid analysis of the quasi-crystalline material (Table 1, column a) indicated that the protein was identical to the structural protein isolated from N. crassa mitochondrial membranes (Table 1, column b).

Extraction of the quasi-crystalline material with ethanol, acetone, chloroform, and 2-chloroethanol removed neutral lipids and phospholipids. Thin-layer chromatography in a non-polar solvent system for the identification of neutral lipids (Figure 2) and in a polar solvent system for the identification of phospholipids (Figure 3) indicated that phosphatidyl ethanolamine was the major lipid component. Di- and triglycerides, free fatty acids, cholesterol, and cholesterol esters were present in trace amounts.

Chloroethanol solubilized a small amount of the structural protein which was separated from the solubilized lipid by gel filtration on LH-20 sephadex equilibrated with chloroethanol (Figure 4). Quantitation based on triangulation of the peak on the recorder paper and comparison with the peak given by a known amount of bovine serum albumin indicated that 36 mg of quasi-crystalline material contained 1.0 mg of structural protein which was soluble in chloroethanol. Thus, 2.8% of the quasi-crystalline material is protein which is soluble in chloroethanol. Fractions from the protein peak gave an absorption spectrum in the ultraviolet region which is characteristic of structural protein (Figure 5). Amino acid analysis of this protein gave a pattern identical with that

for structural protein isolated from N. crassa mitochondrial membranes (Table 1, column c).

Table 1. Amino acid analyses of structural protein.

Amino acid	Residues of amino acid per residue of aspartic acid				
	a[1]	b[2]	c[3]	d[4]	e[5]
Asp	1.00	1.00	1.00	1.00	1.00
Thr	0.62	0.61	0.61	0.57	0.61
Ser	0.62	0.68	0.60	0.61	0.58
Glu	1.14	1.09	1.15	1.13	1.01
Pro	0.58	0.52	0.54	0.44	0.50
Gly	0.91	0.96	0.90	1.32	0.80
Ala	1.01	1.08	1.00	0.95	1.01
Val	0.80	0.69	0.68	0.73	0.78
Ile	0.60	0.53	0.57	0.53	0.62
Leu	0.97	0.93	0.97	0.89	0.94
Tyr	0.34	0.30	0.33	0.30	0.33
Phe	0.45	0.42	0.43	0.40	0.40
Lyx	0.81	0.72	0.85	0.78	0.72
His	0.22	0.22	0.23	0.20	0.24
Arg	0.67	0.60	0.65	0.61	0.68

[1]Column a is an analysis of the quasi-crystalline material.

[2]Column b is an analysis of the structural protein from N. crassa mitochondrial membranes.

[3]Column c is an analysis of the structural protein isolated from the quasi-crystalline material by solubilization in chloroethanol.

[4]Column d is an analysis of the residue remaining after chloroethanol extraction of the quasi-crystalline material.

[5]Column e is an analysis of the protein released from the chloroethanol extraction residue by alkaline hydrolysis.

The absorption spectrum of the lipid fraction (Figure 6) indicated the presence of conjugated trienoic acids (Chapman, 1965:45). The presence of these fatty acids in the quasi-crystalline material was

confirmed by gas-liquid chromatography following transmethylation with boron trifluoride (Figure 7). The relative quantities of these fatty acids were determined by triangulation (Table 2).

Table 2. Relative quantities of the fatty acids from the quasi-crystalline material.

Fatty acid	Percent of total
lauric-myristic	13
palmitic	21
stearic-oleic	10
linoleic	41
linolenic	15

The delipidized quasi-crystalline material remaining after chloroethanol extraction was very insoluble. It could be solubilized in 2% dodecyl sodium sulfate, 6 M guanidine hydrochloride, 99% formic acid, or 8 M urea in 25% formic acid. Gel filtration of the solubilized quasi-crystalline material on G-50 sephadex equilibrated with dodecyl sodium sulfate gave a single peak of ultraviolet absorbing material (Figure 8). Fractions from the peak gave an absorption spectrum with a maximum at 250 nm (Figure 9). Amino acid analysis identified this material as structural protein (Table 1, column d).

Alkaline hydrolysis of the delipidized quasi-crystalline material in aqueous sodium hydroxide or in anhydrous, methanolic potassium hydroxide released the protein. Hydrolysis rendered the quasi-crystalline material completely soluble in chloroethanol. Subsequent gel filtration on G-50 sephadex in dodecyl sodium sulfate or on LH-20 sephadex in chloroethanol gave two peaks of ultraviolet absorbing material (Figure 10). The first peak gave the absorption spectrum in the ultraviolet region

characteristic of structural protein (Figure 11). Amino acid analysis of the protein from these fractions indicated the protein was identical with structural protein isolated from N. crassa mitochondrial membranes (Table 1, column e). The protein released from the delipidized quasi-crystalline material by alkaline hydrolysis was quantitated by triangulation of the peak on the recorder paper and by comparison to the peak given by a known amount of bovine serum albumin. Thirty-six mg of quasi-crystalline material contained 6.2 mg of protein which was released by alkaline hydrolysis after delipidization. From these results it was estimated that the quasi-crystalline material was 20% protein and that 14% of this protein was solubilized by chloroethanol.

Fractions from the second peak gave an absorption spectrum with a maximum at 260 nm (Figure 12). Chemical analysis of these fractions indicated the presence of ribose. Inorganic phosphate tests were negative however oxidation and hydrolysis produced inorganic phosphate. A phosphate ester such as ribose phosphate would give similar results. The 260 nm absorption maximum indicated the presence of a third compound in this peak.

The structural protein released by alkaline hydrolysis from the quasi-crystalline material was further characterized by electrofocusing and electrophoresis. In polyacrylamide electrofocusing between pH 4 and pH 6, the protein focused as a single band at pH 5.2 (Figure 13). The structural protein isolated from N. crassa mitochondrial membranes had the same isoelectric point (Figure 13).

Structural protein released from the quasi-crystalline material by either hydrolysis in aqueous sodium hydroxide or anhydrous, methanolic potassium hydroxide migrated at the same rate in polyacrylamide electro-

phoresis (Figure 14). The hydrolysis procedure and the treatment before electrophoresis determined the amount of aggregated structural protein. This aggregated protein was seen as indistinct bands between the origin and the sharp monomer band. Dialysis of the hydrolyzed quasi-crystalline material against 8 M urea allowed the greatest amount of aggregation.

The quasi-crystalline material was examined by polyacrylamide electrofocusing. Prior to hydrolysis the quasi-crystalline material electrofocused with two bands absorbing at 280 nm (Figure 15). The first band from the origin had little absorption at 260 nm, while the second band from the origin absorbed strongly at 260 nm (Figure 15). The first band electrofocused at pH 5.2 while the second band had an isoelectric point of pH 4.5.

After hydrolysis the quasi-crystalline material electrofocused as a single band which absorbed at 280 nm and not at 260 nm (Figure 16). This single band had an isoelectric point of pH 5.2.

The quasi-crystalline material was quantitated for protein, ribose, phosphate and ethanolamine. Protein represented 20% of the material by weight or 0.016 $\pm$0.002 micromole per mg of material based on a molecular weight of 12,500 daltons. Ribose and phosphate represented 11% and 8% by weight respectively. Ribose and phosphate were almost equimolar: 0.73 $\pm$0.04 and 0.77 $\pm$0.03 micromole per mg of material respectively. Based on ethanolamine content, phosphatidyl ethanolamine represented less than 1% of the material by weight or less than 0.004 micromoles per mg of material.

DISCUSSION

The formation of quasi-crystalline material from the soluble structural protein implies an ordered association of this protein. If this protein is a membrane precursor involved in the self-assembly of membranes, the tendency for an ordered association to occur may be one of the protein's most important characteristics.

The quasi-crystalline material contains lipid, structural protein, and an unidentified component with an absorption maximum at 260 nm.

The lipid component is mainly phosphatidyl ethanolamine containing a variety of fatty acids. Linolenic acid represents 15% of the total fatty acid in the quasi-crystalline material. The absorption spectrum of the lipid component removed by gel filtration on LH-20 in chloroethanol is due to conjugated trienoic acids. Since most naturally occurring polyunsaturated fatty acids do not have their double bonds conjugated, it is possible that linolenic acid is isomerized into conjugated trienoic acids by one of the preparation procedures.

The structural protein represents 20% of the quasi-crystalline material by weight and is homogeneous as indicated by amino acid content, electrophoresis, and electrofocusing. The protein is found in two forms. One form, representing 14% of the total protein, is solubilized in chloroethanol and may be separated from the lipid by gel filtration on LH-20 sephadex. The other form, representing 86% of the total protein, is associated with the unidentified component and may be freed by

alkaline hydrolysis. The protein in the two forms are identical. The isoelectric point of free structural protein is pH 5.2 while the structural protein associated with the unidentified component electrofocuses at pH 4.5.

The presence of equimolar amounts of ribose and a phosphate ester in the unidentified component, as well as the presence of a compound absorbing at 260 nm, strongly suggests that the unidentified component is a nucleotide. The molar ratio of protein to the nucleotide depends on the molecular weight of the protein. The monomer molecular weight of the structural protein has been estimated to be 12,500 daltons (Shannon et al., 1970). On the basis of a molecular weight of 12,500 daltons the molar ratio of nucleotide to protein is 55:1. (The protein content of the quasi-crystalline material is corrected for the 14% not associated with nucleotide.) The association of the structural protein with a polynucleotide is consistent with its release on alkaline hydrolysis.

The presence of the structural protein in the two forms may indicate sequential steps in membrane assembly. The structural protein is presumably synthesized on ribosomes. It may then be complexed with some material such as polynucleotide to keep it soluble until it is transported to a site of membrane assembly. The conversion of the precursor structural protein into a form suitable for membrane assembly may require enzymatic release of the protein from the polynucleotide and subsequent complexing of the protein with phospholipid.

Further studies on membrane assembly using radioactive isotope labeling of the structural protein will elucidate the order of the

sequential steps. Pulse labeling of the structural protein will indicate whether the polynucleotide associated protein or the free protein appears first in the pathway to membrane assembly. The techniques of preparation, separation and identification of the different forms of the structural protein described in this thesis make such further studies feasible.

CONCLUSIONS AND SUMMARY

The purpose of this work was to first develop techniques for characterizing a native precursor to membranes which had been obtained as a quasi-crystalline material; and, to then characterize the material. Hopefully the characterization illuminated if it did not elucidate the problem of *in vivo* membrane assembly.

Solvent systems and analytical techniques compatible with the solvent systems were developed to characterize the membrane precursor. Gel filtration and polyacrylamide gel potentiometric analysis were most useful in the characterization since the necessary solvent systems could be employed. Some analytical techniques were used with no modification. Such techniques were: amino acid analyses, thin-layer chromatography, and gas-liquid chromatography. Other techniques required little modification to accommodate the unique characteristics of the material. Such techniques were: protein assay, phosphate assay and spectral analysis.

The membrane precursor contained structural protein which represented 20% of the material by weight. The structural protein was found both free (14%) and associated with an unidentified material (86%). The isoelectric point of the free protein was pH 5.2. The associated protein electrofocused at pH 4.5. The associated protein was dissociated from the unidentified material by alkaline hydrolysis. The unidentified material contained equimolar amounts of phosphate and ribose as well as a component which had an absorption maximum of 260 nm. The molar ratio

of either phosphate or ribose to protein was 55:1 based on a monomer molecular weight for the protein of 12,500 daltons and also based on the fact that 86% of the protein was associated with the unidentified material.

Phosphatidyl ethanolamine was the major lipid in the membrane precursor. Linoleic acid was the major fatty acid in the phosphatidyl ethanolamine. The characteristic spectrum of the lipid from the membrane precursor was due to conjugated trienoic acids, isomers of linolenic acid. On the basis of the ethanolamine assay the maximum phosphatidyl ethanolamine content of the membrane precursor is 0.004 micromoles per mg of material.

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Fig. 1. Metal-shadowed electron micrograph of the quasi-crystalline material from Neurospora crassa cytosol.

Fig. 2. Thin-layer chromatography of neutral lipids on a silica gel G layer. Solvent: hexane-diethyl ether-acetic acid, 90:10:1. Key: A, mixture of 1,2-diolein, 1,3-diolein, and cholesterol; B. oleic acid; C, triolein; D, cholesterol oleate; E, lipids from the quasi-crystalline material. Visualization was with 5% sodium dichromate in 50% sulfuric acid at 200° for 30 min.

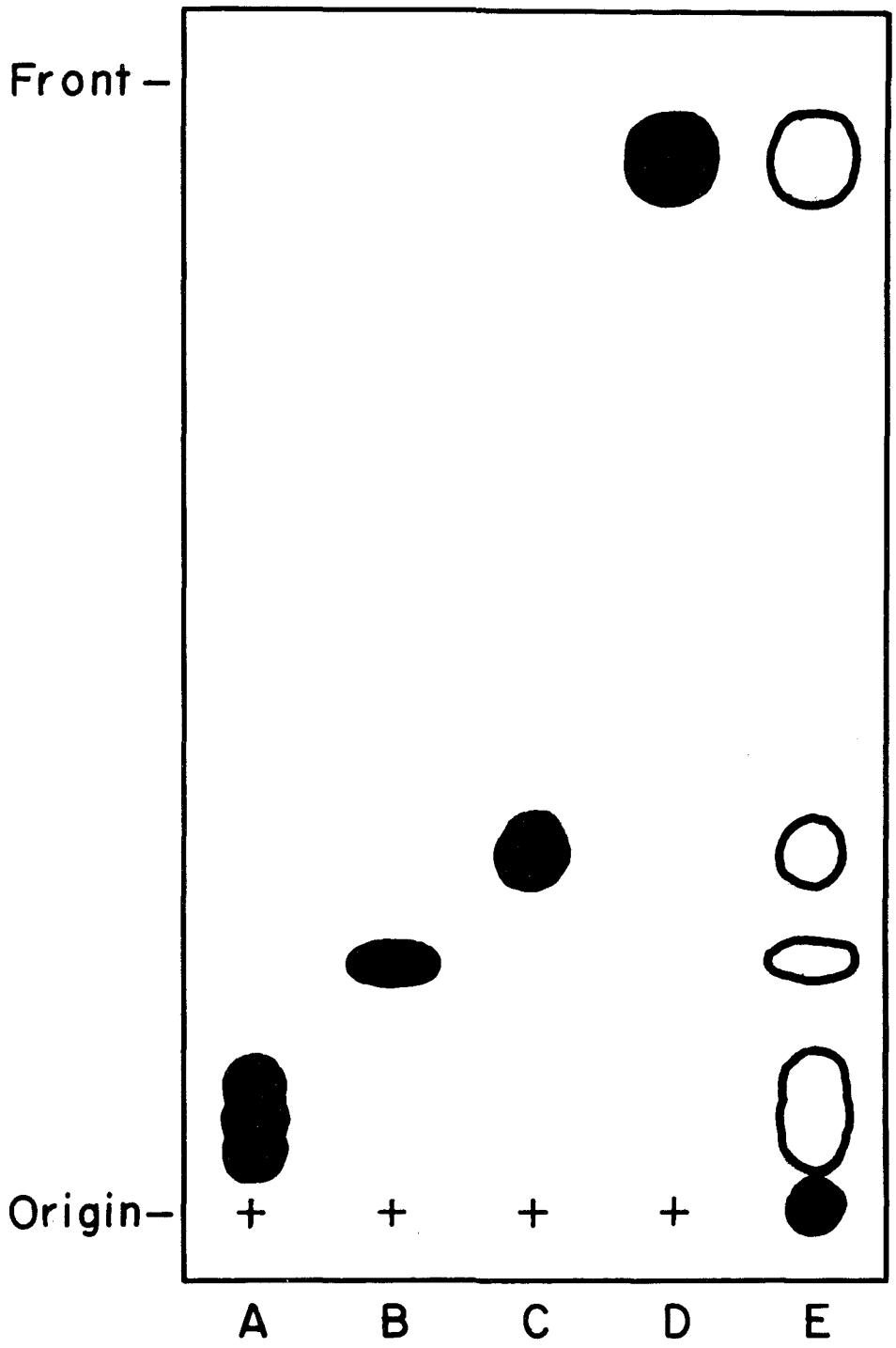
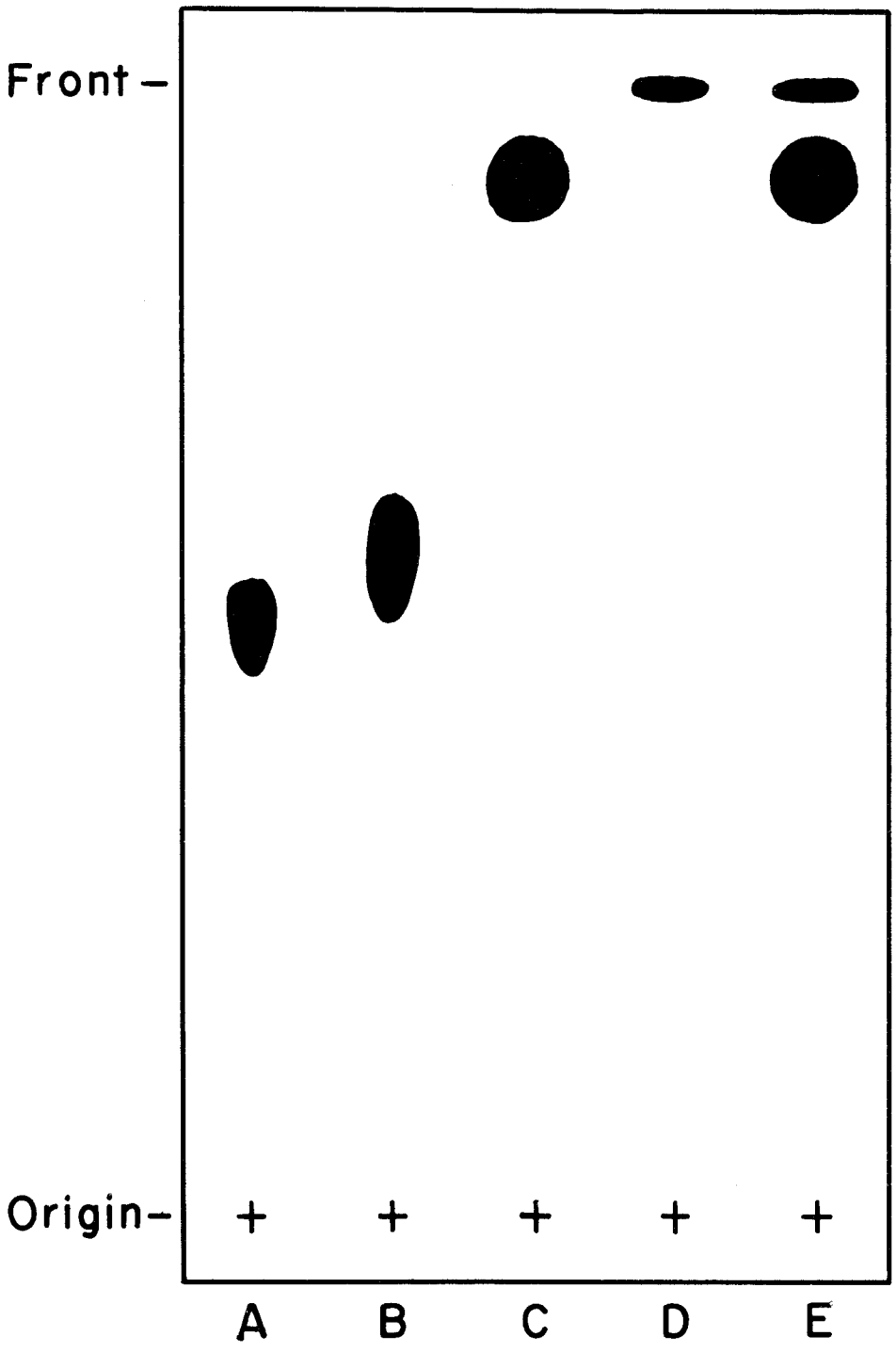

Front
Origin
A
B
C
D
E

Fig. 3. Thin-layer chromatography of phospholipids on a silica gel G layer. Solvent: chloroform-methanol-acetic acid-water, 50:25:8:4. Key: A, phosphatidyl choline; B, Phosphatidyl serine; C, phosphatidyl ethanolamine; D, mixture of diolein, triolein, oleic acid, cholesterol and cholesterol oleate; E, lipids from the quasi-crystalline material. Visualization was with 5% sodium dichromate in 50% sulfuric acid at 200° for 30 min.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Origin
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B
C
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E

Fig. 4. Gel filtration of a chloroethanol extract of the quasi-crystalline material on LH-20 sephadex equilibrated with chloroethanol. The extract from 36 mg of the quasi-crystalline material was applied to the column.

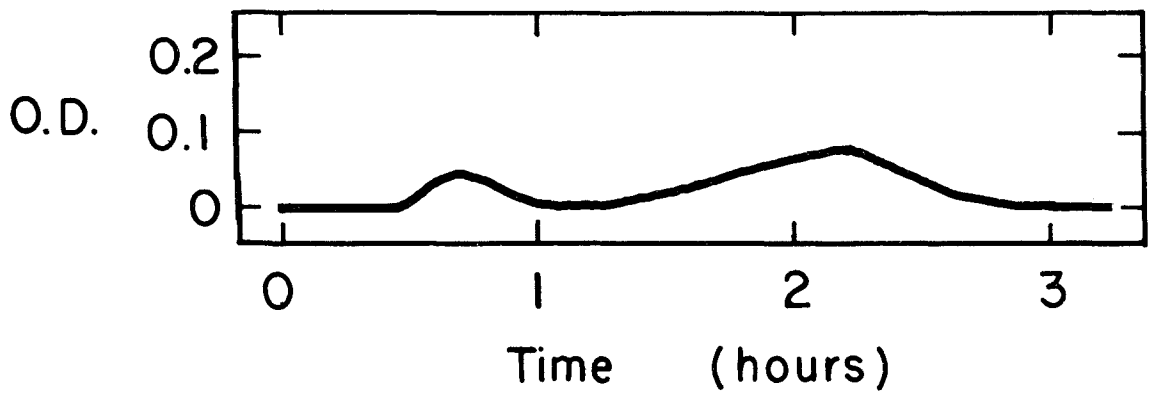

O.D.
0.2
0.1
0
0
1
2
3
Time (hours)

Fig. 5. Absorption spectrum of structural protein from a chloroethanol extraction of the quasi-crystalline material. The concentration of the structural protein was 0.58 mg per ml and the solvent was 2-chloroethanol adjusted to pH 8 with ammonium hydroxide.

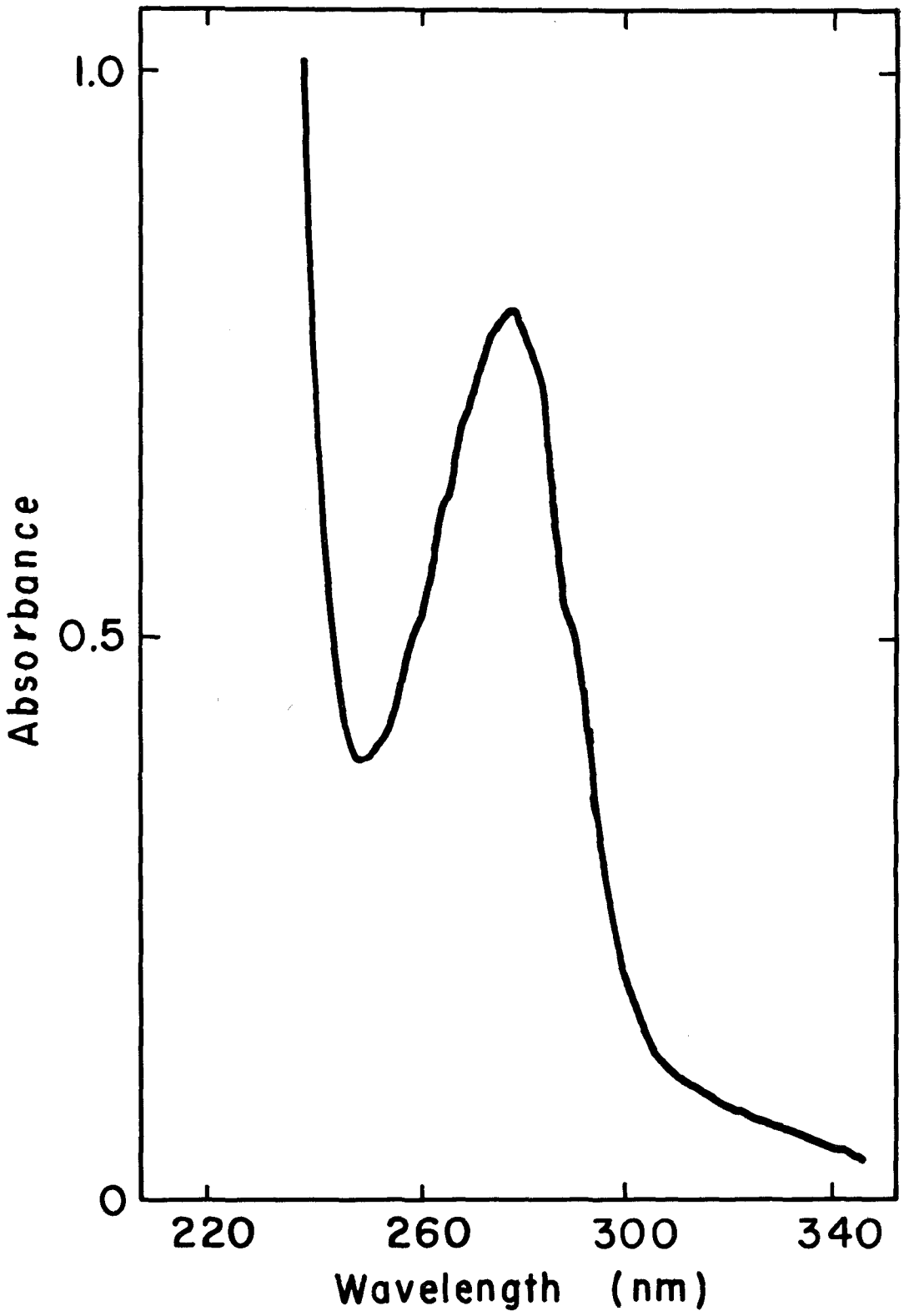

1.0
0.5
0
Absorbance
220
260
300
340
Wavelength (nm)

Fig. 6. Absorption spectrum of lipid from a chloroethanol extraction of the quasi-crystalline material. The solvent was 2-chloroethanol adjusted to pH 8 with ammonium hydroxide.

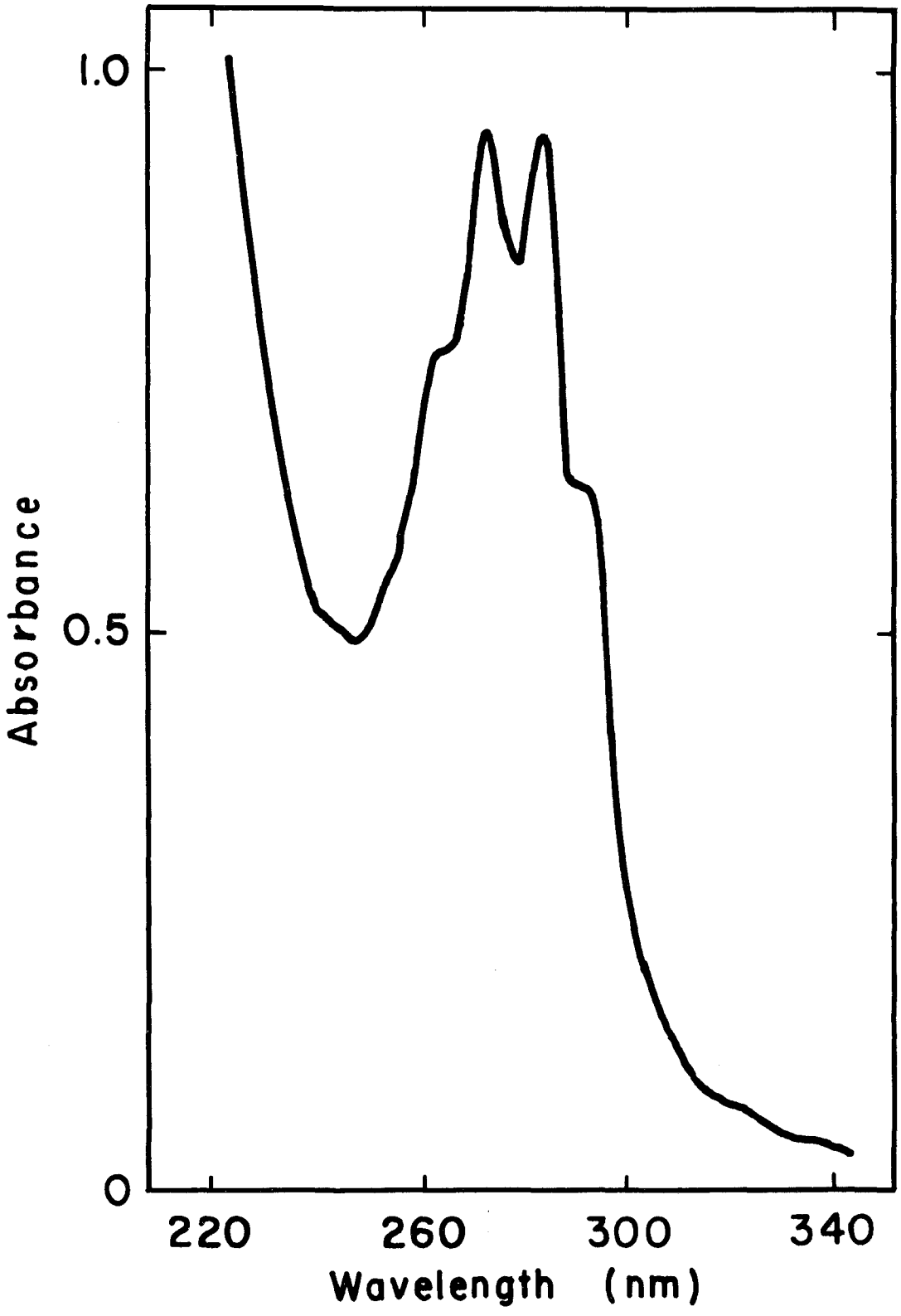

1.0
0.5
0
Absorbance
220
260
300
340
Wavelength (nm)

Fig. 7. Gas-liquid chromatography of fatty acid methyl esters on 10% diethylene glycol succinate and gas-chrom Q. Esters were prepared from the fatty acids in the quasi-crystalline material by transmethylation. The solvent background had been subtracted. Regions marked A, B, C, D, E correspond to laurate-myristate, palmitate, stearate-oleate, linoleate, and linolenate respectively.

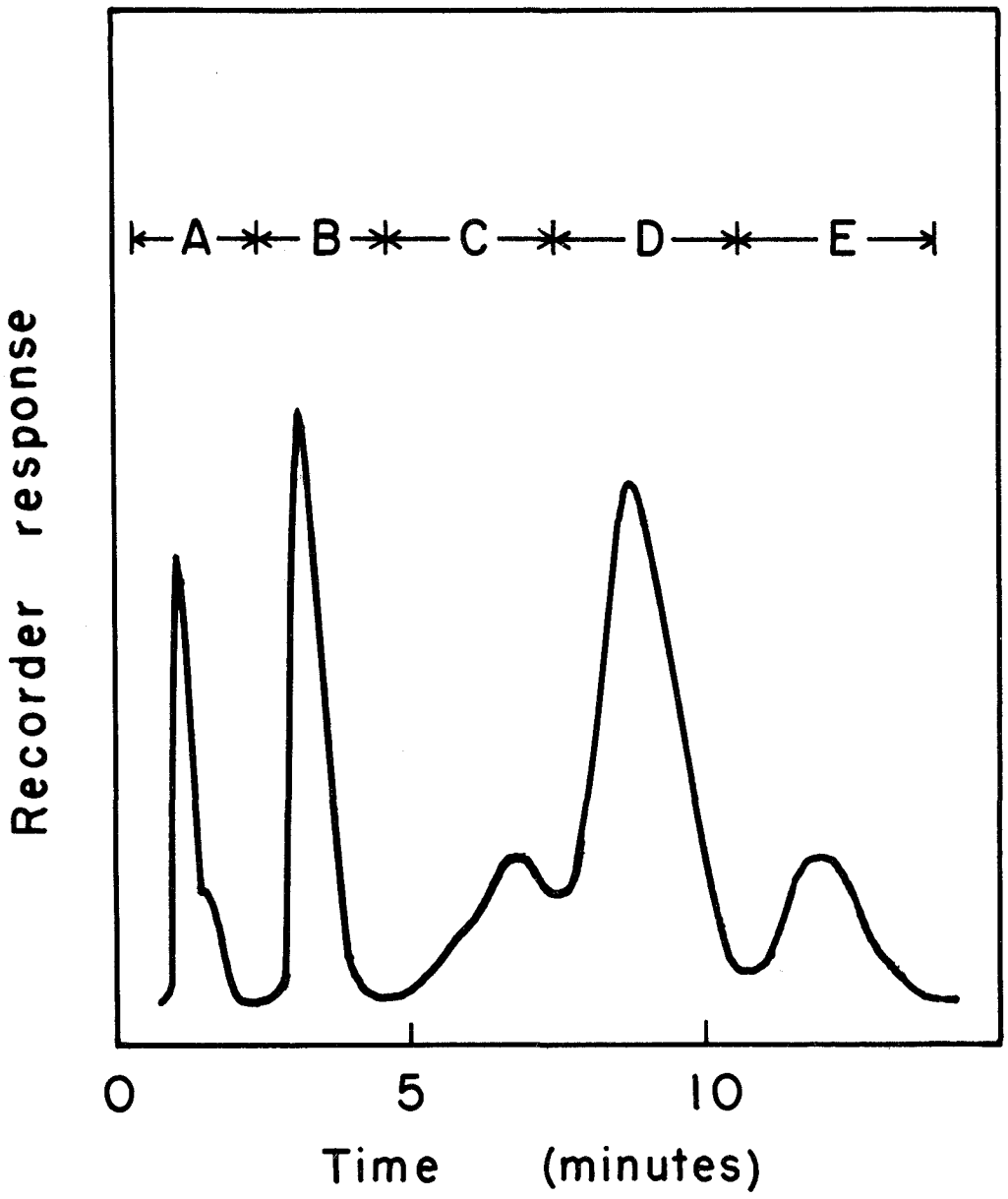

A
B
C
D
E
Recorder response
0
5
10
Time (minutes)

Fig. 8. Gel filtration of delipidized quasi-crystalline material on G-50 sephadex equilibrated with 2% dodecyl sodium sulfate.

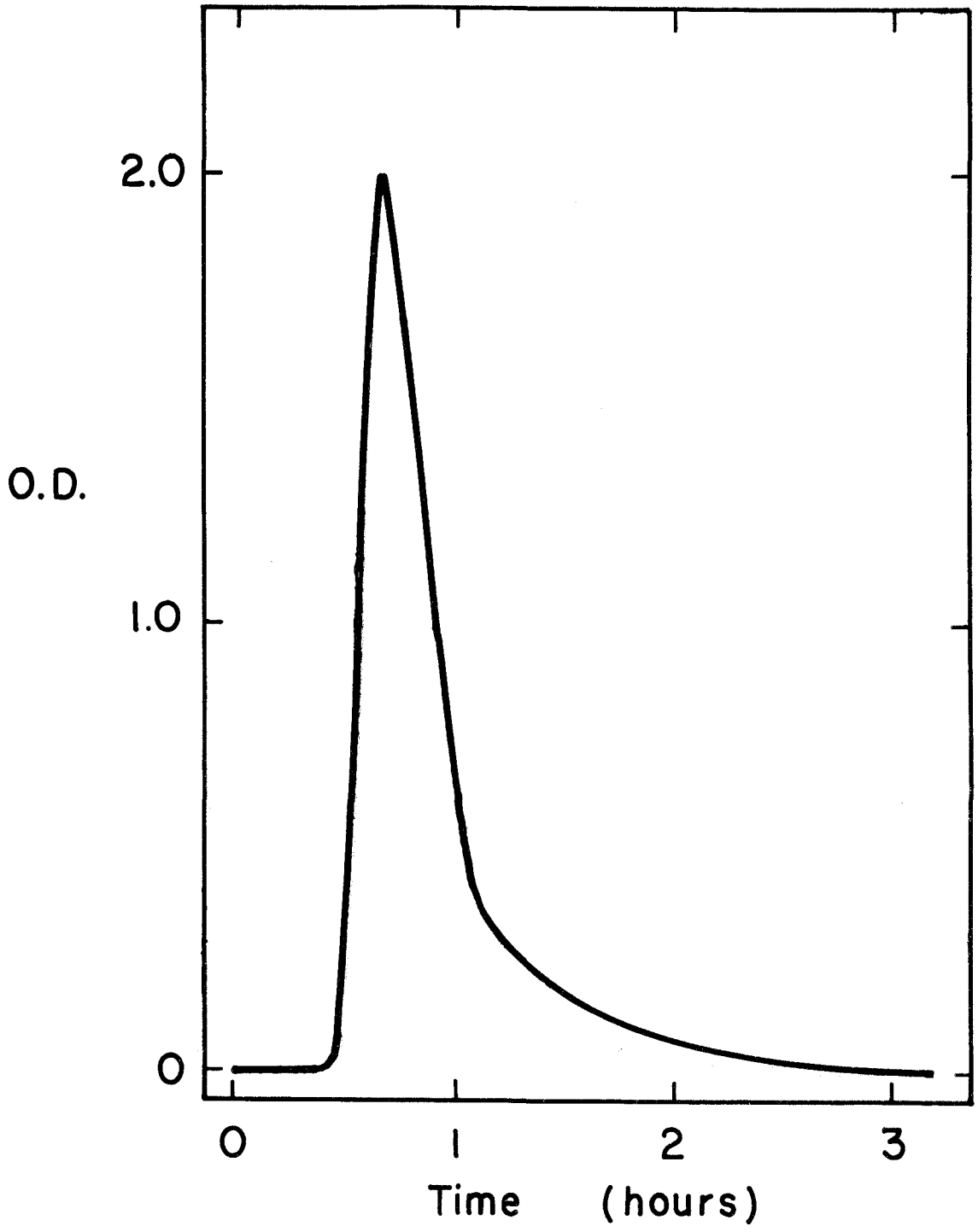

2.0
O.D.
1.0
0
0
1
2
3
Time (hours)

Fig. 9. Absorption spectrum of delipidized quasi-crystalline material solubilized in 2% dodecyl sodium sulfate.

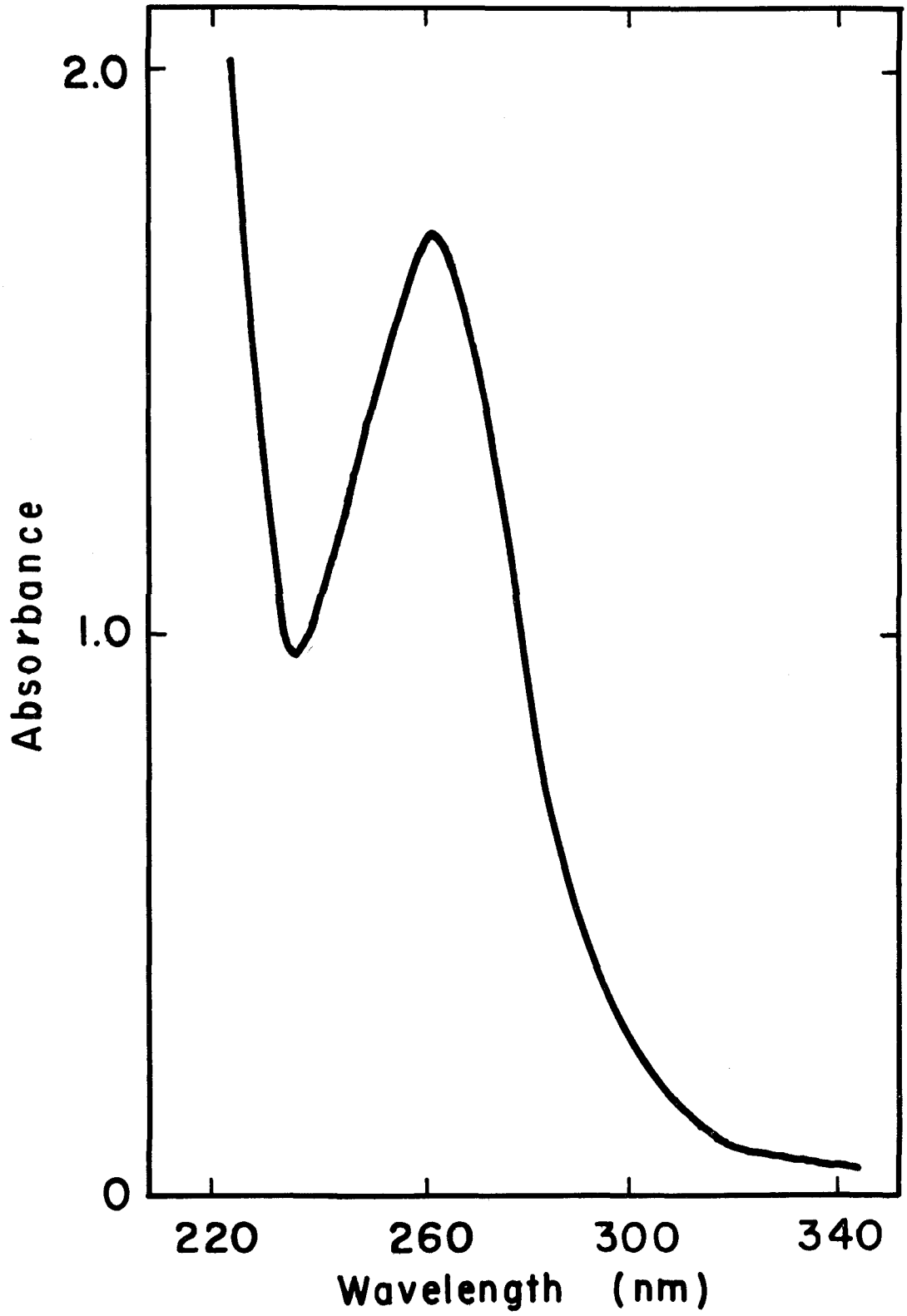

2.0
1.0
0
Absorbance
220
260
300
340
Wavelength (nm)

Fig. 10. Gel filtration, on LH-20 sephadex in chloroethanol, of delipidized quasi-crystalline material treated with 1.0 M sodium hydroxide at 25° for 12 hr. The sample applied to the column represents 36 mg of original quasi-crystalline material.

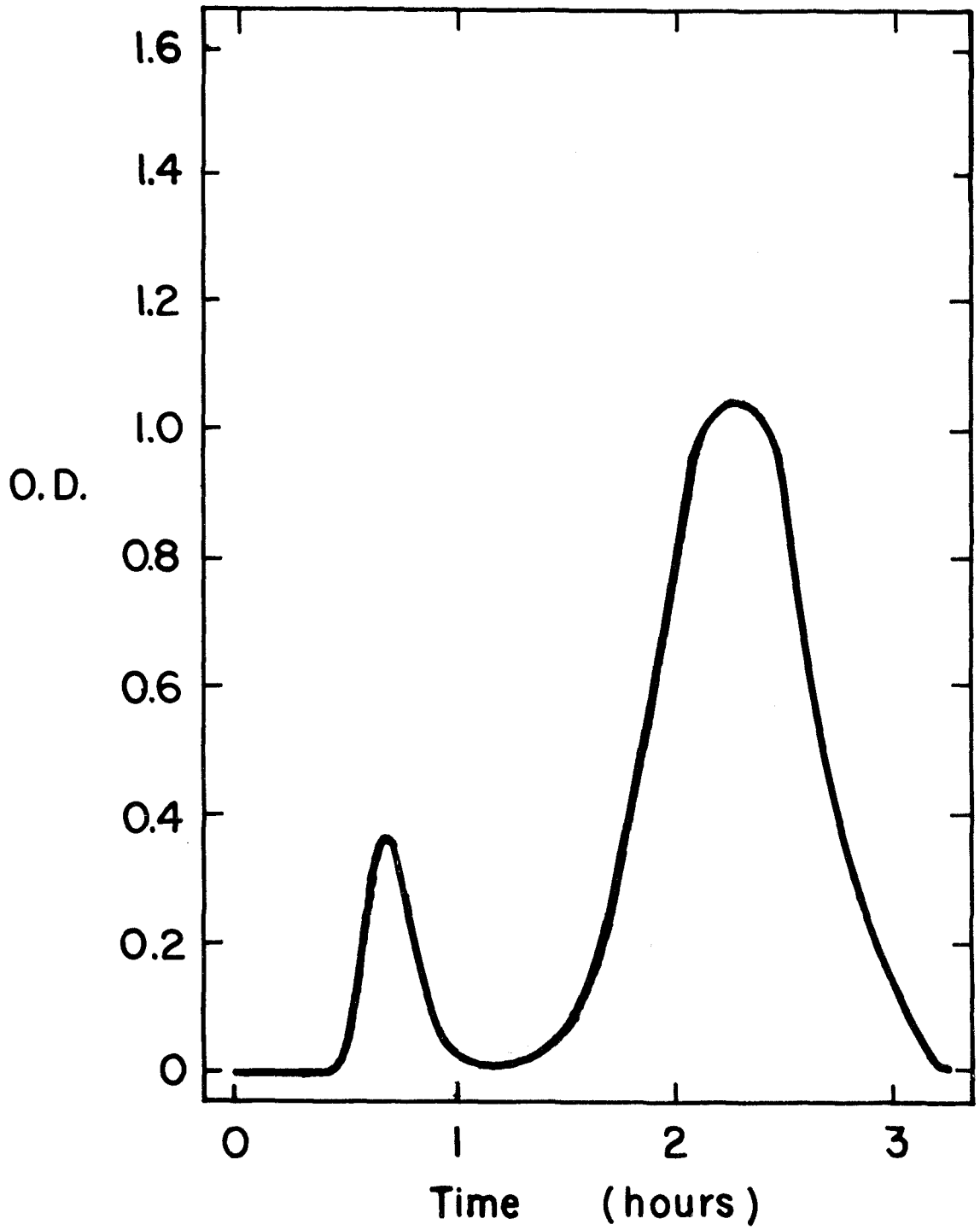

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
O. D.
0
1
2
3
Time (hours)

Fig. 11. Absorption spectrum of the structural protein released from delipidized quasi-crystalline material by alkaline hydrolysis. The concentration of the structural protein was 0.75 mg per ml. The solvent was 2-chloroethanol adjusted to pH 8 with ammonium hydroxide.

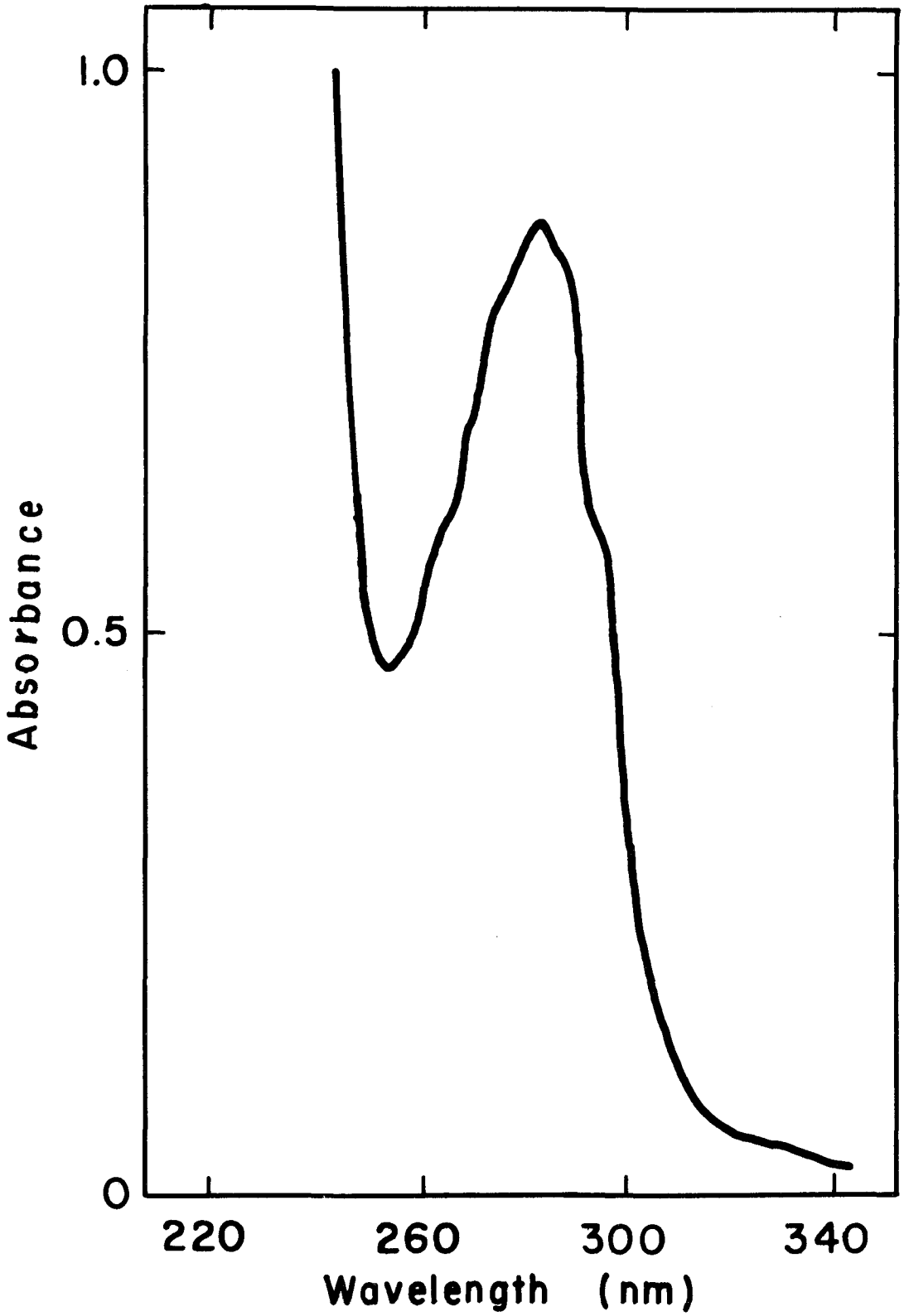

1.0
0.5
0
Absorbance
220
260
300
340
Wavelength (nm)

Fig. 12. Absorption spectrum of the unidentified compound released from the delipidized quasi-crystalline material by alkaline hydrolysis. The solvent was 2-chloroethanol adjusted to pH 8 with ammonium hydroxide.

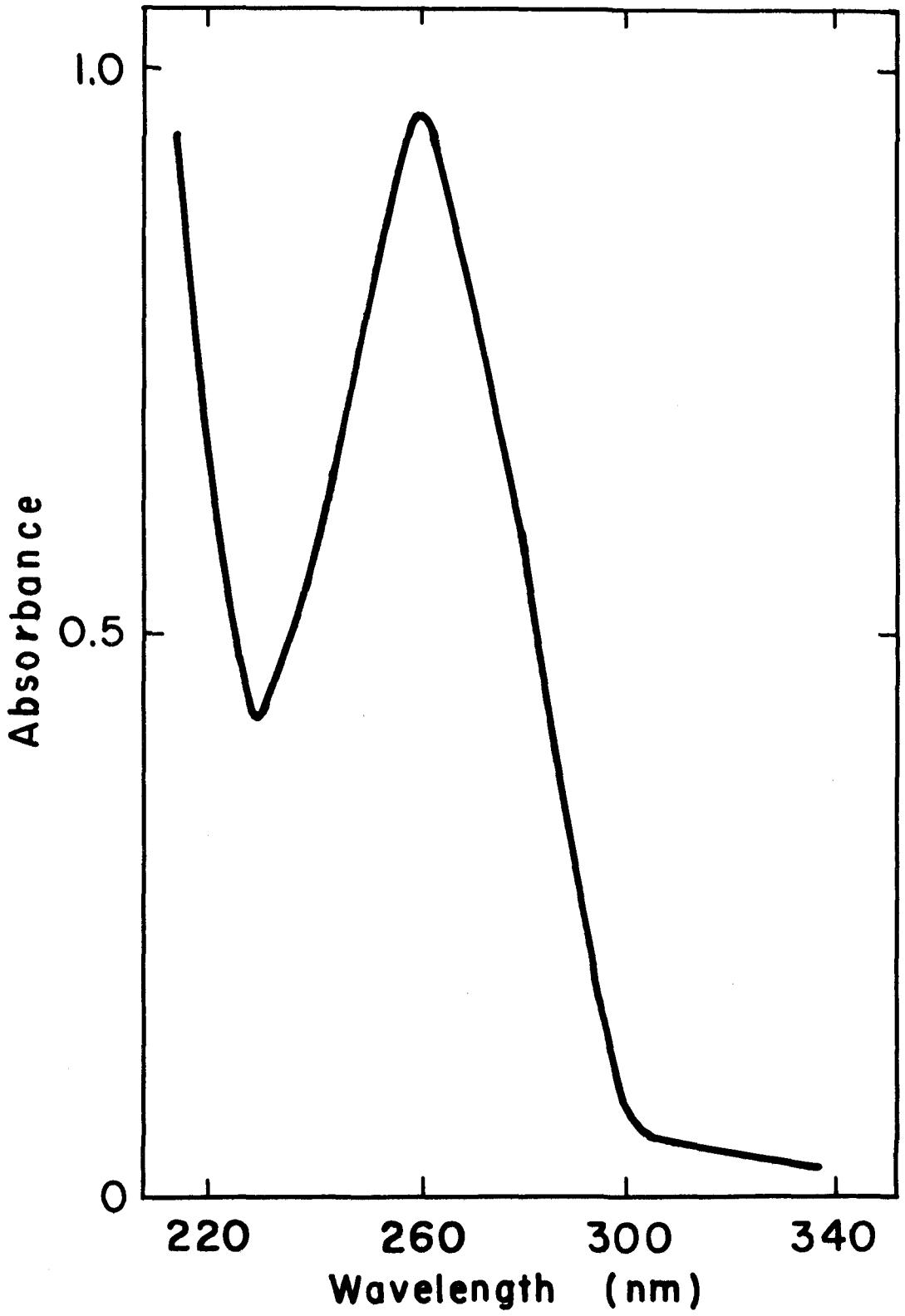

1.0
0.5
0
Absorbance
220
260
300
340
Wavelength (nm)

Fig. 13. Polyacrylamide gel electrofocusing of structural protein. Key: a, structural protein from N. crassa mitochondrial membranes; b, structural protein from the quasi-crystalline material. Samples were applied to the gels in formic acid. Electrofocusing was as described in the methods and procedures section. The gels were fixed in 7% trichloroacetic acid for 12 hr and scanned at a wavelength of 280 nm.

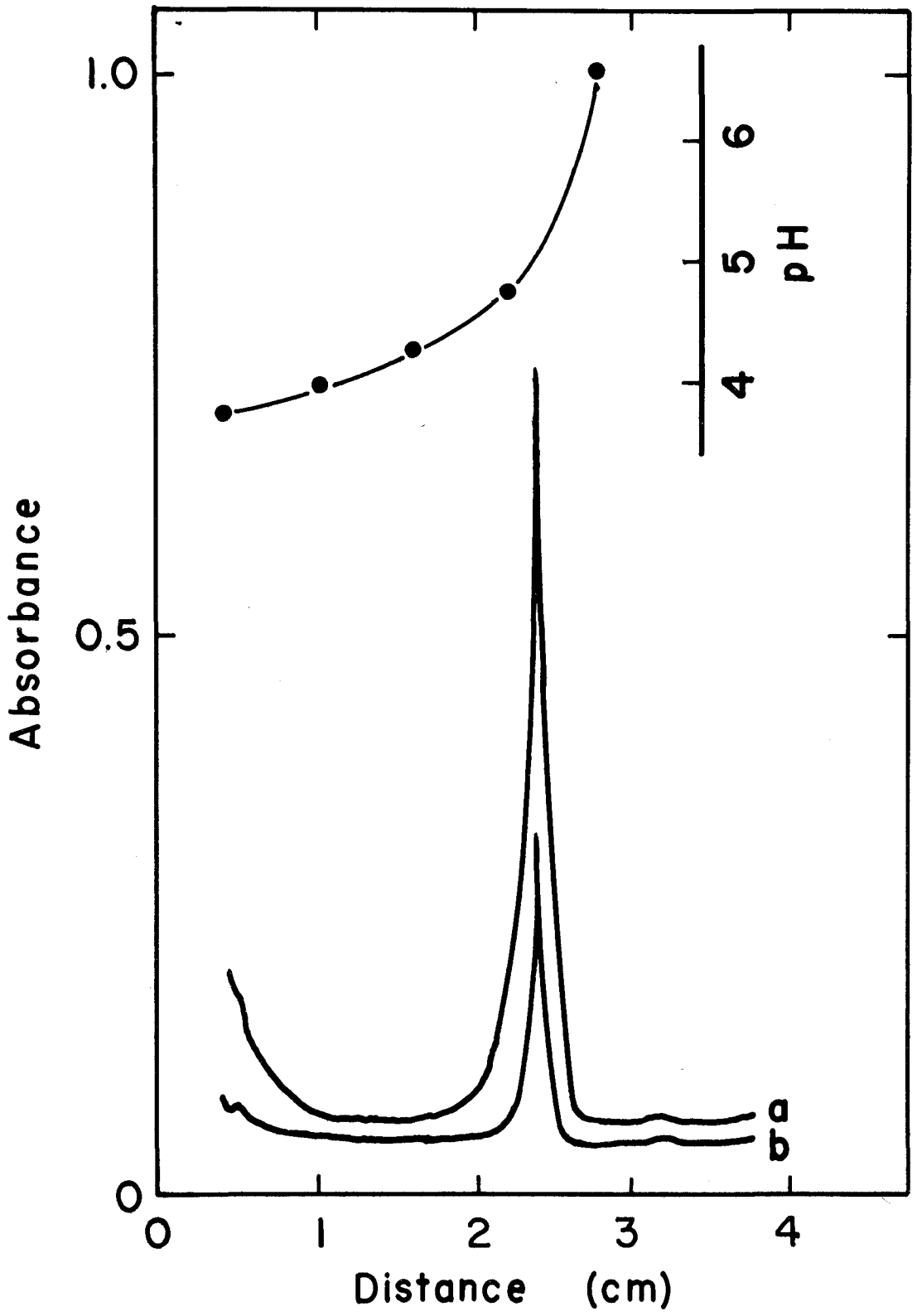

1.0
0.5
0
Absorbance
0
1
2
3
4
Distance (cm)
4
5
6
pH
a
b

Fig. 14. Polyacrylamide gel electrophoresis of structural protein released from the quasi-crystalline material by three hydrolysis procedures. Key: a, structural protein released by hydrolysis in 1.0 M aqueous sodium hydroxide at 25° for 12 hr and dialyzed against 8 M urea for 24 hr before electrophoresis; b, structural protein released by hydrolysis in 0.1 M anhydrous, methanolic potassium hydroxide during reflux for 1 hr and transferred to 8 M urea immediately before electrophoresis; c, structural protein released by hydrolysis in 0.1 M anhydrous, methanolic potassium hydroxide during 6 hr at 25° and transferred to 8 M urea immediately before electrophoresis. The gels were stained with aniline black and scanned at a wavelength of 550 nm after destaining.

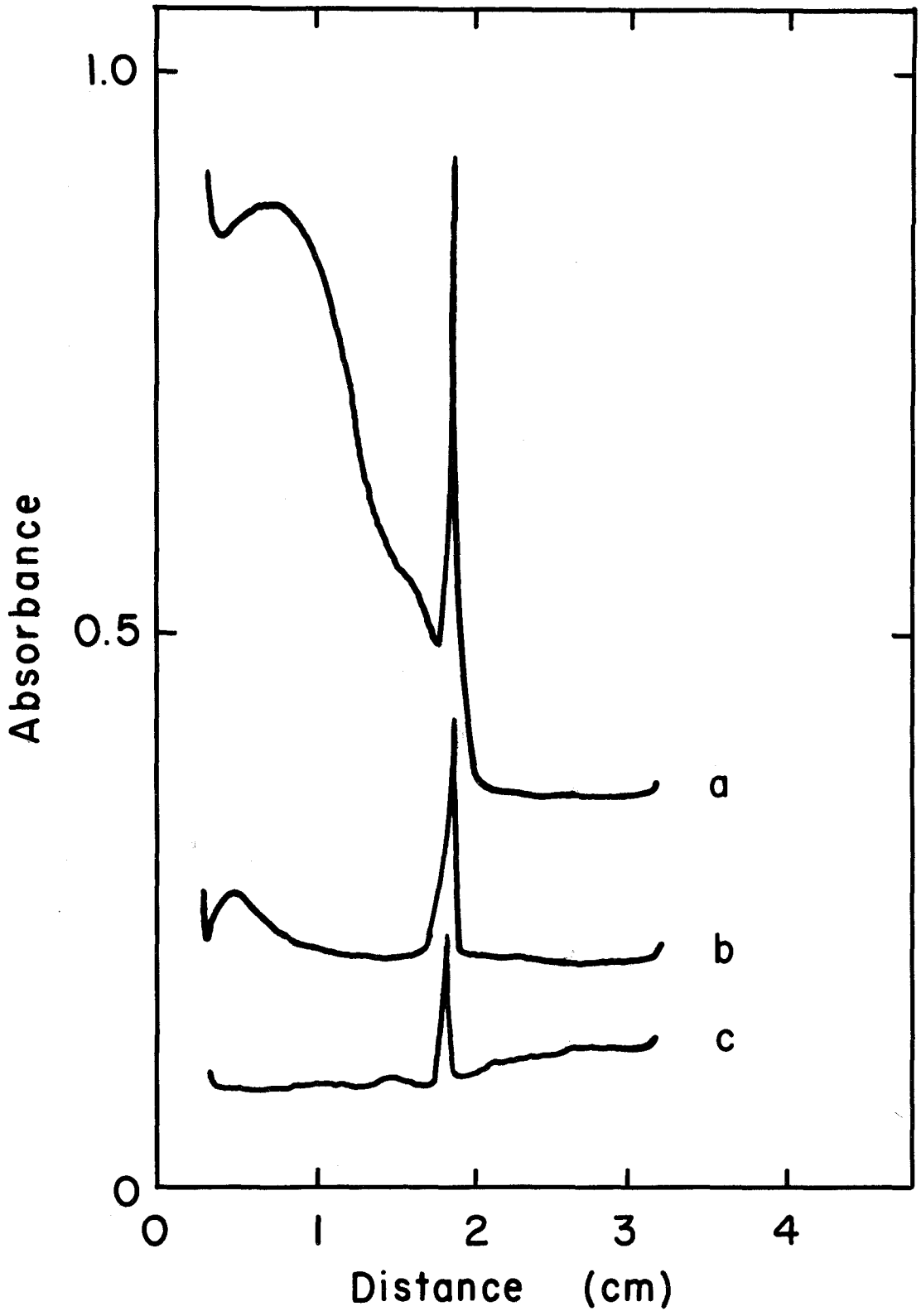

1.0
0.5
0
Absorbance
0
1
2
3
4
Distance (cm)
a
b
c

Fig. 15. Polyacrylamide gel electrofocusing of quasi-crystalline material. Key: a, scan of the gel at wavelength of 280 nm; b, scan of the gel at a wavelength of 260 nm. Samples were solubilized in 6 M guanidine hydrochloride. The gels were scanned immediately after electrofocusing and were not stained or fixed.

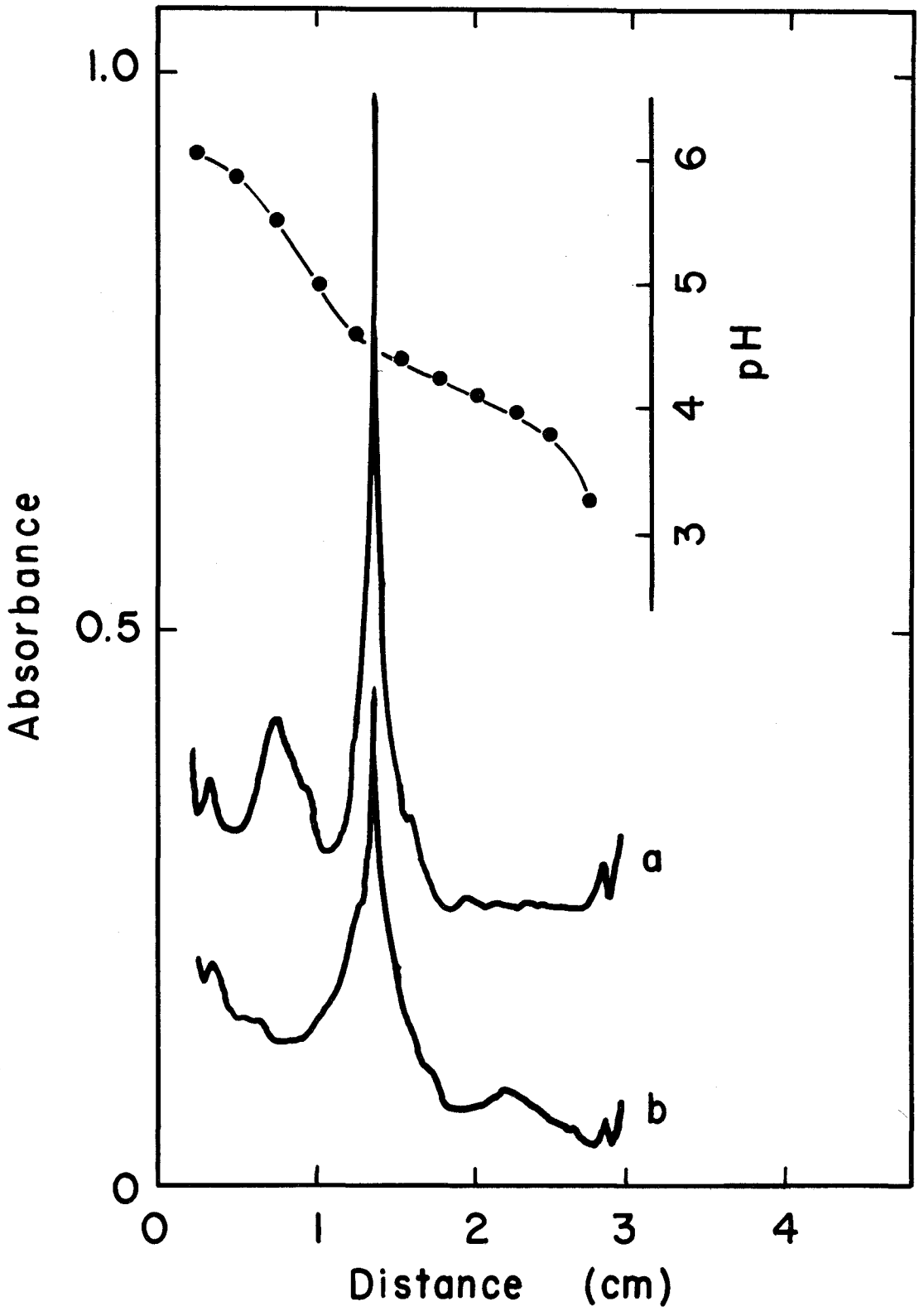
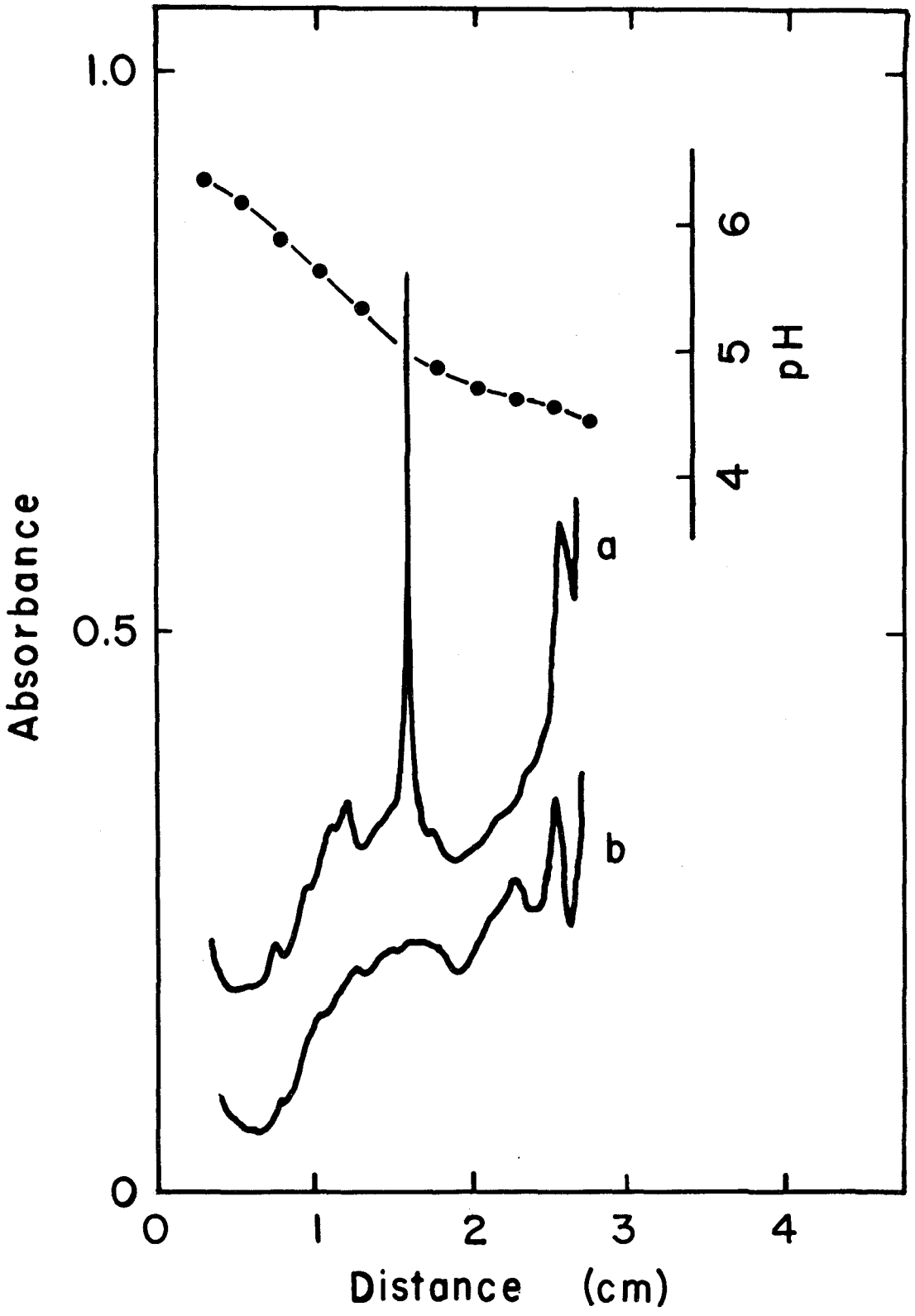

1.0
0.5
0
Absorbance
0
1
2
3
4
Distance (cm)
3
4
5
6
pH
a
b

Fig. 16. Polyacrylamide gel electrofocusing of hydrolyzed quasi-crystalline material. Key: a, scan of the gel at a wavelength of 280 nm; b, scan of the gel at a wavelength of 260 nm. Samples were transferred to 6 M guanidine hydrochloride after hydrolysis. The gels were scanned immediately after electrofocusing and were not stained or fixed.


1.0
0.5
0
Absorbance
0
1
2
3
4
Distance (cm)
a
b
6
5
4
pH

CHARACTERIZATION OF A SOLUBLE FORM OF STRUCTURAL PROTEIN FROM *NEUROSPORA CRASSA*

Clarence Denis Peterson

Department of Zoology

M.S. Degree, May 1971

ABSTRACT

The study of membrane assembly has, until now, consisted of fractionating a membrane into its components or of assembling model membranes from non-physiological components. A native precursor to membranes was obtained as a quasi-crystalline material. It was the purpose of this work to develop the appropriate techniques for characterizing the material as well as to carry out the characterization.

The results of the characterization indicate the quasi-crystalline material contains a single protein, structural protein. This protein is in two forms which may reflect two sequential steps in membrane assembly. One form is solubilized in 2-chloroethanol and isolated by gel filtration. The other form may be isolated on gel filtration only after previous release by alkaline hydrolysis.

This work forms the basis of future study of membrane assembly in that it has developed solvents and compatible techniques for the preparation, identification and characterization of a native precursor of membranes.

COMMITTEE APPROVAL: