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SPECTROPHOTOMETRIC ANALYSIS OF RNA AND DNA USING CETYLTRIMETHYLAMMONIUM BROMIDE

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DISCLAIMER

Spectrophotometric Analysis of RNA and DNA Using Cetyltrimethylammonium Bromide

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Running Title: Analysis of RNA and DNA

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ABSTRACT

A versatile procedure is described for the analysis of DNA and RNA using cetyltrimethylammonium bromide. A determination of optimum conditions for the analysis by UV spectrometry of both RNA and DNA in brain tissue has been carried out. The method is described for the analysis of approximately 100 mg of brain tissue, but may be scaled down by appropriate modifications. The orcinol reaction is shown to give high values for RNA. This method has also been applied to the analysis of nucleic acid in other mammalian organs and to mammalian cells obtained from tissue culture. The method may also be adapted for the determination of radioactivity in nucleic acids.

The RNA and DNA in brain tissue is frequently estimated by the Schmidt-Thannhauser (1) procedure as modified by Munro and Fleck (2). The nucleic acids are precipitated by perchloric acid (PCA). The ribonucleic acid (RNA) has been determined by UV absorption, by the orcinol reaction, or by phosphate; the deoxyribonucleic acid (DNA) has been usually estimated by the diphenylamine reaction (Burton), or by phosphate. The UV determination of DNA was reported by several workers, but the high protein content of this fraction made necessary a correction using a two wavelength reading [Wannemacher, Banks and Wunner (3); Santen and Agranoff (4); Fleck and Begg (5)] or other procedures [Logan, Mannell and Rossiter (6)].

Several investigators have used cetyltrimethylammonium bromide (CTAB) to precipitate nucleic acids from aqueous solution (7), low molecular weight RNA from nuclei (8), and from brain homogenates (9).

This report describes a further modification of the Schmidt-Thannhauser method using CTAB as the initial precipitant for rat brain nucleic acids. Conditions are described which make possible the subsequent quantitative estimation of both RNA and DNA by UV absorption. With minor additional modification, the procedure can be used for the determination of ^{3}H or ^{14}C activity in RNA.

This modified method is presently being used in an investigation of differences in RNA and DNA concentrations in the brain of rats raised in complex and impoverished environments, of changes in RNA during early development of the rat brain, and during the cell cycle of 3T3 tissue culture cells.

METHODS

Reagents

1) Ethylenediaminetetraacetic acid buffer. Ethylenediaminetetraacetic acid, disodium salt, dihydrate (EDTA, Aldrich Chemical Co.), 41.8 g/liter, pH adjusted to 5.9 with KOH.

2) Cetyltrimethylammonium bromide, an aqueous 3% solution of hexadecyltrimethylammonium bromide (CTAB), Technical grade, Eastman Kodak.

3) Ribonucleic acid. Ribonucleic acid purified from Torula (Calbiochem B grade), 1 mg/ml in Tris 0.01 M, pH 8.4.

4) Orcinol reagent. Orcinol monohydrate (Aldrich Chemical Co.) recrystallized from boiling benzene. Four percent solution in 95% ethyl alcohol prepared just before use.

5) Ferric chloride reagent. To 100 ml of conc. HCl is added 0.5 ml 10% $FeCl_3 \cdot 6\ H_2O$ solution.

6) Deoxyribonucleic acid. Calf thymus DNA (A grade, Calbiochem) stock solution 2 mg/ml in 0.01 M Tris, pH 8.6.

7) Diphenylamine reagent. Diphenylamine (Polysciences, Inc.), 1.5 g is dissolved in 100 ml glacial acetic acid, and 1.5 ml of conc. sulfuric acid is added. Just before use, 0.5 ml 1.6% aqueous acetaldehyde is added per 100 ml of reagent.

8) Scintillation solution. 40 ml Permafluor (Packard Instrument Co., Inc., Illinois); 200 ml Bio-Solve (Beckman); 33 ml butyric acid; are diluted to 1 liter with toluene.

9) Ascorbic acid. Twenty percent solution of ascorbic acid (Cal Biochem) in H_2O.

Procedure

The recommended procedure for the analysis of RNA and DNA is described below. Parameters which were investigated are briefly described in the Results section.

Sample preparation. All operations of the sample preparation were carried out at 0-5° using cold solutions. Rat cerebrum, i.e., total brain excluding cerebellum and medulla, was homogenized in EDTA buffer (0.11 M) using a Potter-Elvehjem homogenizer to a concentration of 25 mg/ml. In a 16 x 75 mm culture tube, 4 ml of homogenate was added to 2 ml of 3% CTAB, and the precipitate was allowed to form. After 1 hr, the precipitate was collected by centrifugation in a Sorvall RC-3 centrifuge at 7,000 x g for 15 min. The supernatant was discarded, and the pellet was washed twice with 1 ml H_2O and once with 0.1 N KOAc in absolute ethyl alcohol. The pellet was thoroughly dispersed using a Vortex test tube mixer and then centrifuged between each washing.

When direct comparisons were desired between the PCA and CTAB methods, the tissues were homogenized in distilled water to a concentration of 50 mg/ml. Two ml of homogenate were added to 2 ml of 0.4 N PCA, or to 2 ml of double strength EDTA buffer and 2 ml of 3% CTAB.

In the PCA method the tissues were precipitated with an equal volume of 0.4 N PCA. After 15 min the samples were spun at 3,000 x g for 15 min, the supernatant was discarded, and the pellet was washed with 1 ml of 0.2 N PCA two times, with thorough dispersal, using the Vortex mixer. During the collection of the RNA supernatant and washings, the pellet was spun at 2,500 x g; in all other respects the PCA and CTAB pellets were hereafter treated identically.

RNA fraction. The pellet was dispersed with 100 µl of H_2O and hydrolyzed in 1.1 ml of 0.3 N KOH at 37°C for 1 hr in a shaker bath. After cooling, the alkaline digest was made 0.2 N in acid by the addition of 500 µl of 1.3 N PCA, and allowed to stand for 15 min at 0°C. After centrifugation at 7,000 x g for 15 min, the supernatant was recovered, and the acid-insoluble fraction was washed twice with 500 µl of 0.2 N PCA. The three supernatants comprising the RNA fraction were pooled, and the volume adjusted to 5.0 ml (0.1 N PCA, final concentration).

The RNA content was assayed by absorbance at 260 nm, and calculated on the assumption that an absorbance of 1.00 at 260 nm was equivalent to 32 µg RNA/ml (2). The Dische (10) modification of the orcinol method was used for comparison of RNA content. Torula RNA was used as a standard.

DNA fraction. The acid-insoluble fraction was drained and blotted dry. The pellet was thoroughly dispersed in 1.00 ml of N PCA. The DNA was heated for 20 min at 70°C, cooled, and spun at 7,000 x g for 15 min. An appropriate volume, usually 400 µl, was reacted with diphenylamine [Burton (11)] for 24 hr at room temperature, and the absorbance read at 600 nm. Calf thymus DNA was used as a standard. Absorbance measurements were made on the Gilford 2200 spectrophotometer; spectra were obtained with the Cary 118 spectrophotometer.

Protein. Protein was analyzed by the Lowry method, using bovine serum albumin as a standard (12).

Determination of radioactivity in the RNA fraction. The sample (obtained from rats previously injected with ^{14}C-uridine or ^{3}H-uridine) was carried through the procedure as described above using CTAB until

the 0.3 N KOH hydrolysis, then aliquots were taken and hydrolyzed overnight at 37°C; 500 µl of hydrolysate were then mixed in a vial with 18 ml of scintillation solution and 50 µl of 20% ascorbic acid and counted in a Tricarb Packard scintillation counter.

RESULTS

RNA fraction. In order to establish the optimum length of hydrolysis for maximum recovery of RNA, the time course of alkaline hydrolysis for the tissue pellet precipitated by 1% CTAB or PCA, and for Torula RNA, was investigated. For Torula RNA, 25 min in 0.3 N KOH is sufficient for complete solubilization, but 45 min is necessary for hydrolysis of RNA from a brain pellet (Fig. 1). After 60 min of hydrolysis, the UV absorbance of the hydrolysates obtained by CTAB or PCA precipitation from brain homogenates are nearly comparable, but the protein content is fivefold higher in the PCA hydrolysate. This accounts in large part for the 5% higher UV absorbance of the hydrolysate from the pellet precipitated by PCA. With extended hydrolysis, chromogenic material and protein continue to be extracted but at a faster final rate from the PCA precipitated fraction due to its greater contamination from non-RNA tissue constituents. The spectra of the acidified alkaline hydrolysate of Torula RNA, and RNA from rat cerebrum isolated by the CTAB method and by PCA is shown in Fig. 2. During the alkaline hydrolysis, degradation products, mainly from protein, are released from the tissue along with the ribonucleotides. These contaminants are indicated by the shift in the absorption minimum from 230 nm to higher wavelengths and by the decreased $A_{\frac{260}{230}}$ nm ratio. Table 1 summarizes these data for RNA precipitated by 0.2 PCA and by varying concentrations of CTAB. At very low concentrations

of CTAB, the protein content in the RNA fraction is approximately the same by the PCA method and the CTAB method. With progressively higher concentrations of CTAB, the contamination decreases, as evidenced by the decreasing protein content and decreasing wavelength of minimum absorbance in the 230-235 nm region. With 1% CTAB, the RNA fraction contains 1/5 the protein of the PCA method, and its spectrum more closely resembles that of the Torula RNA hydrolysate (Fig. 2). Brain RNA levels based on orcinol were consistently much higher than estimates based on UV absorbance. This disparity may be due to the chemical composition of brain tissue and the non-specificity of the orcinol reaction.

Rat brain RNA, isolated by the phenol technique, was added to brain homogenates to check recovery. The mean recovery from 14 determinations was 95% for CTAB and 96% using 0.2 N PCA. To test the reliability of the assay, one rat cerebrum was analyzed 14 times. The RNA level was 147.4 $\pm$ 2.9 µg/100 mg brain (mean $\pm$ S.D.).

The spectral data of the RNA fraction from 3T3 cells and from rat liver are very similar whether prepared by the CTAB or PCA method, and are indicative of low contamination (Table 2). RNA content of the 3T3 cells was similar by both precipitation methods. Liver RNA levels were similar whether estimated by UV absorbance or by orcinol.

DNA fraction. To establish the optimal time and temperature of hydrolysis of DNA prior to its estimation by the diphenylamine reaction, we investigated the effect of these variables on DNA obtained by several methods. We found that at 96°C, deoxyribose, as measured by the diphenylamine reaction (11), was released rapidly but was also very labile (Fig. 3).

However, at 70°C, deoxyribose, although more slowly released, reaches maximal levels after 20 min, and appears reasonably stable thereafter. This confirms the report of Hubbard et al. (13). The release of deoxyribose from brain tissue precipitated by CTAB follows very closely that of calf thymus DNA. The PCA pellet requires rigorous agitation for adequate extraction, but still releases deoxyribose more slowly, and yields low values throughout the hydrolysis. For approximately the first 15 min of hydrolysis at 70°, the absorbance at 266 nm of the DNA fraction is similar for the DNA obtained by the CTAB and PCA methods (Fig. 4). After 20 min, protein analyses of the hydrolysates indicate extensive protein contamination in the PCA fraction. This is also shown by comparison of the spectra of the hydrolysates of DNA obtained by the two precipitants with the spectrum of the hydrolysate of calf thymus DNA. The minimum of the PCA precipitated DNA is shifted from approximately 232 to 238 nm (Fig. 5). Accordingly, the estimates of DNA in brain by the PCA method are low based on the diphenylamine reaction, and high based on the uncorrected UV absorbance reading.

The DNA content in brain estimated by diphenylamine is only moderately influenced by the concentration of CTAB used as a precipitant (Table 3), and at CTAB concentrations between 0.25% and 2% is in good agreement with values estimated by the UV absorbance at 266 nm.

Calf thymus DNA was added to brain homogenates to determine the recovery using the CTAB method. The mean recovery of 100 µg of DNA was 98% based on the diphenylamine reaction of 15 samples. To test the reliability of the assay, one rat cerebrum was analyzed 14 times. The DNA content was 105.6 $\pm$ 3.8 µg/100 mg brain (mean $\pm$ S.D.).

The PCA method applied to rat liver gives results similar to those obtained with brain; that is, low values by diphenylamine and high values by uncorrected absorbance at 266 nm (Table 4). In 3T3 cells, however, DNA estimates are more nearly comparable by UV and diphenylamine when PCA is used. Using CTAB as the precipitant, excellent agreement between values based on absorbance at 266 nm and on diphenylamine was obtained for the DNA content of rat liver, and good agreement for 3T3 cells.

Determination of radioactivity in the RNA fraction. When the KOH hydrolysate of the RNA fraction obtained by an initial precipitation with PCA is added to the scintillation fluid, a precipitate is always obtained. However, when CTAB is used as the precipitant, addition of the resulting KOH hydrolysate to the scintillation fluid does not lead to a precipitate.

An additional problem typically encountered with alkaline hydrolysates of tissue samples is a variable chemiluminescence. This luminescence usually disappears on standing at 37° for many hours, but a more effective and reproducible method for its elimination is by the addition of a small amount of ascorbic acid (14). (Fifty µl of β-mercaptoethanol can be substituted for ascorbic acid.) The addition of ascorbic acid or β-mercaptoethanol was tested in our procedure and found to be reliable. Counting efficiencies of 25% were obtained for ^{3}H and 80% for ^{14}C using a Packard Tri-Carb Model 3375 scintillation counter. Agreement was obtained between uridine-labeled brain samples in which the radioactivity was determined by this procedure and duplicate samples which were oxidized to $^{14}CO_2$ or $^{3}H_2O$ in a Packard Oxidizer Model 306.

DISCUSSION

The conditions for the estimation of RNA by the Schmidt-Thannhauser method have been clearly established for liver. Hydrolysis for 1 hr with 0.3 N KOH is sufficient for solubilization of RNA, but only negligible amounts of polypeptide material are extracted. Comparable values are obtained by either UV or orcinol estimations. However, for other tissues corrections may be required for the extraction of non-RNA UV absorbing material. Fleck and Begg (5) found that the estimation of calf thyroid RNA based on $A_{260\ nm}$ would be in error by 12% without a two wavelength correction for the presence of protein in the RNA fraction. In our studies, without a correction, the amount of RNA was estimated to be 5 to 6% more after PCA precipitation than after precipitation by CTAB (Table 1). However, if we used the formula suggested by Balázs and Cocks (15), less than 2% discrepancy was found. However, by either method of precipitation, higher values were obtained using the orcinol reaction.

Numerous conditions have been reported for the extraction and solubilization of DNA [Munro and Fleck (2)]. Conditions must be sufficiently rigorous for completeness of extraction and hydrolysis, yet mild enough to ensure the stability of the deoxyribonucleotides. Wannemacher et al. (3) extracted DNA from various organs by heating at 96°C in 0.5 N PCA for 20 min. Balázs and Cocks (15) found maximal extraction of brain DNA after 20 min at 70°C in N PCA, but lower extraction in 0.6 N and 1.6 N PCA. Hutchison (16) extracted liver for two 20-min periods and found complete recovery from isolated nuclei, but only 85% from tissue homogenates. Our data indicate that 96°C is clearly inappropriate for the estimation of DNA. We found a single extraction with N PCA at 70°C sufficient for

DNA by the CTAB method (but not by the PCA method), due to the more facile extraction of DNA from tissue freed of extraneous material, as Hutchison found with isolated nuclei from liver.

Since the CTAB method results in a negligible amount of protein in the DNA fraction (Table 2), the $A_{266\ nm}$ and Burton diphenylamine assay (11) gave very similar values for DNA content of rat brain and liver, as well as the mouse fibroblast cell culture. Hydrolyzed calf thymus DNA gave an absorbance of approximately 1.0 at 266 nm for 44 μg DNA/ml. This correspondence was linear for DNA concentrations from 10 to 150 μg/ml, and was limited at high DNA concentrations by the non-linearity of the Burton reaction.

Zamenhof *et al.* (17) have reported that the original diphenylamine method gave low, inconsistent values, and that the developing color was purple instead of blue [see also Santen (4)]. We have used the Burton modification of the diphenylamine method (11) and have noticed that the early developed chromophore has an absorption maximum at 570 nm for calf thymus DNA and rat brain DNA prepared by both methods. As the color intensity increases, the maximum shifts, and after 8 hr when the color is 92% of maximal intensity, the absorption maximum is 597 nm. At this time a secondary peak appears at 460 nm; at 24 hr it is 32% of the major peak.

Table 5 summarizes some representative RNA and DNA values for rat brain from various reports. It is difficult to make reliable comparisons, since the nucleic acid levels vary with age and strain of animal, as well as the anatomical area, and these data are often not noted. However, it is obvious that a wide range of values have been reported.

Frequently, besides the concentration of RNA, the determination of the incorporation of radioactive RNA precursors is desirable. Using

the RNA fraction after precipitation of proteins and DNA, followed by reduction of the perchlorate concentration by neutralization with KOH. However, this neutralization step is time consuming if many samples are to be analyzed, and the resultant counting efficiency is low, due to quenching by perchlorate, if a sizable aliquot of the neutralized sample is used. The present method circumvents these drawbacks.

ACKNOWLEDGMENTS

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Table 1

Characteristics of the RNA Fraction Obtained after Precipitation of Brain by PCA or by Various Concentrations of CTAB

	Precipitant									
	PCA	% CTAB								
	0.2 N	.025	.050	.075	0.10	0.25	0.5	1	2	3
Protein (μg)	236	268	190	141	110	84	54	47	42	45
λ_{min} (nm)	234	235	235	234	234	233	232	232	232	231
$\lambda_{max/min}$	2.5	2.1	2.2	2.2	2.3	2.8	3.1	3.1	3.1	3.1
RNA content*										
by UV	105	115	114	112	106	101	99	100	98	94
by orcinol	130	137	141	135	125	121	118	117	120	114

*RNA content is expressed as % of value for RNA precipitated by 1% CTAB. The RNA content at 1% CTAB is 152 μg/100 mg cerebrum, and 160 μg/100 mg using PCA. Protein content is expressed in μg/100 mg cerebrum.

Table 2

Characteristics of the RNA Fraction of Torula RNA and of RNA Obtained from Liver and 3T3 Cells by Precipitation with PCA and CTAB

	Torula	Liver		3T3	
		0.2 N PCA	1% CTAB	0.2 N PCA	1% CTAB
λ_{min} (nm)	230	232	231	233	231
$\lambda_{max/min}$	3.5	3.1	3.3	2.8	3.3
RNA content*					
by UV	-	668 ± 15	661 ± 10	14.8	15.1
by orcinol	-	648 ± 21	665 ± 18	-	-

*The average values with standard deviation are given for 10 replicate analyses of one liver homogenate. The content for liver is expressed in terms of µg/100 mg of tissue. The values for 3T3 cells represent one analysis by each method of approximately 10^6 cells, and is expressed in µg/approximately 10^6 cells.

Table 3

Characteristics of the DNA Fraction Obtained after Precipitation of Brain by PCA or by Various Concentrations of CTAB

	Precipitant									
	PCA	% CTAB								
	0.2 N	.025	.050	.074	0.10	0.25	0.5	1	2	3
Protein (µg)	291	258	232	237	207	104	64	55	53	54
λ_{min} (nm)	238	237	237	236	236	233	232	231	-	231
$\lambda_{max/min}$	1.5	1.4	1.6	1.7	1.7	2.0	2.2	2.2	-	2.2
DNA content*										
by Burton	91	96	95	95	96	98	99	100	102	104
by UV	125	123	108	108	106	101	99	99	107	103

*DNA content is expressed as % of value for DNA precipitated by 1% CTAB. The DNA content at 1% CTAB is 101.6 µg/100 mg cerebrum. Protein content is expressed as µg protein/100 mg cerebrum.

Table 4

Characteristics of the DNA Fraction of Calf Thymus DNA and of Liver and 3T3 Cells after Precipitation by PCA and CTAB

	Calf Thymus	Liver		3T3	
		0.2 N PCA	1% CTAB	0.2 N PCA	1% CTAB
λ_{min} (nm)	231	234	231	233	231
$\lambda_{max/min}$	2.7	2.0	2.4	2.2	2.7
DNA Content*					
by Burton	-	146 ± 3	195 ± 4	12.6	12.7
by UV	-	224 ± 6	200 ± 4	11.6	13.7

*The average values with standard deviation are given for 10 replicate analyses of one liver homogenate. DNA content for liver is expressed as µg/100 mg tissue and for 3T3 cells as µg/10^6 cells.

Table 5

Comparison of Reported RNA and DNA Values of Rat Brain for Several Ages

Reference	μg/100 mg wet weight of tissue RNA	DNA	Strain	Brain area	Age (days)
Brasel et al. (19)	-	172	Sprague Dawley	Whole brain	20
Geel et al. (18)	176	85	Long Evans	Cortex	25
Present study	193	97	S_1 male	Cortex	30
	191	115		Whole brain minus Ce & Med	30
	225	484		Cerebellum (Ce) + Medulla (Med)	30
	200	200		Whole brain	30
Ferchmin et al. (20)	212	-	"Albino"	Whole brain minus Ce & Med	32
Balázs et al. (15)	13.6*	6.7*	Carshalton	Brain	35
Brasel et al. (19)	-	125	Sprague Dawley	Whole brain	44
Santen et al. (4)	22.0*	16.9*	Holtzman	Whole brain	Young adult
May et al. (21)	155	70	Not described	Cerebral cortex	Cortex weighed 300-450 mg
Mandel et al. (22)	14.8*	8.35*	Not described	Cerebral gray	Adult
	11.1*	8.5*		Brain	
Zamenhof et al. (17)	-	70	Sherman	Whole brain	3 months
Zamenhof et al. (23)	-	72	Sprague Dawley	Whole brain minus Ce	3 months
Ferchmin et al. (20)	163	-	"Albino"	Whole brain minus Ce & Med	90
Rosenzweig et al. (24)	141	67	S_1 male	Cortex	100
	131	119		Whole brain	100

(Continued)

Table 5 (Cont.)

Reference	µg/100 mg wet weight of tissue RNA	DNA	Strain	Brain area	Age (days)
Present study	146	85	S_1 male	Cortex	105
	142	100		Whole brain minus Ce & Med	105
	147	370		Cerebellum (Ce) + Medulla (Med)	105
	143	170		Whole brain	105

*These results are expressed in terms of RNA-P or DNA-P (in µg P/100 mg wet weight of tissue). It is difficult to convert to µg RNA because various workers have used conversion values ranging from 10 to 13 [Chargaff (25), Santen (4), and others].

FIGURE CAPTIONS

Fig. 1. Time course of hydrolysis of Torula RNA (□ — - —□) and of the RNA precipitated from rat brain by CTAB (o——o) or PCA (Δ-----Δ). Apparent RNA was estimated by absorbance at 260 nm. To convert to apparent μg RNA/100 mg, the absorbance values should be multiplied by the volume of the fraction--5 ml--and by the absorbance of RNA--32 μg RNA/od_{260}. Protein in the RNA fraction from rat brain precipitated by CTAB (●——●) and by PCA (▲-----▲) was determined by the Lowry method.

Fig. 2. Spectra of the 60 min, 0.3 N KOH (37°C) hydrolysate after acidification--the RNA fraction--obtained from rat brain precipitated by CTAB (————), and by PCA (--------). Also shown is the spectra obtained upon hydrolysis of Torula RNA under similar conditions (—— - ——).

Fig. 3. The time course of hydrolysis at 70°C in 1 N PCA of rat brain DNA isolated by CTAB (o——o), and by PCA (Δ-----Δ). Calf thymus DNA was hydrolyzed at 70°C in 1 N PCA (□—— - ——□) and at 96°C in 0.5 N PCA (x—— - ——x). After hydrolysis for the indicated time, the sample was chilled, centrifuged, anc the supernatant analyzed by the diphenylamine reaction.

Fig. 4. Time course of hydrolysis of DNA precipitated by CTAB (o——o) or PCA (Δ-----Δ). Apparent hydrolysis of DNA was estimated by absorbance at 266 nm. Protein in the DNA fraction from rat brain precipitated by CTAB (●——●) and by PCA (▲-----▲) was determined by the Lowry method.

Fig. 5. Spectra of the 20 min, 1 N PCA (70°C) hydrolysate obtained from rat brain precipitated by CTAB (————), and by PCA (------). Also shown is the spectra obtained upon hydrolysis of calf thymus DNA under similar conditions (—— - ——).

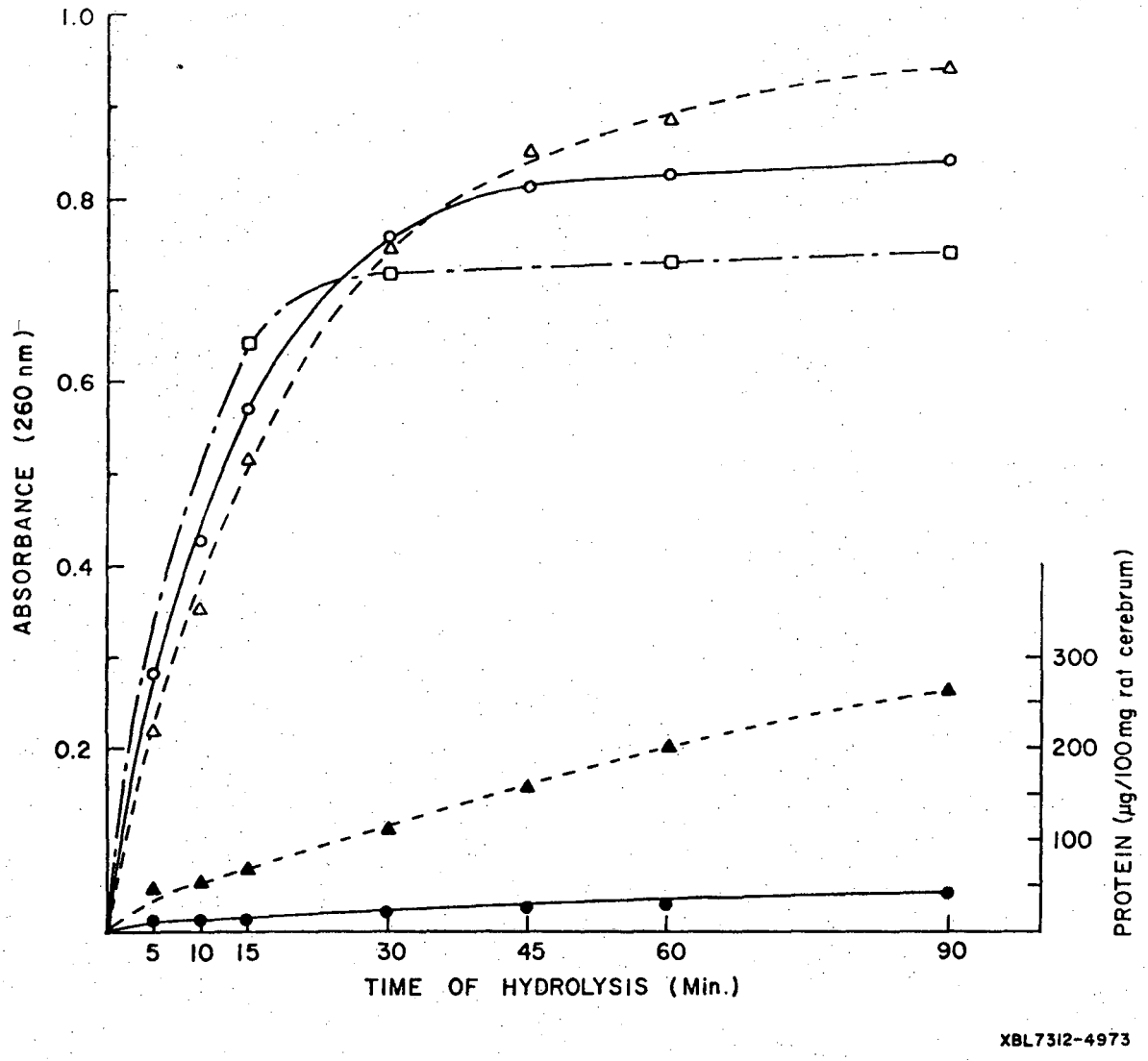
1.0
0.8
0.6
0.4
0.2
ABSORBANCE (260 nm)
300
200
100
PROTEIN (µg/100 mg rat cerebrum)
5
10
15
30
45
60
90
TIME OF HYDROLYSIS (Min.)
XBL7312-4973

Fig. 1.

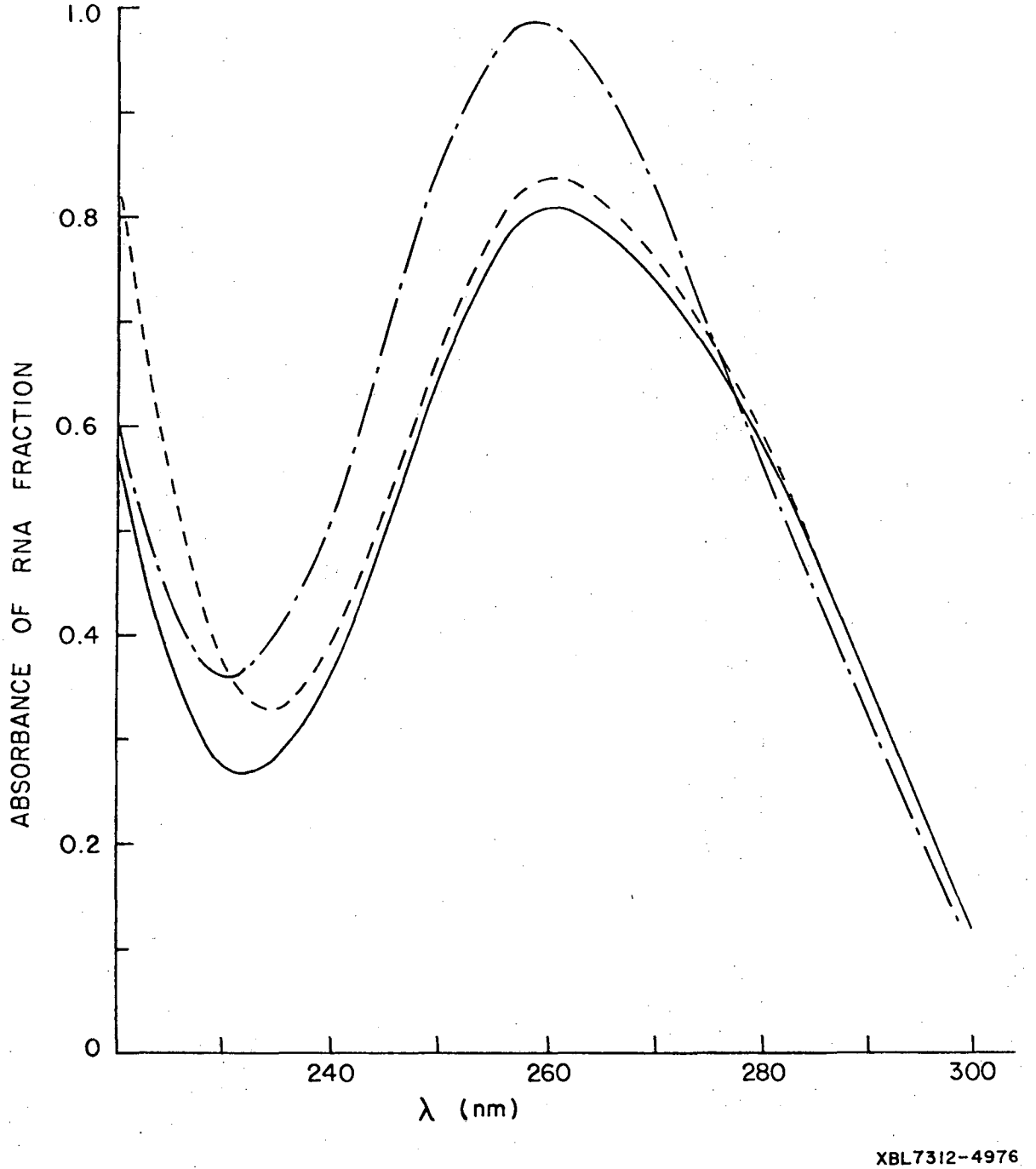
1.0
0.8
0.6
0.4
0.2
0
ABSORBANCE OF RNA FRACTION
240
260
280
300
λ (nm)
XBL7312-4976

Fig. 2.

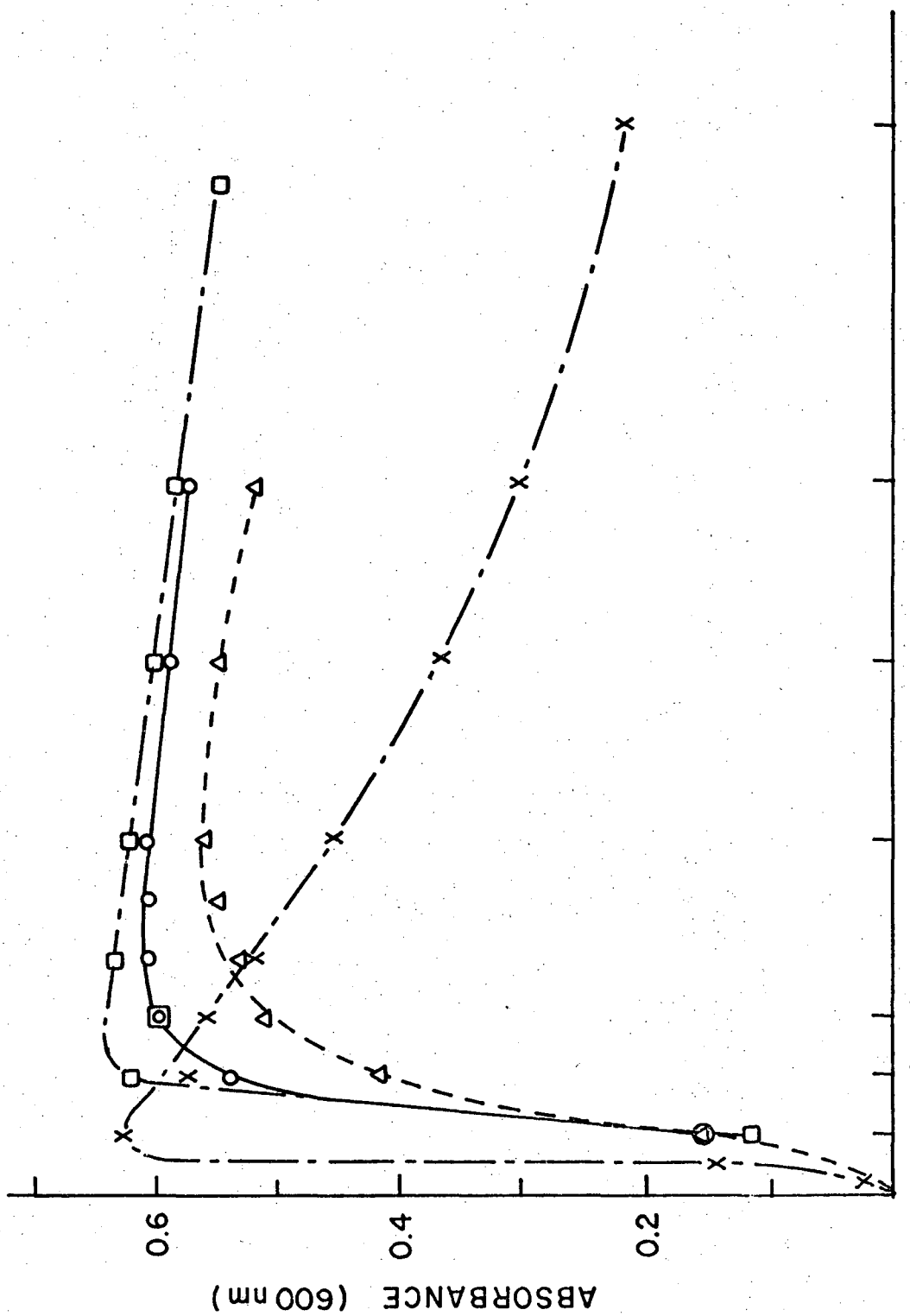
ABSORBANCE (600 nm)
0.6
0.4
0.2
5
10
15
30
45
60
90
TIME OF HYDROLYSIS (Min.)
XBL7312-4974

Fig. 3.

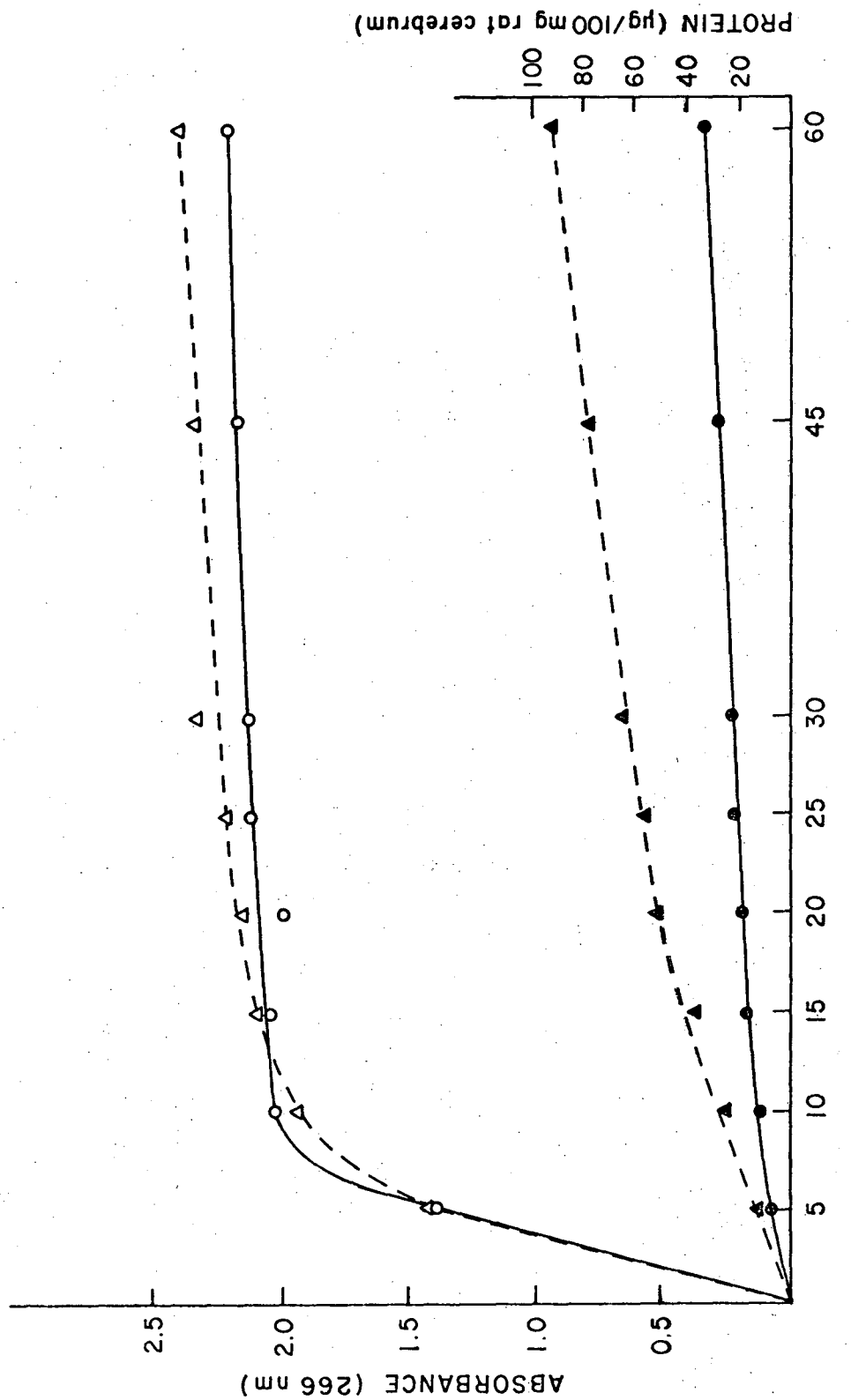
ABSORBANCE (266 nm)
2.5
2.0
1.5
1.0
0.5
TIME OF HYDROLYSIS (Min.)
5
10
15
20
25
30
45
60
PROTEIN (μg/100 mg rat cerebrum)
100
80
60
40
20
XBL7312-4972

Fig. 4.

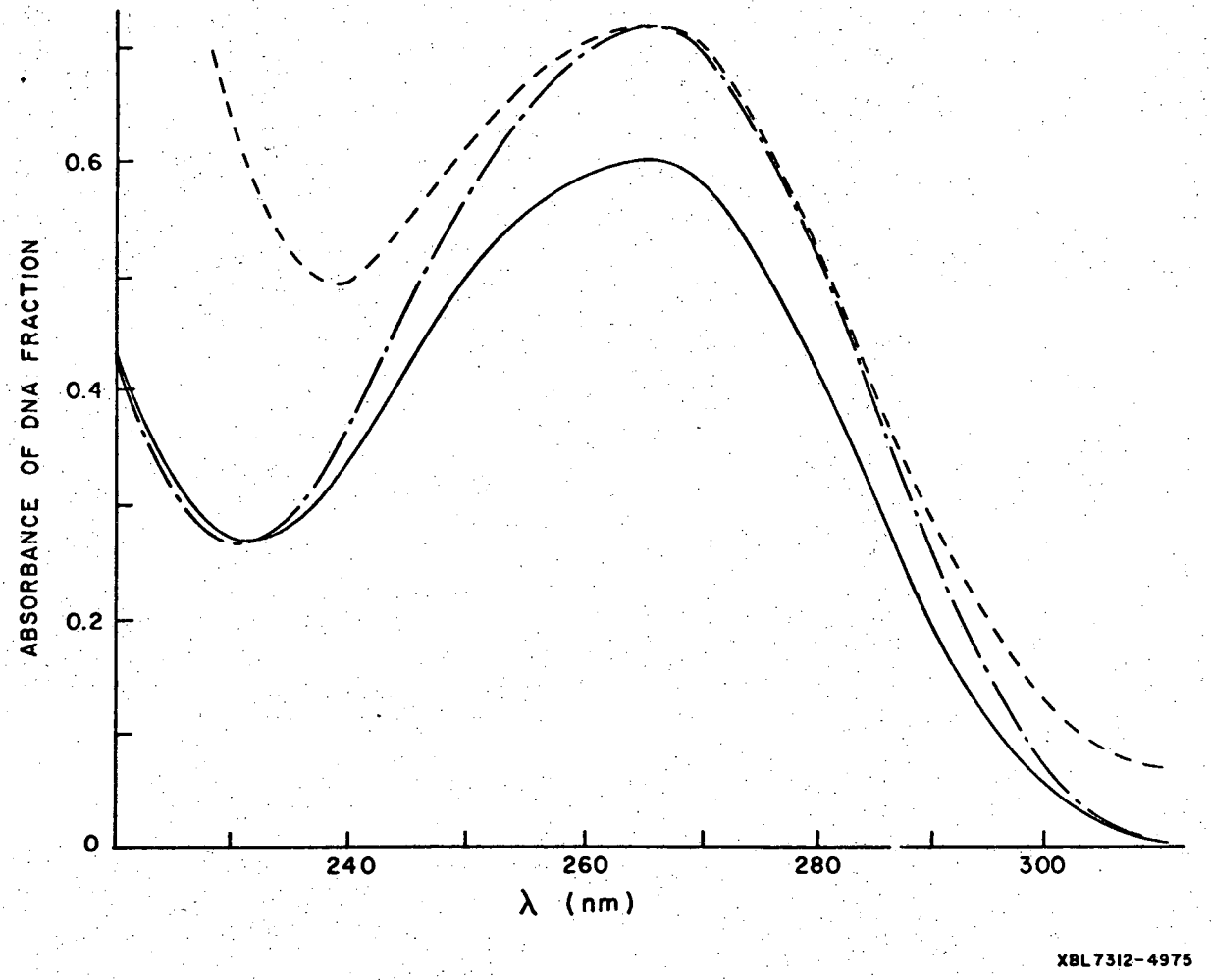
ABSORBANCE OF DNA FRACTION
0.6
0.4
0.2
0
240
260
280
300
λ (nm)
XBL7312-4975

Fig. 5.

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