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SIMPLE METHOD FOR THE SIMULTANEOUS DETERMINATION OF HISTAMINE, POLYAMINES AND HISTONE H1

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SUMMARY

Histamine, putrescine, spermidine, spermine and histone H1 in tissue extracts were separated step by step on a small cellulose phosphate column (3 × 0.6 cm). Borate buffers (pH 8.5) of different ionic strength were used for the separation. Histamine was determined fluorimetrically by reaction with *o*-phthalaldehyde. Other substances were determined spectrophotometrically by reaction with 2,4,6-trinitrobenzene sulphonate or fluorimetrically by reaction with fluorescamine. Quantitative determinations can be carried out on 0.1 nmol of histamine, about 1 nmol of polyamines and 0.5 μg of histone H1 by fluorimetry, and 10–20 nmol of polyamines and 50 μg of histone H1 by spectrophotometry. The method is simple, does not require costly equipment and allows the analysis of many samples simultaneously.

INTRODUCTION

Numerous studies have shown that polyamines interact with nucleic acids and that polyamines have a variety of stimulatory effects on DNA, RNA and protein syntheses[1,2]. On the other hand, histones, which are complexed with DNA and support the structure of DNA, have been considered to supress activity in the transcription of DNA[3,4]. In spite of these interesting opposing functions of polyamines and histones, there have been few studies on the relationship between polyamines and histones. A role has also been suggested for histamine in rapidly growing tissues such as foetal and wound tissues and a number of tumours[5,6]. Hence the simple and simultaneous determination of these substances, which are interesting factors in the processes of growth, development and pathological state, would be of value.

Among several types of histones, selective solubilization of histone H1 with 10% trichloroacetic acid was reported by Kinkade and Cole[7]. I found that histone H1 can be selectively solubilized also by 0.4 *M* perchloric acid. In this paper, a simple and rapid method for the simultaneous determination of histamine, polyamines and histone H1 in animal tissues is described. A method for the separation of histamine and polyamines on a CM-cellulose column (10 × 0.6 cm) was reported previously[8]; the method presented here has some advantages over the previous method.

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EXPERIMENTAL

Materials

Cellulose phosphate was obtained from Brown (Berlin, NH, U.S.A.). Calf thymus histone H1 isolated by the method of De Nooij and Westenbrink[9] and calf thymus DNA were obtained from Sigma (St. Louis, MO, U.S.A.). These were used as references without correction for moisture. Other reagents were purchased from Wako (Osaka, Japan). Borate buffer (0.2 *M*) was prepared from 0.2 *M* orthoboric acid and 0.2 *M* sodium carbonate solutions. Phosphate buffer (0.1 *M*) was prepared from 0.1 *M* sodium dihydrogen orthophosphate and 0.1 *M* disodium hydrogen orthophosphate solutions. Male rats (Wistar) and mice(ddI) and redistilled water were used.

Preparation of tissue extracts for cellulose phosphate column chromatography

Animals were decapitated and tissues were removed rapidly and stored in a dry-ice box (1–3 days). The tissues were homogenized in more than 10 volumes of 0.4 *M* perchloric acid. The homogenate (less than 5 ml) was centrifuged (5000 *g*, 5 min). The precipitate was subjected to the DNA assay as described below. The supernatant was adjusted to pH 5–6 with 2 *M* potassium hydroxide solution in an ice-bath with magnetic stirring. The potassium hydroxide solution was added through a fine tube with monitoring with a pH meter. Then the precipitate was removed by centrifugation (5000 *g*, 5 min) and the supernatant was used for chromatography.

Preparation of cellulose phosphate column

The capacity and/or particle size of cellulose phosphate differs depending on the manufacturer. In this study, cellulose phosphate obtained from Brown (capacity 0.99 mequiv./g) was used. The cellulose phosphate was swollen in water and the fine particles were removed by decantation and discarded. Then the exchanger was washed with 0.1 *M* sodium hydroxide solution, water, 0.1 *M* hydrochloric acid and water on a glass filter and stored in water at 2–4°C. After the exchanger had been packed into a column (3 × 0.6 cm), the column was washed with 0.1 *M* sodium hydroxide solution (5 ml) and water (10 ml) and equilibrated with 0.03 *M* phosphate buffer (pH 6.2) by passing 5 ml of the buffer.

Column chromatography

The neutralized supernatant (less than 5 ml) was applied to the column described above. The separation procedure is summarized in Table I. After washing the column with buffers 1, 2 and 3, histamine, putrescine, spermidine, spermine and histone H1 were eluted step by step from the column in this order with the borate buffers containing different amounts of sodium chloride. In the separation of histone H1 only, the column was washed with buffer 1 (3 ml) and buffer 6 (8 ml), then histone H1 was eluted with buffer 7.

Determination of histamine, putrescine, polyamines and histone H1

A portion of each eluate (3 ml), which was diluted with the elution buffer if necessary, was used. Histamine was determined fluorimetrically by reaction with *o*-phthalaldehyde (OPA). Putrescine, polyamines and histone H1 were determined spectrophotometrically by reaction with 2,4,6-trinitrobenzene sulphonate (TNBS) or

TABLE I

SEPARATION PROCEDURE IN CELLULOSE PHOSPHATE COLUMN CHROMATOGRAPHY

Preparation of buffers is described under *Materials*.

Elution step and buffers (NaCl concentration in the buffers)	*Elution volume (ml)*	*Separation*
(1) 0.03 *M* phosphate buffer, pH 6.2	3.0	NH_3, many primary amines, amino
(2) 0.06 phosphate buffer, pH 6.2	5.0	acids and peptides (discarded
(3) 0.1 *M* borate buffer, pH 8.5 (0.025 *M*)	1.5	fraction)*
	3.0	Histamine, 1-methylhistamine
	3.0	for blank
(4) 0.2 *M* borate buffer, pH 8.5 (0.2 *M*)	3.0	Putrescine, cadaverine, agmatine
(5) 0.2 *M* borate buffer, pH 8.5 (0.4 *M*)	8.0	Spermidine
(6) 0.2 *M* borate buffer, pH 8.5 (0.6 *M*)	8.0	Spermine
(7) 0.2 *M* borate buffer, pH 8.5 (0.8 *M*)	5.0	Histone H1

* The following authentic substances were eluted completely in this fraction: tryptophan, 5-methoxytryptophan, tyrosine, phenylalanine, dopa, histidine, lysine, arginine, ornithine, adrenaline, noradrenaline, dopmaine, metanephrine, normetanephrine, tyramine, serotonin, tryptamine, 5-methoxytryptamine, melatonin, glutathione, histidylglycine and carnosine.

fluorimetrically by reaction with fluorescamine (FA). The procedures for these reactions are essentially the same as those described previously[8].

(a) OPA reaction

The sample (3 ml) was mixed with 2 *M* sodium hydroxide solution (0.4 ml), then OPA reagent (0.1 % in methanol, prepared fresh daily) (0.2 ml) was added and mixed with shaking. After the reaction mixture had been allowed to stand for 5 min at room temperature, 3.5 *M* orthophosphoric acid (0.4 ml) was added to the mixture. The fluorescence was measured at 440 nm with excitation at 360 nm.

(b) TNBS reaction

TNBS reagent (100 mg in 150 ml of dimethyl sulphoxide) (1 ml) was added to the sample (3 ml). The reaction was carried out at 50°C for 10 min and terminated by cooling the reaction mixture in water. Then the absorbance at 420 nm ($A_{420}^{1\,cm}$) was measured.

(c) FA reaction

While the sample solution (3 ml) was being vigorously shaken on a vortex mixer, 1 ml of FA in dioxan (15 mg per 100 ml) was added rapidly by means of a syringe at room temperature. The reaction was carried out for about 5 sec. The fluorescence was measured at 475 nm with excitation at 390 nm.

Determination of DNA

The precipitate described above was used for the determination of DNA. The procedure for the extraction of DNA was based on that of Schmidt and Thannhauser[10] and DNA was determined by the diphenylamine reaction as modified by Richards[11] using calf thymus DNA as a reference.

RESULTS

Column chromatography

Many possible interfering substances such as ammonia, amino acids, amines and peptides were eluted from the column in the washing step (Table I). However, the histamine fraction contained a significant amount of unidentified substances in addition to 1-methylhistamine, if present. Therefore, the OPA reaction was used for the determination of histamine, because the reaction is specific and sensitive to histamine[12]. Known interfering substances in the reaction, such as histidine, arginine, glutathione, hystidylglycine, carnosine, agmatine and spermidine, were separated from histamine by chromatography. 1-Methylhistamine (10 nmol in the volume of the reaction mixture) showed no fluorescence in the OPA reaction. Although cadaverine and

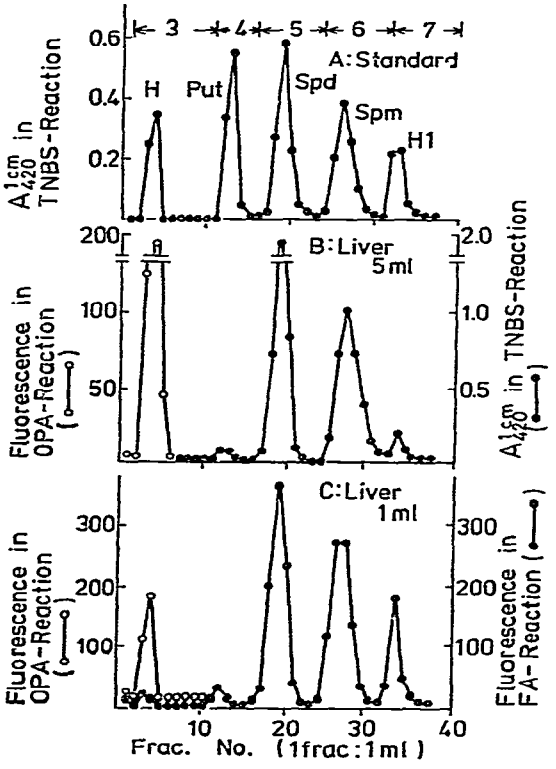

Fig. 1. Separation of histamine, putrescine, polyamines and histone H1 on a cellulose phosphate column. Each substance was eluted step by step with buffers 1–7 (Table I). Fractions (1 ml) were collected, diluted to 3 ml with each elution buffer and subjected to the OPA, TNBS or FA reaction. A, 0.4 *M* $HClO_4$ (4 ml) containing authentic histamine, putrescine, spermidine, spermine (100 nmol each) and calf thymus histone HI (10 nmol) was neutralized with KOH and the supernatant was applied to the column as described under Experimental. B, Neutralized extract from rat liver (5 ml) corresponding to about 0.5 g of the tissue was applied. In the OPA reaction, the control fluorescence intensity was 100 for 1 nmol of histamine (reagent blank: 3). C, The same sample as B (1 ml) corresponding to about 0.1 g of the liver was applied. Control fluorescence intensities in the OPA and FA reactions were 100 for 0.2 nmol of histamine (reagent blank: 14) and 100 for 10 nmol of putrescine (reagent blank: 3), respectively.

TABLE II

AMINO ACID ANALYSIS OF HISTONE H1 OBTAINED FROM RAT TISSUES BY CELLULOSE PHOSPHATE COLUMN CHROMATOGRAPHY

Histone H1 was eluted from the cellulose phosphate column with a small volume of 0.1 *M* NaOH (1.5 ml) after other amines were eluted. The sample was hydrolysed in 1 *M* $HClO_4$ at 98–100°C for 48 h. The hydrolysed sample was neutralized with KOH to pH 2–3 and its supernatant was subjected to amino acid analysis.

Amino acid	*Amino acid composition (mole-%)*				
	*Reference**	*Thymus*	*Spleen*	*Liver*	*Kidney*
Lysine	23.3	24.4	23.4	22.5	23.4
Histidine	0.0	0.0	0.0	0.0	0.0
Arginine	1.1	1.0	0.8	1.5	1.4
Cysteic acid	0.0	0.0	0.0	0.0	0.0
Aspartic acid	4.1	3.0	2.9	4.2	3.5
Threonine	4.0	3.4	3.4	4.5	4.5
Serine	7.6	7.7	7.9	6.8	7.1
Glutamic acid	5.0	4.5	4.3	5.1	4.5
Proline	4.9	5.2	4.6	4.7	5.0
Glycine	8.6	8.4	8.3	9.7	9.5
Alanine	28.7	29.0	28.7	26.9	27.5
Half-cystine	0.0	0.0	0.0	0.0	0.0
Valine	3.6	3.9	4.2	3.4	3.5
Methionine	0.0	0.0	0.0	0.0	0.0
Isoleucine	Trace	Trace	Trace	Trace	Trace
Leucine	5.5	5.7	5.6	5.7	5.9
Tyrosine	1.8	2.0	2.7	2.4	2.1
Phenylalanine	2.0	1.8	3.2	2.7	2.1

* Calf thymus histone H1 obtained from Sigma.

agmatine were eluted together with putrescine, neither amine is present in detectable amounts in animal tissues[13–15]. Fig. 1 shows the separation patterns of histamine, putrescine, polyamines and histone H1. Each substance was completely separated from others. The base values (values in the fractions not containing these substances) were almost the same as those of the reagent blank in both the OPA and TNBS reactions. In the FA reaction, the base values were usually higher than that of the reagent blank. Therefore, the value of the fraction for the blank (see Table I) was subtracted from the values of the fractions of putrescine, polyamines and histone H1 in the FA and TNBS reactions.

Amino acid analysis of histone H1

When 0.1 *M* sodium hydroxide solution was passed through the column after histone H1 had been eluted, no substance that reacts with TNBS was observed in the eluate. Therefore, histone H1 was eluted from the column with a small volume of 0.1 *M* sodium hydroxide solution (1.5 ml) after spermine had been eluted and subjected to the amino acid analysis (Table II). The results indicate that the amino acid composition of histone H1 in rat tissues obtained by the present method is in good agreement with that of calf thymus histone H1. The absence of histidine, cystine and methionine appears to be accepted as evidence for freedom from other proteins and other histones. This result is similar to that obtained by Kinkade and Cole[7], who extracted histone H1 directly from calf thymus with 10% (0.6 *M*) trichloroacetic acid.

TABLE III

RELATIVE FLUORESCENCE AND ABSORBANCE OF HISTAMINE, PUTRESCINE, SPERMIDINE, SPERMINE AND HISTONE H1 IN OPA, FA AND TNBS REACTIONS

Amine	*Reaction*	*nmol in reaction mixture*	*Fluorescence intensity*	$A^{*1\,cm}_{420}$ **
Histamine	OPA	1.0	100 (3)*	
		0.2	100 (15)	
Putrescine	FA	5.0	31 (1)	
Spermidine	FA	5.0	38 (1)	
Spermine	FA	5.0	43 (1)	
Histone H1	FA	0.5 (10 μg)	100 (1)	
Putrescine	TNBS	50		0.50 (0.04)*
Spermidine	TNBS	50		0.54 (0.04)
Spermine	TNBS	50		0.51 (0.04)
Histone H1	TNBS	5 (100 μg)		0.34 (0.04)

* Reagent blank.
** Measured using water as a reference.

Relative fluorescence and absorbance of histamine, putrescine, polyamines and histone H1 in OPA, FA and TNBS reactions

Table III shows the relative fluorescence intensities and absorbances of histamine, putrescine, polyamines and histone H1 in each reaction. Calf thymus histone H1 (molecular weight 21,000) was used as a standard.

The absorbances of putrescine and polyamines in the TNBS reaction were similar. The molar absorbance of histone H1 was about seven times higher than those of putrescine and polyamines. Assuming that the absorbance of putrescine and polyamines is derived from the reaction of TNBS with two amino groups in their molecules, it is suggested that about 14 amino groups of a histone molecule react with TNBS. Similarly, the molar fluorescence intensity of histone H1 in the FA reaction is more than 25 times higher than those of putrescine and polyamines, suggesting that more than 50 amino groups (*i.e.*, most of the lysine residues) of histone H1 react with FA.

Fluorescence intensities of FA derivatives of putrescine and polyamines and their pH dependence have been reported by Veening *et al.*[16]. They reported that the fluorescence intensities of the FA derivatives of spermidine and spermine were markedly lower than that of putrescine (less than 30%). Similar results were also observed in a previous study[8], whereas in this study the fluorescence intensities of the FA derivatives of polyamines were higher than that of putrescine. This difference can be explained by the difference in the ionic strength in the reaction mixture, *i.e.*, larger amounts of sodium chloride in the reaction mixture enhanced the fluorescence intensities of the FA derivatives of polyamines.

TABLE IV

SENSITIVITY, LINEARITY AND STABILITY

Amine	*Reaction*	*Sensitivity (nmol)* *	*Linearity (nmol)* **	*Stability (h)* ***
Histamine	OPA	0.15	0–100	1
Putrescine	FA	0.9	0–50	3
Spermidine	FA	0.7	0–50	3
Spermine	FA	0.6	0–50	3
Histone H1	FA	0.025 (0.5 μg)	0–1	1
Putrescine	TNBS	22	0–150	1
Spermidine	TNBS	20	0–150	1
Spermine	TNBS	21	0–150	1
Histone H1	TNBS	3.3 (70 μg)	0–30	1

* Amount of amines that gave a fluorescence intensity or absorbance 5 times higher than that of the reagent blank.

** Range of concentration of amines that gave a linear relationship between fluorescence intensity or absorbance and the concentration of amines.

*** Minimum time during which the fluorescence intensity or absorbance remained constant.

Sensitivity, linearity and recovery

Table IV shows the sensitivity and linearity of the proposed method. The calibration graphs for calf thymus histone H1 in the TNBS and FA reactions are shown in Fig. 2 as an example. When tissue extracts contain each substance in an amount greater than the sensitivity shown in Table IV, then the substances can be determined accurately. The stability of the products produced by the OPA, FA and TNBS reactions is also shown in Table IV. Various amounts of histamine, putrescine, polyamines and calf thymus histone H1 were added to each 5 ml of homogenate of rat liver (50 mg). The mixture was subjected to chromatography and each substance was determined. A linear relationship was observed between the amount of each substance recovered and that added. The recoveries of each amine and histone H1 were more than 90% and 85%, respectively.

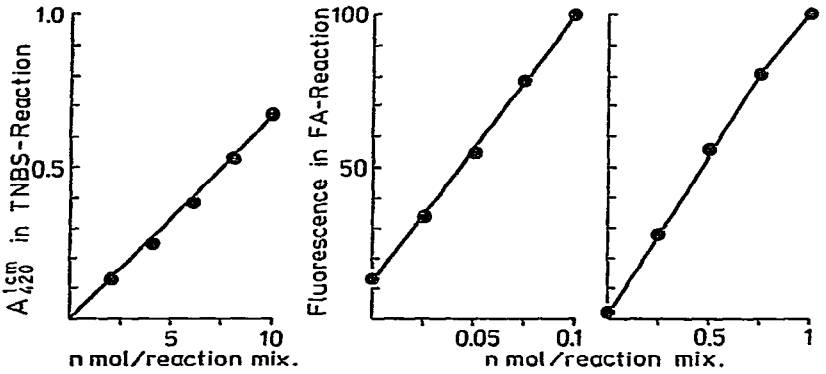

Fig. 2. Calibration graphs for calf thymus histone H1 in the TNBS and FA reactions.

Levels of histamine, putrescine, polyamines, histone H1 and DNA in rat tissues

In Table V the levels of histamine, putrescine, polyamines, histone H1 and DNA in the tissues of rats weighing about 120 g (37 days old) and 200 g (58 days old) determined by the proposed method are given. Histamine was determined by the OPA reaction and the other substances by the TNBS reaction. The values were compared with published data determined by various methods as follows: enzymatic isotopic assay for histamine by Beaven *et al.*[17] and for putrescine by Harik *et al.*[15], high-performance liquid chromatography for putrescine and polyamines by Marton *et al.*[14], thin-layer chromatography for polyamines by Abe and Samejima[18], paper electrophoresis for putrescine and polyamines by Inoue and Mizutani[19] and Jänne *et al.*[20,21] and my previous method based on CM-cellulose column chromatography[8] for histamine, putrescine and polyamines. The values for histamine, putrescine and polyamines obtained in this work were generally within the range of those reported by other investigators, whereas the values for putrescine in the kidney were higher than those obtained by others. In the experiment using a longer column or mild conditions for the elution of putrescine, an unknown substance was observed in the putrescine fractions obtained from the kidney, liver and brain. This unknown substance, which is present in a significant amount in the kidney, disappeared when the tissue extracts were incubated in 1 *M* perchloric acid at 98–100°C for 12 h.

With respect to histone H1, comparable published data could not be found. Therefore, the contents of histone H1 were compared with those of DNA. The DNA

TABLE V

LEVELS OF HISTAMINE, PUTRESCINE, POLYAMINES, HISTONE H1 AND DNA IN RAT TISSUES

Mean values from 3 rats.

Tissue	*Histamine (nmol/g)*		*Putrescine (nmol/g)*		*Spermidine (nmol/g)*		*Spermine (nmol/g)*	
	37 days old	*58 days old*	*37 days old*	*58 days old*	*37 days old*	*58 days old*	*37 days old*	*58 days old*
Brain	0.53	0.55	21	23	366	352	187	175
Liver	4.03	6.06	43	25	936	686	624	615
Kidney	2.47	2.87	78	65	419	361	575	573
Spleen	9.03	25.8	69	56	1075	966	623	636
Thymus	78.3	198	153	103	2290	1859	635	581

	Histone H1 (nmol/g)		*DNA (mg/g)*		*H1/DNA ratio*		*DNA data from literature*	
	37 days old	*58 days old*	*37 days old*	*58 days old*	*37 days old*	*58 days old*	*mg P/g*	*mg/g*
Brain	4.0	3.5	1.1	1.1	3.6	3.2	0.12*; 0.15**	1.0***
Liver	11.8	8.6	1.6	1.4	7.4	6.1	0.25; 0.22	3.0
Kidney	21.5	16.1	3.1	2.7	6.9	6.0	0.38; 0.34	
Spleen	83.7	83.2	12.3	11.4	6.8	7.3	1.29	
Thymus	172	134	27.9	24.9	6.2	5.4	2.64	

* Schneider and Klug[22].

** Schmidt and Thannhauser[10].

*** Karsten and Wollenberger[23].

values determined in the present study are very similar to those reported by other investigators (shown in Table V). The ratios of histone H1 to DNA indicate that histone H1 is correlated well with DNA levels in the tissues considered except for the brain. It is known that in the determination of DNA in the brain, contamination by sugars derived from a large amount of glycoproteins interferes in determinations by the diphenylamine method. Karsten and Wollenberger[23] reported a method for the determination of DNA that included pronase treatment in the procedure. They reported that the DNA content of the rat brain is one third that of the liver. This ratio agrees with that of histone H1 in the brain and liver. The contents of histone H1 in tissues of mice (35 days old) were as follows; brain, 7.3; liver, 20; kidney; 27; spleen, 190; and thymus, 223 nmol/g. These values are significantly higher than those in rats but their proportions in the various tissues are nearly parallel to those in rats.

DISCUSSION

The chromatin of eukaryotes contains nearly equal weights of histone and DNA. Among the five main types of histones, there is one H1 for two each of the other histones. This molecular composition is nearly invariant among different organisms, and these histones are complexed with DNA and support the structure of DNA[3,24]. It has been also shown that histone synthesis begins in a phase with DNA synthesis and ends with the completion of DNA synthesis[25,26]. These results indicate that the content of histone is parallel to that of DNA among animal tissues. Of the five main types of histones, histone H1 was selectively determined by the present method. The amino acid composition of histone H1 obtained by the present method was in good agreement with that of calf thymus histone H1, and the contents of histone H1 among various rat tissues were parallel to those of DNA. These results indicate that the values for histone H1 obtained by the present method are reasonable.

Many methods for the determination of putrescine and/or polyamines have been developed and were compared by Marton *et al.*[14] and Harik *et al.*[15]. The methods of Marton *et al.*[14,27] are highly sensitive. The sensitivity of the present method is similar to those of other methods[13,16,18,19,28]. In general, however, the previous methods are tedious, time consuming, unsuitable for a large number of samples and expensive. The method described in this paper is a simple one in which common instruments are used and allows the analysis of many samples simultaneously; this is the first method for the simultaneous determination of histone H1, histamine, putrescine, spermidine and spermine.

A method was reported previously for the separation of histamine and polyamines on a CM-cellulose column (10 × 0.6 cm)[8]. The present method has some advantages over the previous one: (i) each amine is eluted from the column with a smaller volume of buffer; (ii) the separation procedure can be carried out without the use of a fraction collector; therefore, (iii) it is possible to analyse many samples simultaneously; (iv) higher fluorescence intensities of spermidine and spermine in the FA reaction (2–3 times) were obtained by the use of elution buffers containing larger amounts of sodium chloride; (v) a lower base value (or reagent blank) in the TNBS reaction was obtained by the use of elution buffers of lower pH.

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REFERENCES

1 S. S. Cohen, *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs, NJ, 1971.
2 C. W. Tabor and H. Tabor, *Annu. Rev. Biochem.*, 45 (1976) 285.
3 R. D. Kornberg, *Annu. Rev. Biochem.*, 46 (1977) 931.
4 V. G. Allfrey, in D. M. P. Phillips (Editor), *Histones and Nucleohistones*, Pleunm Press, London, New York, 1971, p. 241.
5 G. Kahlson and E. Rosengren, *Physiol. Rev.*, 48 (1968) 155.
6 D. Russell and S. H. Snyder, *Proc. Nat. Acad. Sci. U.S.*, 60 (1968) 1420.
7 J. M. Kinkade, Jr., and R. D. Cole, *J. Biol. Chem.*, 241 (1966) 5790.
8 Y. Endo, *Anal. Biochem.*, 89 (1978) 235.
9 E. H. De Nooij and H. G. K. Westenbrink, *Biochim. Biophys. Acta*, 62 (1962) 608.
10 G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.*, 161 (1945) 83.
11 G. M. Richards, *Anal. Biochem.*, 57 (1974) 369.
12 P. A. Shore, A. Burkhalter and V. H. Cohn, Jr., *J. Pharmacol. Exp. Ther.*, 127 (1959) 182.
13 H. Tabor and C. W. Tabor, *Anal. Biochem.*, 55 (1973) 457.
14 L. J. Marton, O. Heby, C. B. Wilson and P. L. Y. Lee, *FEBS Lett.*, 41 (1974) 99.
15 S. I. Harik, G. W. Pasternak and S. H. Snyder, *Biochim. Biophys. Acta*, 304 (1973) 753.
16 H. Veening, W. W. Pitt, Jr., and G. Jones, Jr., *J. Chromatogr.*, 90 (1974) 129.
17 M. A. Beaven, S. Jacobsen and Z. Horáková, *Clin. Chim. Acta*, 37 (1972) 91.
18 F. Abe and K. Samejima, *Anal. Biochem.*, 67 (1975) 298.
19 H. Inoue and A. Mizutani, *Anal. Biochem.*, 56 (1973) 408.
20 J. Jänne, A. Raina and M. Siimes, *Acta Physiol. Scand.*, 62 (1964) 352.
21 J. Jänne, *Acta Physiol. Scand.*, Suppl., 300 (1967) 1.
22 W. C. Schneider and H. L. Klug, *Cancer Res.*, 6 (1964) 691.
26 U. Karsten and A. Wollenberger, *Anal. Biochem.*, 46 (1972) 135.
24 R. D. Kornberg, *Science*, 184 (1974) 868.
25 D. M. Prescott, *J. Cell Biol.*, 31 (1966) 1.
26 T. W. Borun, M. D. Scharff and E. Robins, *Proc. Nat. Acad. Sci. U.S.*, 58 (1967) 1977.
27 L. J. Marton and P. L. Y. Lee, *Clin. Chem.*, 21 (1975) 1721.
28 C. W. Gehrke, K. C. Kuo, R. W. Zumwalt and T. P. Waalkes, *J. Chromatogr.*, 89 (1974) 231.