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Note

Determination of polyamines by ion-exchange thin-layer chromatography and video-densitometry

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By the combination of thin-layer ion-exchange chromatography with video-densitometry [1, 2] several methods have recently been developed for the determination of amino acids and for the measurement of the activity of enzymes catalyzing amino acid transformation.

Since amino acids can be readily separated from polyamines by ion-exchange column chromatography [3,4], a thin-layer ion-exchange technique has been developed for the determination of basic amino acids and natural polyamines. Combined with video-densitometry [5] the method is suitable for the quantitative determination of polyamines in tissue extracts without any previous purification.

EXPERIMENTAL

Rat tissue samples were homogenized with a Potter-type Teflon homogenizer immediately after removal from the animals in 3 volumes (w/v) of 10% trichloroacetic acid, then centrifuged for 10 min at 5000 *g*. The clear supernatant was used for the determination of polyamines.

Tissue extracts of 20—40 μl were applied to Fixion 50X8 ion-exchange thin-layer chromato-sheets (Na^+) (Chinoin, Budapest, Hungary and Chromatronix, Palo Alto, CA, U.S.A.). As a reference 1—10-μl samples of a stock solution containing 50 nmole/l putrescine·2HCl, 20 nmole/l spermidine·3HCl, 10 nmole/l spermidine·4HCl, 25 nmole/l ornithine and 25 nmole/l arginine were also applied to the chromato-sheets. All the amino acids and polyamines were purchased from Sigma (Heidelberg, G.F.R.). The chromato-sheets were then run for about 4 h at room temperature in a solvent composed of 200 mmole/l potassium hydrogen phosphate and 2 mole/l sodium chloride. The pH of the solution was adjusted to 7.5 with sodium hydroxide. Trichloroacetic acid present in the

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extract does not alter the results of the determinations, because it runs to the top of the sheet. After chromatography the sheets were dried and developed with ninhydrin reagent containing cadmium acetate [6]. The concentration of polyamines and amino acids was then determined by measuring the density of the ninhydrin spots [1] in a Telechrom-S OE 976 video-densitometer (Chinoin). The detector of the equipment is a vidicon tube which performs electronically the three-dimensional integration of an illuminated chromatogram. In this process the x and y axes correspond to the geometry of the spot, whereas the z axis corresponds to the density. Thus, the density count of a large faint spot may be identical with that of a small darker spot.

RESULTS AND DISCUSSION

As it is seen in Fig. 1, the basic amino acids and natural polyamines (putrescine, spermidine and spermine) are readily separated on the cation-exchange chromato-sheet. The R_F values of the compounds are listed in Table I.

Resolution could be improved by over-run chromatography, i.e. by placing a filter paper strip horizontally on the top of the plate over its whole length. Care had to be taken for smooth contact of the paper and the plate. The optimal running time for the determination of natural polyamines was 8—10 h, but in this case the basic amino acids run off the sheet.

TABLE I

CONCENTRATION OF POLYAMINES IN RAT TISSUES

The data were controlled by measuring the dansyl derivatives of these compounds by fluorimetry. Less than 10% difference was found between the results of the two methods. Values are the means of 10 samples from CFY rats.

	R_F	Polyamines (nmole/g tissue)		
		Liver	Kidney	Brain
Methylamine	0.75	—	—	—
Ornithine	0.66	85—157	48— 63	33— 40
Arginine	0.38	Trace	108—159	29— 52
Putrescine	0.26	25— 34	11— 17	14— 32
Spermidine	0.11	470—620	390—460	630—750
Agmatine	0.08	—	—	—
Spermine	0.05	410—550	330—410	380—450

Under the conditions described in Experimental, the method is suitable for the separation and quantitation of 2—60 nmoles of natural polyamines and basic amino acids. With the original optics of the video-densitometer (ENK TV lens, 12.5 mm, 1:1.3) the error of the determination is within ± 3%. The relationship between concentration and density is illustrated in Fig. 2.

Sensitivity could be increased up to 0.1 nmole of polyamines by using a Fujinon C 6X17.5 zoom optics (Fuji Photo Optical Ltd., Tokyo, Japan) attached to the Bosch TV camera of the equipment. In this case the error increases to about ± 5%.

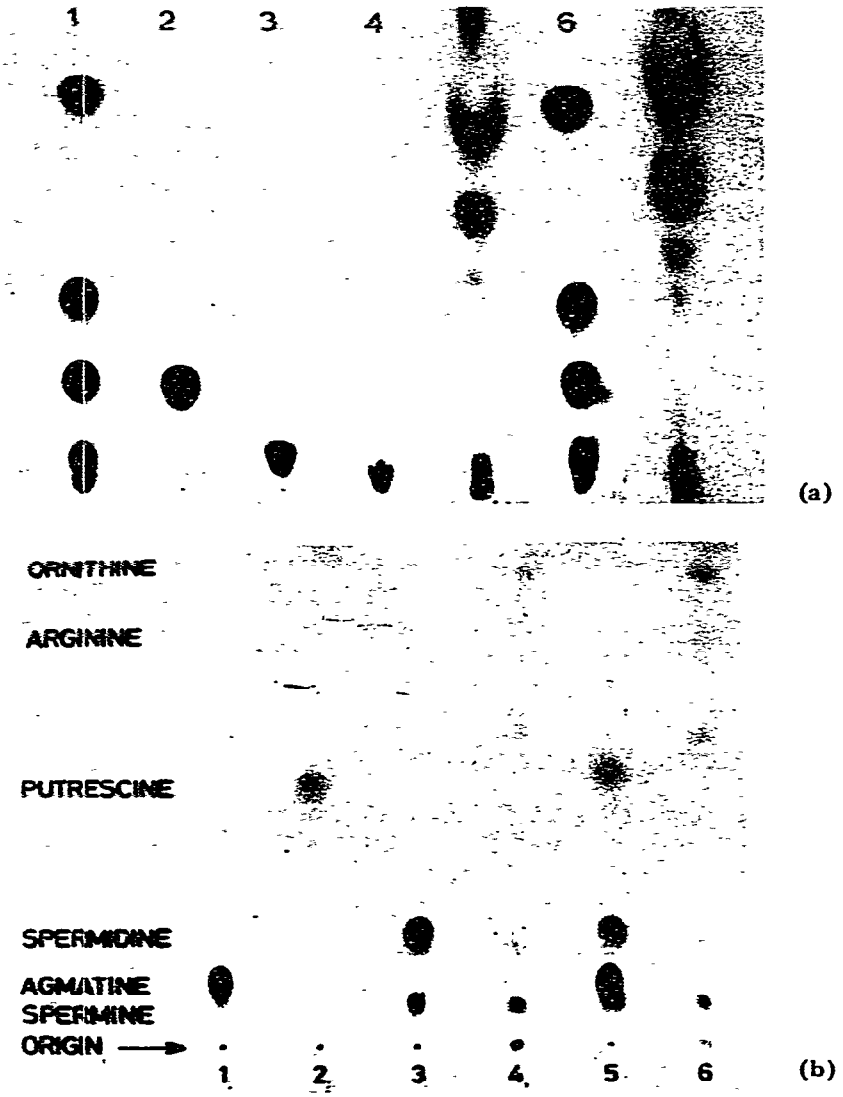

Fig. 1. Ion-exchange chromatography of basic amino acids and natural polyamines from rat liver extracts at room temperature. Solvent: 200 mmol/l potassium phosphate buffer containing 2 mole/l NaCl (pH 7.5). (a) Determination of basic amino acids and polyamines. Running time = 4 h. 1 and 6, mixture of reference compounds; 2, putrescine; 3, spermidine; 4, spermine; 5 and 7, liver extract. (b) Determination of natural polyamines. Running time = 8—10 h. 1, agmatine; 2, ornithine and putrescine; 3, spermidine and spermine; 4 and 6, liver extract; 5, mixture of reference compounds.

The data in Table I demonstrate the results of determining the polyamine content of various rat tissues. The measurements were carried out with individual tissue extracts obtained from ten CFY rats weighing between 150 and 200 g.

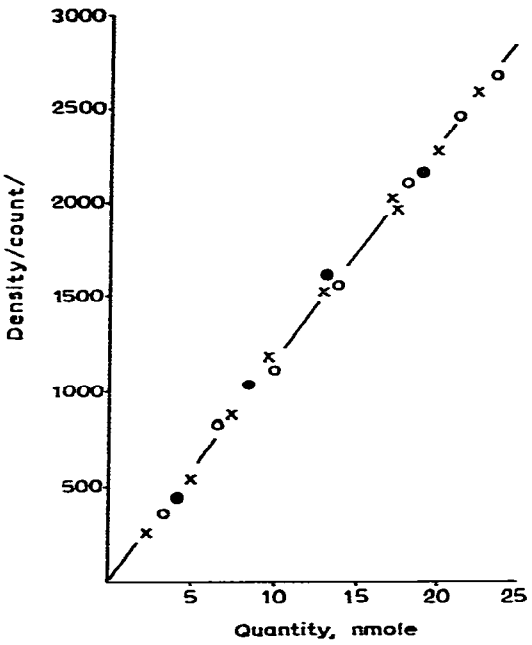

Fig. 2. The change in density of ninhydrin spots of various polyamines as a function of the concentration of polyamine standards. •, putrescine; o, spermidine; x, spermine.

The relatively wide range of these values can be attributed to biological variation, as has also been found in other laboratories [7,8].

Recently a similar method has been published by Pongor and Kramer [9] for the determination of biogenic amines on Fixion 50X8 chromato-sheets.

Our preliminary data convincingly suggest that the analytical technique reported in the present paper may supply satisfactory data both on the diamine and the polyamine content of other tissues than those described here.

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REFERENCES

1 P. Előd i and T. Karsai, J. Liquid Chromatogr., 3 (1980) 809.
2 T. Karsai and P. Előd i, Acta Biochim. Biophys. Acad. Sci. Hung., 14 (1979) 123.
3 J.A. Sturman and G.E. Gaull, Pediatr. Res., 8 (1974) 231.
4 V.R. Villanueva and R.C. Adlakha, Anal. Biochem., 91 (1978) 264.
5 T. Dévényi, Acta Biochim. Biophys. Acad. Sci. Hung., 11 (1976) 1.
6 T. Dévényi, Acta Biochim. Biophys. Acad. Sci. Hung., 5 (1976) 435.
7 S.I. Harik, J. Neurochem., 33 (1979) 1131.
8 L. Selmeci, E. Pösch, G. Simon and L. Hársing, Biochem. Biophys. Res. Commun., 87 (1979) 323.
9 S. Pongor and J. Kramer, J. High Resolut. Chromatogr. Chromatogr. Commun., 3 (1980) 93.