

CHROM. 7845

## Note

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### Fractionation of RNA on a metal ion-equilibrated cation exchanger

#### IV. Chromatographic profiles of RNA: tissue and source variation

V. SHANKAR and P. N. JOSHI

*Department of Chemistry, University of Poona, Poona 411 007 (India)*

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In view of the diverse data existing on the effects of tissue and source variation on the chromatographic behaviour of RNAs from a wide variety of sources<sup>1–4</sup>, employing isolation techniques based on various approaches, it was felt necessary to study the chromatographic behaviour of RNA isolated from different tissues and sources on an IR-120 ( $Al^{3+}$ ) column.

#### EXPERIMENTAL

##### *Profiles of RNA from the same source with variation in tissue*

For these studies, liver, kidney and brain of buffalo were chosen as the starting materials for RNA isolation. RNAs were isolated and deproteinized by the method of Sevag *et al.*<sup>5</sup>. In each instance, the RNA obtained was native and fairly pure.

The preparations obtained were dissolved in acetate buffer (pH 4.0, 0.05 M) and the solutions were chromatographed on an IR-120 ( $Al^{3+}$ ) column as described earlier<sup>6</sup>.

It was observed that all of the RNA preparations were completely retained and quantitatively eluted. The eluted fractions, each of 10 ml, were assayed for their RNA content by the thymol–iron(III) chloride–hydrochloric acid method<sup>7</sup>. The percentage retention of RNA and the percentage elution of the total adsorbed RNA are given in Table I. Fig. 1 shows the elution profiles.

##### *Profiles of RNA from the same tissue with variation in source*

For these studies, RNAs from the livers of buffalo, pigeon and frog were isolated by the method of Sevag *et al.*<sup>5</sup>. The preparations were tested for their purity and nativeness. Homogeneous solutions of these preparations in acetate buffer (pH 4.0, 0.05 M) were then chromatographed on an IR-120 ( $Al^{3+}$ ) column as described earlier<sup>6</sup>. Fractions, each of 10 ml, were assayed for their RNA content by the thymol–iron(III) chloride–hydrochloric acid method<sup>7</sup>.

TABLE I

EFFECT OF TISSUE VARIATION ON THE CHROMATOGRAPHIC PROFILES OF RNA FROM BUFFALO TISSUES ON AN IR-120 ( $Al^{3+}$ ) COLUMN

Tissue	Retention of RNA (%)	Elution (%)	Profiles
Liver	100	100	6 fractions ( $F_1-F_6$ )
Kidney	100	95	6 fractions ( $F_1-F_6$ )
Brain	100	105	6 fractions ( $F_1-F_6$ )

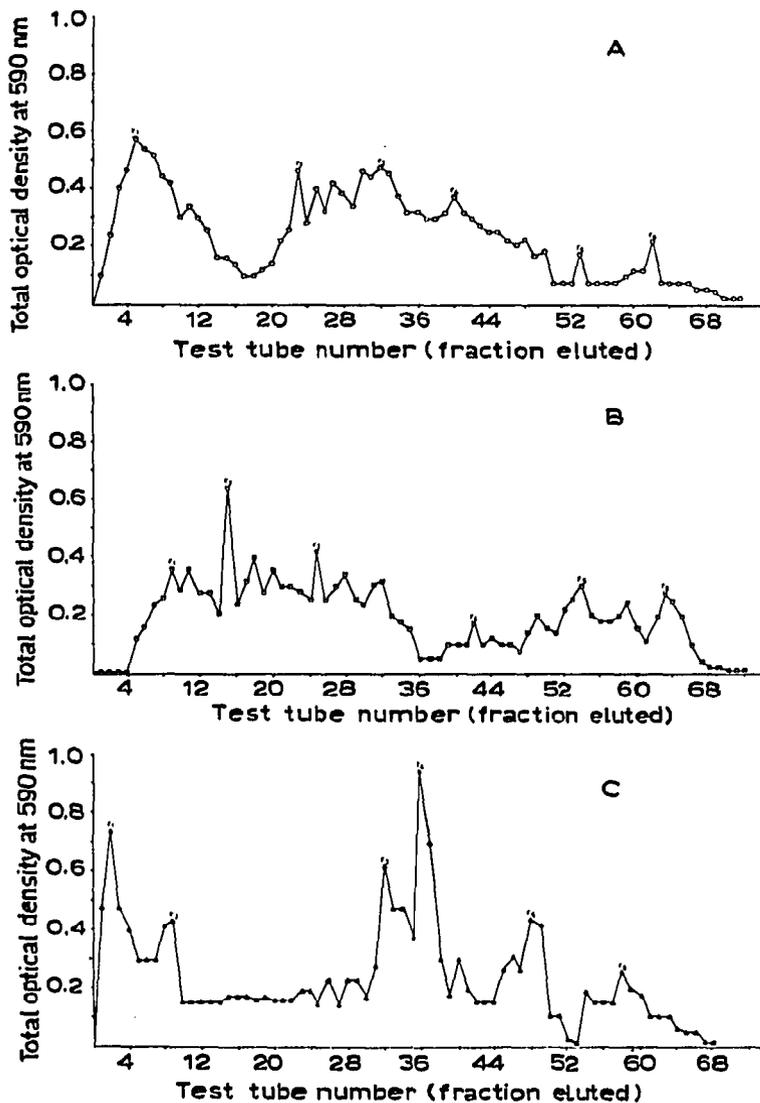


Fig. 1. Effect of tissue variation on the chromatographic profiles of RNA on an IR-120 ( $Al^{3+}$ ) column. Tissue: A, liver; B, kidney; C, brain.

TABLE II

EFFECT OF SOURCE VARIATION ON THE CHROMATOGRAPHIC PROFILES OF LIVER RNAs ON AN IR-120 ( $Al^{3+}$ ) COLUMN

Source	Retention of RNA (%)	Elution (%)	Profiles
Buffalo	100	100	6 fractions ( $F_1-F_6$ )
Pigeon	100	102	6 fractions ( $F_1-F_6$ )
Frog	100	98	6 fractions ( $F_1-F_6$ )

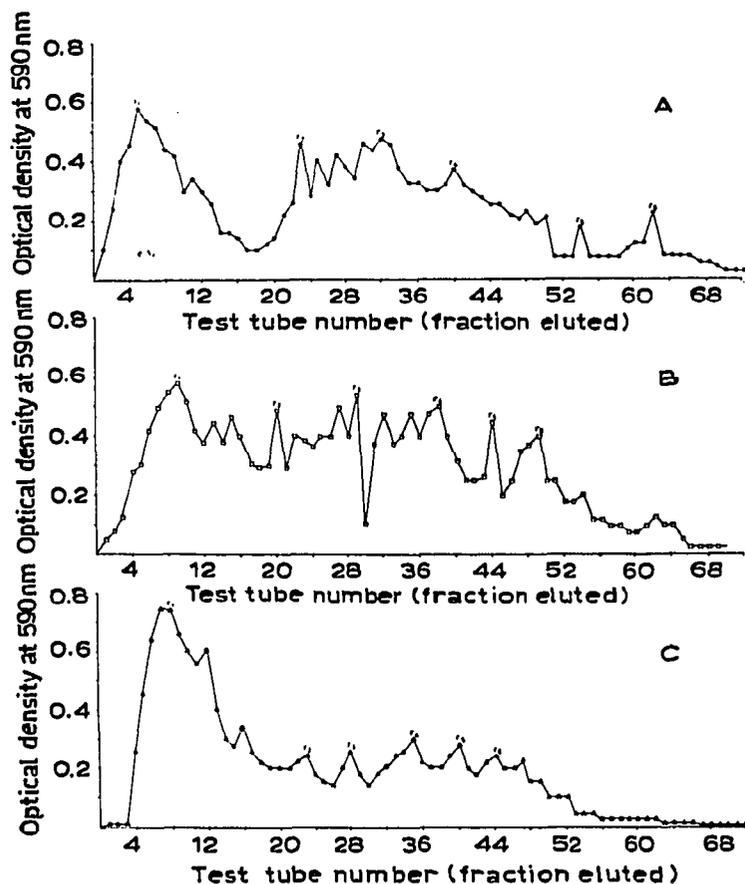


Fig. 2. Effect of source variation on the chromatographic profiles of RNA on an IR-120 ( $Al^{3+}$ ) column. Source: A, buffalo; B, pigeon; C, frog.

Table II gives the percentage retention and the percentage elution of the total adsorbed RNA. Fig. 2 shows the elution profiles.

## RESULTS AND DISCUSSION

From Table I, it is clear that RNAs from different tissues (same source) are

completely retained on an IR-120 ( $Al^{3+}$ ) column and are quantitatively eluted, each giving the characteristic profiles yielding six fractions. It is also clear from Fig. 1 that the elution profiles of RNA from buffalo liver, kidney and brain are qualitatively (location of elution) and quantitatively (percentage distribution) distinct.

As with tissue variations (same source), it is clear from Table II that RNAs from different sources (same tissue) are completely retained and completely eluted into six characteristic fractions. Fig. 2 shows that there is a qualitative and quantitative difference in the profiles with the variation in source.

As the methods of isolation and deproteinization are the same, one would expect the profiles to be similar, unless there are inherent differences in the RNA species from different tissues (same source) and different sources (same tissue).

It is also important to note that all of the RNA preparations studied above, irrespective of either tissue or source variation, had about 10% of protein associated with them. However, in spite of almost the same amount of protein being associated with all the RNA preparations studied, there are distinct qualitative and quantitative differences in the chromatographic profiles.

The difference in the elution profiles as a result of a change in either the source or tissue has been reported by several workers. Creaser and Spencer<sup>1</sup> showed that a certain fraction of Novikoff hepatoma preparation, when chromatographed on ECTEOLA-cellulose, shows a fraction that is completely missing in a rat liver RNA preparation. Considerable differences were found in the elution profiles of yeast and *E. coli* tRNA on DEAE-cellulose columns<sup>2</sup>. Matsuzaki<sup>8</sup> also showed a difference between the elution patterns of aspartyl and seryl tRNAs of silk gland on MAK columns and those of *E. coli*. If a system of such low complexity as that of *E. coli* RNA shows so much diversity, it is difficult to expect similar elution profiles for RNA from a wide variety of sources and tissues. Taylor *et al.*<sup>3</sup> screened a large number of tRNAs from different tissues and organs from a large number of animal species and compared the chromatographic profiles of <sup>14</sup>C- and <sup>3</sup>H-labelled aminoacyl tRNAs on MAK columns. However, they observed no detectable differences in the profiles except for a peak of seryl tRNA present in kidney but absent in liver. They observed significant differences in the chromatographic profiles of liver and Ehrlich ascites tumour tRNAs.

Taylor *et al.*<sup>9</sup> studied the behaviour of mammalian, avian and bacterial tRNAs on MAK columns and found detectable differences amongst them. Further studies<sup>10</sup> supported the above results. A comparison<sup>11</sup> of mitochondrial and cytoplasmic aminoacyl tRNAs of rat liver on MAK columns revealed profound differences in the elution profiles. Marked differences in the elution profiles of seryl tRNAs from beef and of rabbit liver and brain were observed in reversed-phase chromatography by Hatfield and Portugal<sup>12</sup>. Employing a MAK column to examine the chromatographic behaviour of RNAs from avian tumour virus BAI strain A and virus transformed cells, Trávníček and Říman<sup>4</sup> observed certain differences in the elution profiles of some tRNAs. Volkers and Taylor<sup>13</sup> also noted the differences in the chromatographic profiles of tRNAs of rat liver and Morris hepatoma using reversed-phase chromatography.

The present observations that there are profound differences in the elution profiles of RNA from the different tissues and sources studied can be explained in the light of the above results as follows:

(a) The differences could be due either to enzymatic modification or to differential gene transcription as noted by Holland *et al.*<sup>14</sup>.

(b) The differences may be due to different functional states of different tissues from different sources. Depending upon the requirement, certain species of RNA are synthesized while simultaneously suppressing the synthesis of some other types of RNAs.

(c) The differences in the elution profile could be due to differences in the conformation of RNAs from different tissues and from different sources. An IR-120 (Al<sup>3+</sup>) column, like a MAK column, has been reported to effect fractionation on the basis of secondary structure<sup>15,16</sup>.

Thus the differences in the elution profiles studied could be due to several factors. At present there are no data to indicate decisively the factor or factors that govern the separation of RNA on an IR-120 (Al<sup>3+</sup>) column.

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#### REFERENCES

- 1 E. H. Creaser and J. H. Spencer, *Biochem. J.*, 76 (1960) 171.
- 2 P. L. Berquist, B. C. Baguley, J. M. Robertson and R. K. Ralph, *Biochim. Biophys. Acta*, 108 (1965) 531.
- 3 M. W. Taylor, C. A. Buck, G. A. Granger and J. J. Holland, *J. Mol. Biol.*, 33 (1968) 809.
- 4 T. Trávníček and J. Říman, *Biochim. Biophys. Acta*, 199 (1970) 283.
- 5 M. G. Sevag, D. B. Lackmann and J. Smolens, *J. Biol. Chem.*, 124 (1938) 425.
- 6 V. Shankar and P. N. Joshi, *J. Chromatogr.*, 90 (1974) 99.
- 7 N. B. Patil, S. V. Bhide and N. R. Kalc, *Carbohydr. Res.*, 29 (1973) 513.
- 8 K. Matsuzaki, *Biochim. Biophys. Acta*, 114 (1966) 222.
- 9 M. W. Taylor, G. A. Granger, C. A. Buck and J. J. Holland, *Proc. Nat. Acad. Sci. U.S.*, 57 (1967) 1712.
- 10 M. W. Taylor, *Cancer Res.*, 29 (1969) 1681.
- 11 C. A. Buck and M. M. K. Nass, *J. Mol. Biol.*, 41 (1969) 67.
- 12 D. Hatfield and F. H. Portugal, *Proc. Nat. Acad. Sci. U.S.*, 67 (1970) 283.
- 13 S. A. S. Volkers and M. W. Taylor, *Biochemistry*, 10 (1971) 488.
- 14 J. J. Holland, M. W. Taylor and C. A. Buck, *Proc. Nat. Acad. Sci. U.S.*, 58 (1968) 2437.
- 15 V. Shankar and P. N. Joshi, *J. Chromatogr.*, 95 (1974) 65.
- 16 M. V. Hegde, unpublished results.