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Benzoylated Diethylaminoethyl Cellulose Chromatography of Tumor and Nontumor Transfer RNA[1]

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SUMMARY

tRNA isolated from rabbit liver, Ehrlich ascites tumor cells, and L-cells was chromatographed on benzoylated diethylaminoethyl cellulose. With nonacylated tRNA, it was possible to resolve three species of tyrosyl-tRNA, four or five species of lysyl-tRNA, and five species of seryl-tRNA. The elution patterns of acylated tRNA's were also examined. Two isoaccepting phenylalanyl-tRNA's were resolved. When acylated tRNA's from these sources were cochromatographed, no qualitative differences were detectable, although quantitative differences were present.

INTRODUCTION

The existence of chromatographic differences between specific species of isoaccepting transfer RNA's isolated from differentiated cells or tissues from animals and plants has been reported (2, 10, 19). Such differences have also been described after phage T2 infection in *Escherichia coli* (12), after herpesvirus infection of BHK-21 cells (17), during early embryogenesis of the sea urchin (22, 24), and in various tumor cells (10, 13). Sueoka and Kano-Sueoka (18) have recently published an excellent review of the relationship between RNA, cell differentiation, and neoplasia. Most of this work has been carried out with either MAK[2] chromatography or reversed phase chromatography. However, recent improvements in the resolution of tRNA by chromatography on BD-cellulose (9, 21) prompted a reexamination of some of the earlier data. By the use of chromatography on BD-cellulose, we have been able to obtain further resolution of mammalian tRNA and wish to present data reporting the presence of isoaccepting species of tRNA not previously noted in mammalian tissues.

MATERIALS AND METHODS

BD-cellulose Chromatography. BD-cellulose was prepared as described by Gillam *et al.* (9). All columns (25 x 2.5 cm) were run at 4°. Packed columns were washed with 2 M NaCl solution until no further material absorbing at 260 mμ was eluted and then equilibrated with 0.4 M NaCl solution, 0.01 M $MgCl_2$, and 0.05 M sodium acetate, pH 5.0. All subsequent solutions were made up in sodium acetate buffer, pH 5.0, and 0.01 M $MgCl_2$. Samples of tRNA were loaded onto the column in 0.4 M NaCl solution. Unacylated tRNA was eluted with a linear gradient of 500 ml of 0.4 M NaCl solution and 500 ml of 1.0 M NaCl solution. The flow rate was 0.5 ml/min. At the completion of this gradient, 100 ml of 1 M NaCl, containing 10% ethanol, were washed through the column. Fractions of 10 ml were collected, concentrated, and acylated as described below. Acylated tRNA was chromatographed with 5 mg of *E. coli* tRNA (Schwarz BioResearch, Inc., Orangeburg, N. Y.) as carrier. The elution conditions used depended on the species of tRNA, and are described in the respective figures. Fractions were collected, precipitated with alcohol, air dried, and counted as described previously (19).

Amino Acid Acceptor Activity. One-ml aliquots from each column fraction were withdrawn and precipitated with 3 ml 95% alcohol in the presence of glycogen. The precipitate was resuspended after drying in 0.1 ml sterile water. To this was added 0.05 ml of a reaction mixture containing 0.04 M MgCl, 0.02 M sodium cacodylate buffer (pH 7.4), 2×10^{-5} M 19 amino acids excepting the labeled amino acids 0.1 μCi ^{14}C-labeled or 0.5 μCi ^{3}H-labeled amino acid, and 0.05 ml synthetase. The reaction mixture was incubated for 10 min at 37°, and the reaction was stopped by the addition of 2 ml sodium acetate buffer, pH 5.2. The charged tRNA was precipitated 3 times with alcohol, and the final precipitate was resuspended for counting in 0.1 M NaOH, 0.5 ml Nuclear Chicago Solvent, and 10 ml toluene scintillation fluid.

Preparation of tRNA and Aminoacyl-tRNA Synthetase. RNA was prepared as described previously (10). All tRNA preparations were incubated in the presence of electrophoretically pure DNase (Worthington Biochemical Corporation, Freehold, N. J.) at a concentration of 1 μg/ml. The tRNA's were stripped of amino acids by incubation for 60 min at 37° in 0.01 M Tris, pH 8.5. The tRNA's were finally freed of glycogen and contaminating nucleotides by eluting from a BD-cellulose column with 1.0 M NaCl solution, containing 20% ethanol.

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[2]The abbreviations used are: MAK, methylated albumin-Kieselguhr; BD, benzoylated diethylaminoethyl cellulose.

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Aminoacyl-tRNA synthetases were prepared from rabbit liver as previously described (10) and stored at −70° in the presence of 10% glycerol.

Cell Culture. L-cells were grown as monolayers in 32-oz bottles in Eagle's minimal essential medium with 10% calf serum. Cells were harvested by trypsinization, and tRNA was prepared as described above.

Ehrlich ascites tumor was grown in the peritoneal cavity of mature mice and transferred at 8- to 10-day intervals.

Rabbit livers and mouse livers were purchased from Pel-Freeze Biologicals, Rogers, Ark.

Radioactive Compounds. The source and specific activities of the labeled amino acids were as follows: New England Nuclear Corp., Boston, Mass.: L-lysine-^{14}C (247 mCi/mmole), L-lysine-^{3}H (3.96 Ci/mmole), L-serine-^{14}C (123 mCi/mmole), L-phenylalanine-^{14}C (370 mCi/mmole, L-phenylalanine-^{3}H (4.25 mCi/mmole), L-tyrosine-^{14}C (475 mCi/mmole), L-tyrosine-^{3}H (16.5 Ci/mmole). Schwarz Bio-Research: L-serine-^{3}H (1 Ci/mmole).

RESULTS

Fractionation of Nonacylated tRNA. For examination of the distribution of species of tRNA from different sources, 500 A_{260} units of tRNA were loaded onto a BD-cellulose column and eluted with a linear gradient of 0.45 M to 1.0 M NaCl solution. The remaining tRNA was eluted from the column with the 1.0 M NaCl buffer containing 10% alcohol. Ten-ml fractions were collected, and 1-ml samples were analyzed for their ability to accept specific tRNA's.

Since we had previously reported differences in the elution patterns of different tumor tyrosyl-tRNA's on columns (10), it was of interest to examine the elution profile of tyrosine-tRNA from rabbit liver and Ehrlich ascites tumor on a BD-cellulose column. Chart 1 compares the pattern from these 2 sources. Whereas on MAK column chromatography we had reported 1 peak of tyrosyl-tRNA from mouse liver and a major and minor peak from Ehrlich ascites tumor, 3 possible species of tyrosyl-tRNA are detectable by BD-cellulose in both the above sources. No differences in the number of isoaccepting species of tyrosyl-tRNA from rabbit liver and Ehrlich ascites cells were detectable. We have previously reported no differences in the elution profile of rabbit liver and mouse liver tRNA's (19).

Gillam *et al.* (9) have reported that yeast phenylalanine-tRNA elutes from BD-cellulose only in the presence of ethanol. Dudock *et al.* (7) have found similar results with wheat germ phenylalanine-tRNA, and Fink *et al.* (8) have observed the same with rat liver phenylalanine-tRNA. On examining the tRNA eluting with 1.0 M NaCl solution and 10% ethanol for acceptance activity other than phenylalanine, we have found that both lysyl- and seryl-tRNA's are present in small amounts in this fraction. Table 1 presents the distribution of phenylalanyl-, tyrosyl-, seryl-, and lysyl-tRNA eluted from BD-cellulose with different salt concentrations. A small quantity of phenylalanyl-tRNA elutes with 1.0 M NaCl solution. A detailed analysis of lysyl- and seryl-tRNA profiles from Ehrlich ascites tumor and rabbit liver indicated that a number of separate species are resolvable on BD-cellulose.

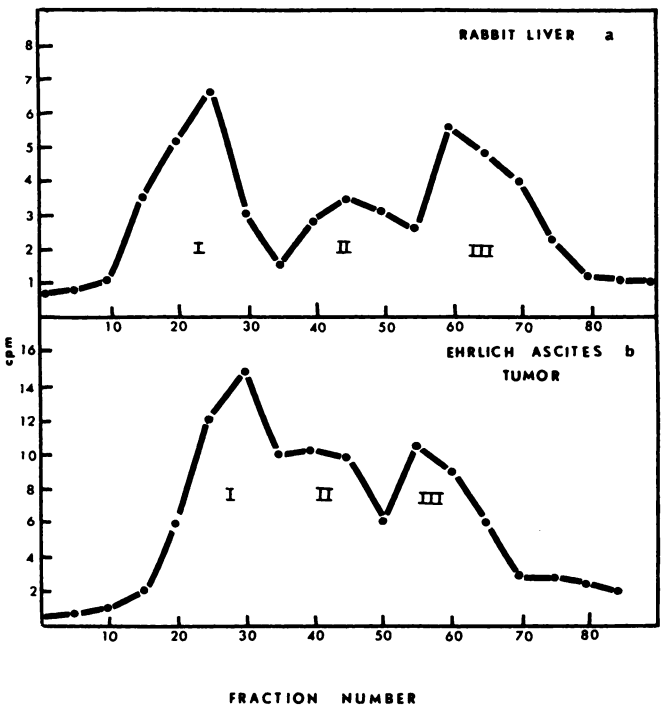

Chart 1. Elution profile of tyrosine-tRNA from (*a*) rabbit liver and (*b*) Ehrlich ascites tumor; gradient used was 0.4 M NaCl to 1.0 M NaCl, containing 0.01 M $MgCl_2$ and 0.05 M sodium acetate buffer, pH 5.0; 10-ml fractions were collected; tyrosine-^{3}H, cpm X 10^3/ml.

Table 1

Aminoacyl-accepting activity of tRNA fractions eluting at different salt concentrations from BD-cellulose

Rabbit liver tRNA, 100 A_{260} units, was loaded onto a BD-cellulose column in 0.2 M NaCl buffer. The column was sequentially washed with 0.6 M NaCl, 0.6 M to 1.0 M NaCl gradient, 1.0 M NaCl, and finally with 2.0 M NaCl and 10% ethanol. Fractions were collected, combined, and assayed for amino acid acceptor activity.

Fraction	Salt concentration	Total amino acid acceptor activity (μmoles) Phe	Tyr	Ser	Lys
I	0.5 M NaCl	<1	190	2719	3914
II	0.6 M 1.0 M NaCl	45	132	1672	88
III	1.0 M NaCl	204	69	3120	2964
IV	2.0 M NaCl + ethanol	1408	40	874	658

Chart 2 illustrates the profile of lysine-tRNA from Ehrlich ascites tumor and L-cells. Lysyl-tRNA elutes over a much longer gradient than tyrosyl-tRNA and appears to contain 3 or 4 species that elute with the salt gradient and 1 major species eluting with 1 M NaCl solution in 10% ethanol. A similar number of species have been found with rabbit liver lysyl-tRNA. Further analysis is needed to verify the exact number of species.

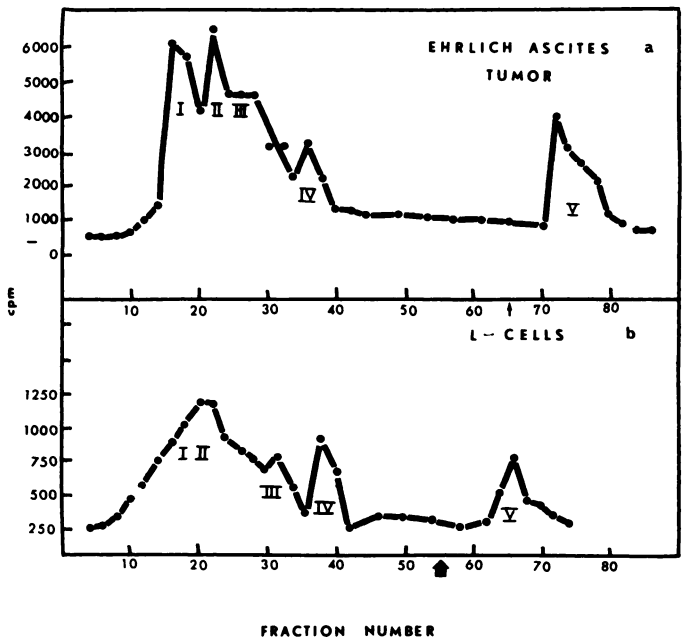

Chart 2. Elution profile of lysine-tRNA from (*a*) Ehrlich ascites tumor and (*b*) L-cells; gradient used was the same as in Chart 1, except that on completion of the salt gradient 1.0 M NaCl, 0.01 M $MgCl_2$, 0.05 M sodium acetate buffer, pH 5.0, containing 10% alcohol, was used to elute the remaining tRNA (*arrow*).

Chart 3 illustrates the BD-cellulose chromatographic profile of serine-tRNA from L-cells. The chromatographic elution profiles of such seryl-tRNA's from Ehrlich ascites tumor cells and rabbit liver are qualitatively identical. However, there do appear to be quantitative differences in the proportions of the different species, the ratio of Peak 5 to other peaks being greater in L-cells than in either Ehrlich ascites tumor or rabbit liver. Whereas Peak 5 appears to be a major species in L-cells, it is a minor species in rabbit liver and Ehrlich ascites tumor.

Aminoacyl-tRNA's. In a previous communication (19), we reported that qualitative differences exist in the elution profiles of phenylalanyl-, seryl-, and tyrosyl-RNA's of tumor and nontumor tRNA. These differences were detected by utilizing cochromatography of ^3H- and ^{14}C-labeled RNA's on a MAK column. Similar types of experiments were carried out on a BD-cellulose column with different gradient conditions, depending on the aminoacyl-tRNA being studied. In these studies, we compared specific species of tRNA from rabbit liver and Ehrlich ascites tumor cells. Each chromatographic run was repeated a number of times to confirm the general distribution of species. Five species of lysyl-tRNA (Chart 4) are detectable by this method. Three are major species that elute early in the salt gradient (0.4 M NaCl to 1.2 M NaCl), one a minor species that elutes late (at approximately 1.0 M NaCl), and finally a second minor species of lysyl-tRNA which elutes with 10% ethanol. This distribution is identical to lysine-tRNA (Chart 2). The ^{14}C:^3H ratio is constant throughout the elution profile, suggesting neither quantitative nor qualitative differences. No previous differences have been reported between lysyl-tRNA from Ehrlich ascites tumor and liver.

Five possible species of seryl-tRNA can be distinguished (Chart 5); however, the elution profile is not identical to that of serine-tRNA. The separation of the major peaks into 3 separate species is inconclusive, and it has not been possible to resolve clearly the first 3 species. No qualitative

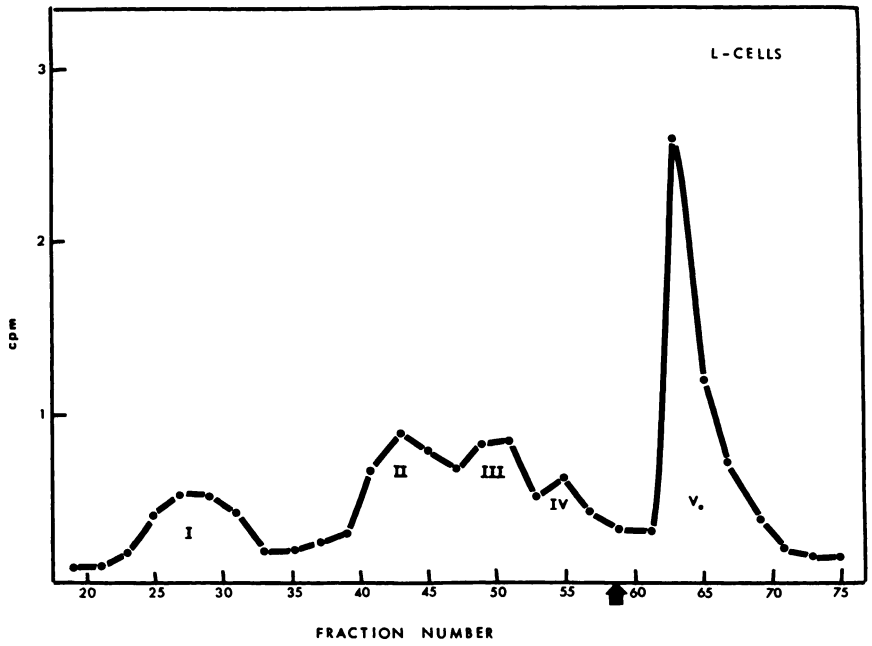

Chart 3. Elution profile of serine-tRNA from L-cells. Conditions as in Chart 2; serine-^3H, cpm X 10^3/ml; *arrow*, point of addition of 10% alcohol.

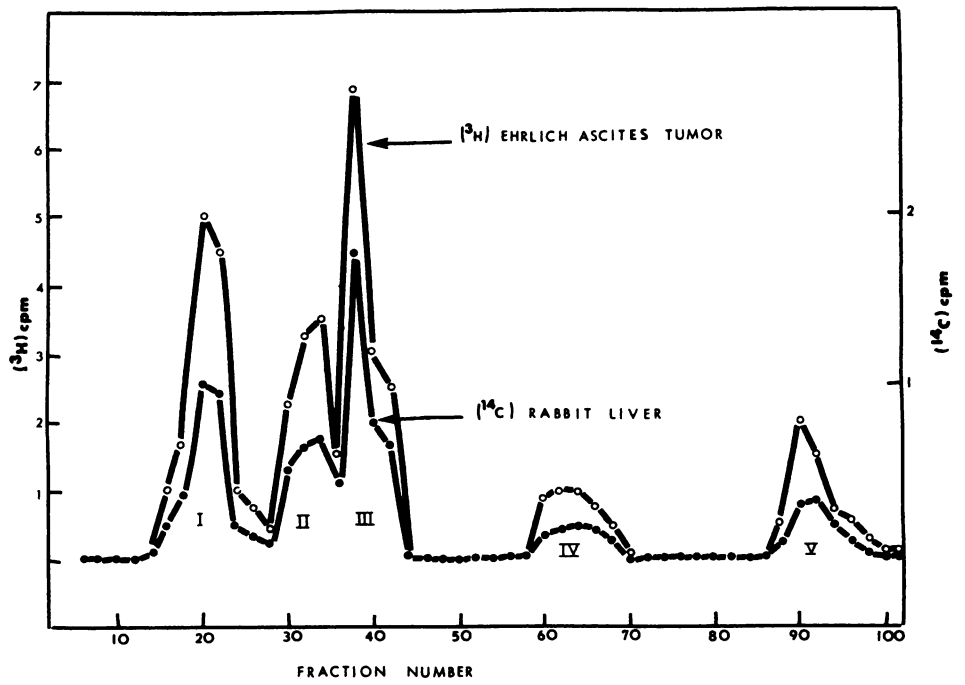

Chart 4. Elution profile of lysyl-tRNA from rabbit liver and Ehrlich ascites tumor cells. Gradient used was 0.4 M NaCl to 1.2 M NaCl, containing 0.01 M $MgCl_2$, and 0.05 M sodium acetate, pH 5.0, followed by 10% alcohol in 1.0 M NaCl sodium acetate buffer, pH 5.0; cpm along the *ordinates* are expressed in thousands.

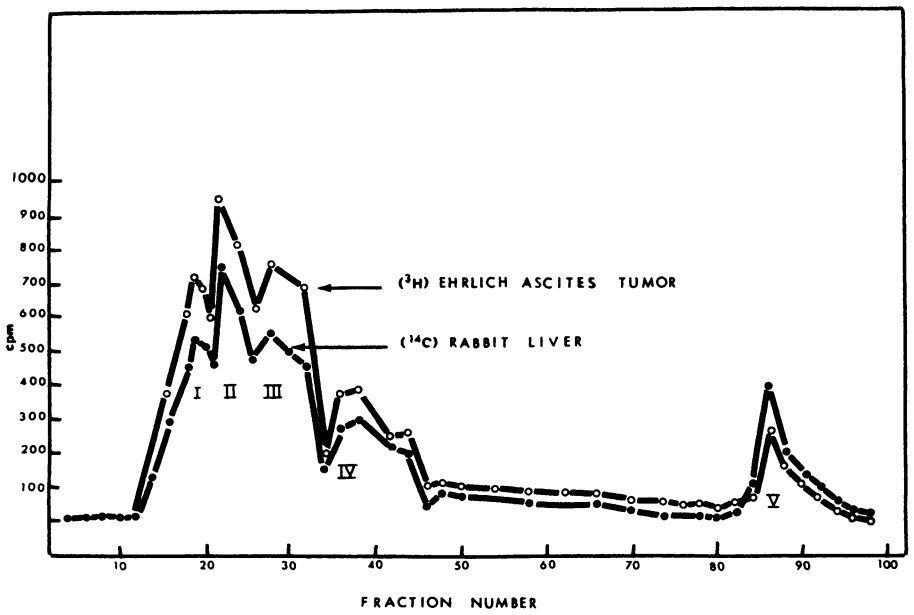

Chart 5. Elution profile of seryl-tRNA from rabbit liver and Ehrlich ascites tumor cells. Conditions as in Chart 4.

differences can be discerned between seryl-tRNA from Ehrlich ascites tumor and rabbit liver. However, when ^3H:^{14}C ratio is compared in the various peaks, certain species are present in greater proportion in rabbit liver than in Ehrlich ascites tumor. (Table 2). Quantitative variations in Peak 5 have also been noted in chromatography of serine-tRNA. A minor difference has previously been reported between seryl-tRNA from Ehrlich ascites tumor and rabbit liver by MAK column chromatography (20).

Tyrosyl-tRNA and phenylalanyl-tRNA are bound more tightly by the BD-cellulose than other tRNA's and elute from the column only with a combined ethanol-salt gradient.

Table 2

^3H:^{14}C ratio in species of seryl-tRNA

Seryl-t-RNA-^3H from Ehrlich ascites tumor and seryl-tRNA-^{14}C from rabbit liver were cochromatographed on BD-cellulose as described in Chart 5. The ^3H:^{14}C ratio was calculated for all species.

Peak	Ratio
I	1.30
II	1.30
III	1.34
IV	1.25
V	0.75

At least 2 distinct species of tyrosyl-tRNA can be separated by using a shallow ethanol-salt gradient (Chart 6). As in the case of tyrosine-tRNA, no differences were detectable. Likewise, at least 2 distinct species of phenylalanyl-tRNA are discernible in both rabbit liver and Ehrlich ascites tumor (Chart 7). We have noted a persistant quantitative difference in the ratio of Peak 1 to Peak 2 between Ehrlich ascites tumor phenylalanyl-tRNA and rabbit liver phenylalanyl-tRNA.

DISCUSSION

Wimmer *et al.* (21) and Gillam *et al.* (9) have previously shown that BD-cellulose chromatography provides a very simple and reliable means of analytically examining the distribution of certain species of tRNA. The basis for this separation of the isoaccepting species is unknown, but it is probably a reflection of both the presence of unusual bases in the tRNA and different molecular conformation of the isoaccepting tRNA's.

Yang and Novelli (23), on the basis of the elution profiles of aminoacyl-tRNA's from a mouse plasma cell tumor on reverse phase chromatography, have reported that the number of isoaccepting species of specific transfer RNA's is much greater in mammalian cells than in *E. coli* or yeast. We have shown in this study that there are at least 2 or 3 distinct species of tyrosyl-tRNA, 2 phenylalanyl-tRNA's, 5 seryl-tRNA's, and 4 to 5 lysyl-tRNA's. These results confirm in part the recent findings of Yang and Novelli (23) and Nishimura and Weinstein (14) who reported a similar number of separable species of tRNA's by other chromatographic means. We have not examined the binding of these species to ribosomes in the presence of polynucleotide triplets, but it is possible that each of the seryl-tRNA species may recognize a district triplet codon, since 6 codons are known for serine. However, Nishimura and Weinstein (14) have demonstrated that the "wobble mechanism" by which a single tRNA recognizes more than 1 codon (6), applies to 2 tyrosyl-tRNA's and 2 phenylalanyl-tRNA's isolated from rat liver.

The functional significance of multiple species of specific tRNA is unknown. Many theories have been proposed which suggest that the presence or absence of such species may play a role in regulation of protein synthesis [see review by Sueoka and Kano-Sueoka (18)]. Although both quantitative and qualitative differences in tRNA's have been reported, no confirmation of the above hypothesis is available (2, 10, 12, 17, 19, 24).

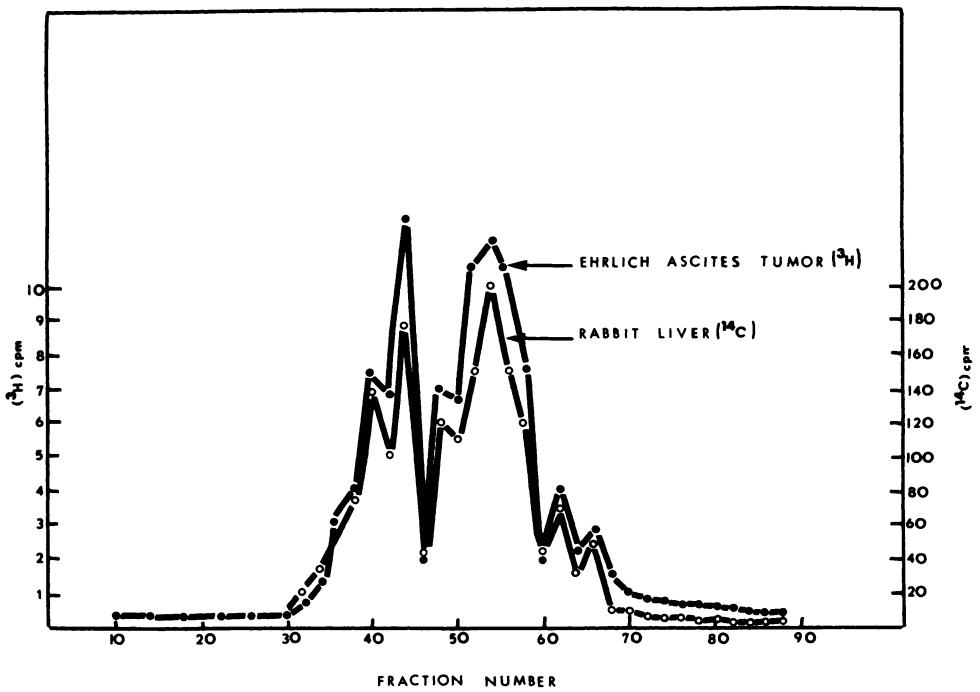

Chart 6. Elution profile of tyrosyl-tRNA from rabbit liver and Ehrlich ascites tumor cells. Gradient used was 0.4 to 1.0 M NaCl, 0 to 5% ethanol, containing 0.01 M $MgCl_2$ and 0.05 M sodium acetate, pH 5.0; cpm along the *left ordinate* is expressed in hundreds.

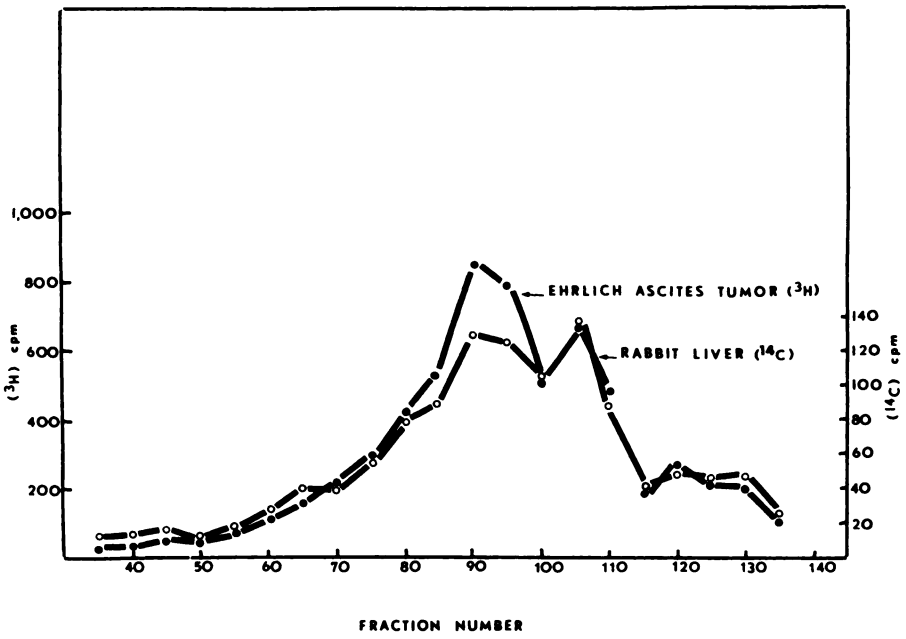

Chart 7. Elution profile of phenylalanyl-tRNA from rabbit liver and Ehrlich ascites tumor. Combined salt gradient of 0.4 M NaCl to 1.0 M NaCl, 0 to 15% ethanol, containing 0.01 M $MgCl_2$ and 0.05 M sodium acetate buffer, pH 5.0.

We have previously reported that seryl- and phenylalanyl-tRNA from Ehrlich ascites tumor elutes slightly earlier in a salt gradient from a MAK column than normal rat liver (20). The results presented here would suggest that this difference may have been due to the presence of an excess of Species V in rabbit liver seryl-tRNA and Peak 2 in phenylalanyl-tRNA compared to the homologous species in Ehrlich ascites tumor. Most of the differences noted by MAK column chromatography are of this type (3, 19). Quantitative differences in tRNA species may therefore appear as "shifts" in elution profiles by MAK column chromatography. We have been unable to detect any major qualitative differences by BD-cellulose chromatography.

Our data do not exclude the possibility that many of our chromatographic peaks are the result of experimental artifacts due to aggregation (16) or partial denaturation (1). However, this appears unlikely in view of the findings of others (14, 23). The observation that the same number of species are present in charged and uncharged tRNA would suggest that the number of isoaccepting species are not due to aggregation, RNase degradation, or loss of CCA end groups. However, the possibility that some of our minor species may be of mitochondrial origin was not ruled out in the present study (5).

The presence of multiple isoaccepting species of transfer RNA may not have any functional significance in regulation or differentiation but might rather reflect mutational changes that have occurred in nonessential regions of the tRNA molecule analogous to the evolutionary changes noted in protein molecules (11). Single alterations in nucleotide sequences at places other than the enzyme recognition site, the anticodon region of the molecule, or the ribosome-binding site may have no functional (selective) value but may chromatograph as separate species. This could be a result of extensive redundancy of tRNA genes. Ritossa *et al.* (15) have reported that there may be at least a 10-fold redundancy in the number of cistrons for specific tRNA's in *Drosophila melanogaster.* In similar studies, Brown and Weber (4) concluded that there is a redundancy in the DNA coding for tRNA in *Xenopus laevis.*

The data presented in this paper confirm the findings of Gillam *et al.* (9) that BD-cellulose chromatography may be utilized as an analytical tool in examining isoaccepting species of transfer RNA. MAK chromatography is a useful method for screening tRNA differences, but the results presented in this paper suggest that changes in the relative abundance of isoaccepting species of tRNA may appear as altered profiles on MAK columns. A more definitive study, such as codon recognition, and purification of individual species with base composition and sequence analyses, are required to give clear answers to this question. Such studies are now being undertaken in this laboratory.

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