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Chromatographic Alterations in Transfer RNA's accompanying Speciation, Differentiation and Tumor Formation

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Specific aminoacyl-tRNA's from different mammalian organs and species have been compared by methylated albumin column chromatography. The elution profile of such chromatographs were usually similar, both between tRNA's from different organs in the same animal, and across widely divergent mammalian species. Species differences were found in the elution profile of avian leucyl- and tyrosyl-tRNA's, but not among other tRNA's.

In contrast to the similarities of methylated albumin–kieselguhr elution profiles of specific tRNA's from most mammalian organs, the elution profile of Ehrlich ascites tumor cells phenylalanyl-, seryl-, glycyl- and tyrosyl-tRNA differed from normal mouse organ tRNA. An extra peak of tyrosyl-tRNA has also been found in HeLa cells and adeno-7 virus transformed cells, but not in all tumor cells. The tyrosyl-tRNA of mammalian and chick fibroblasts in cell culture, and fibroblasts in the body exhibited a markedly different elution profile from the tyrosyl-tRNA of epithelial cells in culture and of normal organs. The tRNA differences discovered by *in vitro* charging have all been confirmed *in vivo*. Possible reasons for these variations are discussed.

1. Introduction

We have recently reported (Buck, Granger, Taylor & Holland, 1967) that different strains of enteroviruses—Mengo viruses and bovine enteroviruses—replicate with as much as a thousand-fold difference in efficiency in different animal cells. These infections lead ultimately to cytopathic effects and cell death even in the cases of inefficient or abortive infection. We suggested that such abortive infection might be explained by an inability of certain cells to provide an efficient translation mechanism for the infecting viral mRNA. Quantitative differences in available species of transfer RNA or aminoacylating enzymes, or an alteration in the coding properties of a particular species of tRNA, could alter the rate or quality of translation of the viral genome; we have recently been searching for such differences. The role that such differences in tRNA might have in controlling cellular protein synthesis during differentiation has been discussed by Ames & Martin (1964), Stent (1964), Taylor, Buck, Granger & Holland (1967), and Strehler, Hendley & Hirsch (1967). The

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possibility of control of macromolecular synthesis at the translation level has been further supported by the findings of alterations of existing tRNA's or the *de novo* formation of new tRNA's following T2 phage infection in *Escherichia coli* (Kano-Sueoka & Sueoka, 1966), in herpes virus infection of BHK21/C13 cells (Subak-Sharpe, Shepherd & Hay, 1966) and during sporulation of *Bacillus subtilis* (Kaneko & Doi, 1966).

Utilizing methylated albumin–kieselguhr chromatography, we examined the elution profiles of specific aminoacylated tRNA's isolated from different mammalian organs and species. In a preliminary report (Taylor *et al.*, 1967), we noted a remarkable similarity among most specific aminoacyl-tRNA's examined between organs, although large differences were apparent between certain specific tRNA's from tumor cells (Ehrlich ascites tumor cells and HeLa) when compared to normal cells. In this paper we report a more complete study of aminoacyl-tRNA's from widely divergent mammalian species, cells in tissue culture, and various tumors.

2. Materials and Methods

(a) *Source of transfer RNA*

Transfer RNA was isolated from the liver, brain, kidney and muscle of rabbit, mouse, cow, chicken and hamster. Normal and malignant human material used as a source for tRNA was kindly supplied by Dr S. Kaplan, Childrens' Hospital, Los Angeles, Dr R. Davis, U.S. Naval Hospital, San Diego, and Dr G. Sinykin, Hoag Memorial Hospital, Newport Beach.

(b) *Cell lines*

All cell cultures were propagated in Eagle's medium containing 7% calf serum. Primary cell cultures were obtained by treating minced tissues with 0·25% trypsin for 30 to 180 min at 37°C. Resulting cell suspensions were washed, placed in Eagle's medium and incubated to form monolayers on glass.

The following continuous cell lines were used: HeLa, Madin strain of bovine kidney, and mouse L-cells. Transformed cell lines derived from hamster tumors induced by adenovirus-7, Schmidt–Ruppin strain of Rous sarcoma virus, and simian virus 40 were purchased from Flow Laboratories, Rockville, Md. Ehrlich ascites tumor cells and sarcoma-1 cells were harvested from the peritoneum of mice 6 to 8 days after inoculation. Primary cell cultures were prepared from hamster, chick and mouse embryos, and from human amnion.

(c) *Preparation of tRNA and aminoacyl-tRNA synthetases*

Transfer RNA was prepared by blending the minced organs in phenol in the presence of 0·01 M-Tris, pH 7·0, 0·001 M-EDTA, 0·15 M-NaCl. The aqueous layer was separated from the phenol layer by centrifugation, chloroform extracted and alcohol precipitated 3 times. The 1 M-NaCl-soluble material was reprecipitated with 95% ethanol, and treated with electrophoretically pure DNase (Worthington). The final tRNA concentration was adjusted to 2 mg/ml. and stored in sterile distilled water at −70°C.

The aminoacyl-tRNA synthetases were prepared from homogenates of organs and cell cultures suspended in 0·01 M-Tris, pH 7·5, 0·01 M-KCl, 0·001 M-$MgCl_2$, and 6×10^{-3} M-mercaptoethanol. A 30,000 *g* supernatant was passed through Sephedex G100 equilibrated with the same buffer and the void volume collected. This was done to free the enzyme fraction from contaminating amino acids and endogenous tRNA. The enzyme fraction was retained at −70°C without detectable loss of activity.

(d) *Preparation of aminoacyl-tRNA*

In vitro: The transfer RNA's were charged *in vitro* with either ^{3}H- or ^{14}C-labeled amino acid according to a modification of the procedure of Yamane & Sueoka (1963). The tRNA's were acylated in a reaction mixture containing 0·1 M-Tris, pH 7·5, 0·01 M-KCl,

0·001 M-$MgCl_2$, 6×10^{-3} M-mercaptoethanol with each 0·3 ml. containing 2 μmole ATP (neutralized), 1 μmole CTP, 0·5 μc ^{14}C- or 5 μc ^{3}H-labeled amino acids, 2 mg protein from the enzyme fraction, and 0·2 mg tRNA. The reaction mixture was incubated for 10 min at 37°C. The reaction was stopped by the addition of 3 ml. 0·05 M-sodium acetate–0·15 M-sodium chloride buffer (pH 5·2) and deproteinized by mixing with pH 5·2 buffered phenol. The aqueous layer was precipitated 3 times with ethanol, dried and resuspended in 0·01 M-Tris, pH 7·0, before loading on to the methylated albumin–kieselguhr column.

In vivo: The cells were washed 3 times with Eagle's balanced salts solution, and overlayered with the same medium supplemented with either ^{14}C- or ^{3}H-labeled amino acid. After 5 min incubation at 37°C the radioactive medium was removed and the RNA extracted directly from the cells with a 1 : 1 mixture of 0·05 M-sodium acetate–0·15 M-NaCl, pH 5·2, and buffer-saturated phenol. The tRNA was prepared as described above. Ehrlich ascites tumor cells and sarcoma-1 cells were harvested from the peritoneum of mice 6 to 8 days after intraperitoneal injection of the tumor inoculum. Such cells were washed 3 times in sterile saline and resuspended in Eagle's balanced salts solution supplemented with ^{3}H- or ^{14}C-labeled amino acid. After 10 min incubation at 37°C the cells were harvested and used as a source of tRNA.

(e) *Column chromatography*

A methylated albumin–kieselguhr column (20 cm × 1 cm) was prepared as described by Sueoka & Yamane (1962). The aminoacyl-tRNA was loaded in 0·02 M-Tris, pH 7·0, with 0·9 mg of *E. coli* tRNA as carrier. The tRNA were eluted from the MAK† column with a linear saline gradient at an initial concentration of 0·2 M-NaCl–0·02 M-Tris, pH 7·0, and a final concentration of 0·65 M-NaCl in 0·02 M-Tris buffer, pH 7·0. The total volume of elution fluid was 130 ml. Optical density was measured at 260 mμ in a Gilson recording fraction collector, the 1-ml. fractions under the optical density curve being collected. The tRNA was precipitated with carrier glycogen by addition of 2 vol. of ethanol, dried and resuspended in 0·1 ml. 0·1 M-NaOH, 0·5 ml. Nuclear–Chicago solvent. The fractions were brought to an appropriate volume with 15 ml. of a toluene –PPO–, POPOP scintillation medium and counted in a Beckman liquid-scintillation counter.

(f) *Source of chemicals*

The source and specific activities of the labeled amino acids was as follows: New England Nuclear: L-[^{14}C]alanine (117 mc/m-mole), L-[^{14}C]glycine (116 mc/m-mole), L-[^{14}C]isoleucine (234 mc/m-mole), L-[^{14}C]leucine (225 mc/m-mole), L-[^{14}C]serine (120 mc/m-mole), L-[^{14}C]-phenylalanine (393 mc/m-mole), L-[^{14}C]threonine (156 mc/m-mole), L-[^{14}C]tyrosine (393 mc/m-mole), L-[^{14}C]valine (208 mc/m-mole), DL-[^{3}H]alanine (40 c/m-mole), L-[^{3}H]leucine (5 c/m-mole), L-[^{3}H]tyrosine (40 c/m-mole). Schwarz Bioresearch: L-[^{14}C]lysine (198 mc/m-mole), L-[^{3}H]glycine (2·1 c/m-mole), L-[^{3}H]isoleucine (0·385 c/m-mole), L-[^{3}H]lysine (0·48 c/m-mole), L-[^{3}H]serine (1·2 c/m-mole), L-[^{3}H]phenylalanine (2·5 c/m-mole), L-[^{3}H]-threonine (0·575 c/m-mole), L-[^{3}H]valine (0·87 c/m-mole). Nuclear Chicago: L-[^{14}C]tyrosine (457 mc/m-mole), L-[^{3}H]tyrosine (49 c/m-mole). *E. coli* tRNA was purchased from Schwarz Bioresearch; yeast tRNA was purchased from General Biochemicals. Yeast enzyme fraction was kindly supplied by Dr C. McLaughlin.

3. Results

Figure 1 demonstrates that in our system enzyme is in excess and the extent of phenylalanine [^{3}H]RNA synthesis is proportional to tRNA concentration. Figure 2 demonstrates that the aminoacylation of the tRNA under our conditions is completed by five minutes, both when a homologous and a heterologous enzyme are used.

To examine whether large relative differences in specific tRNA content existed between different mammalian organs and species, we examined the ratio of [^{14}C]-aminoacyl-tRNA's to [^{3}H]phenylalanine tRNA from the sources listed in Table 1. In

† Abbreviation used: MAK, methylated albumin–kieselguhr.

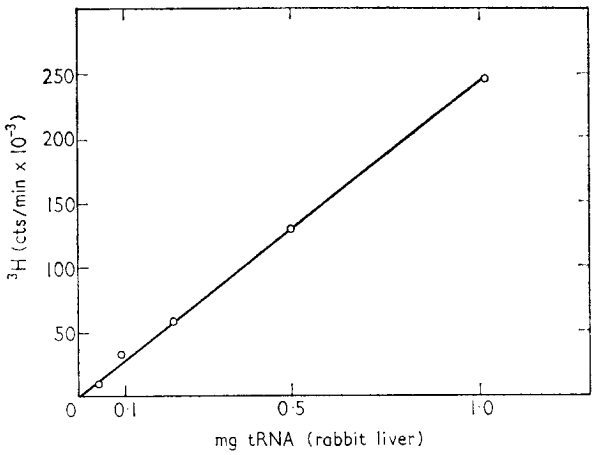


FIG. 1. Incorporation of [^{3}H]phenylalanine into varying amounts of rabbit liver tRNA. Reaction mixture as described in Materials and Methods. Note linearity over 0·05 to 1 mg tRNA.

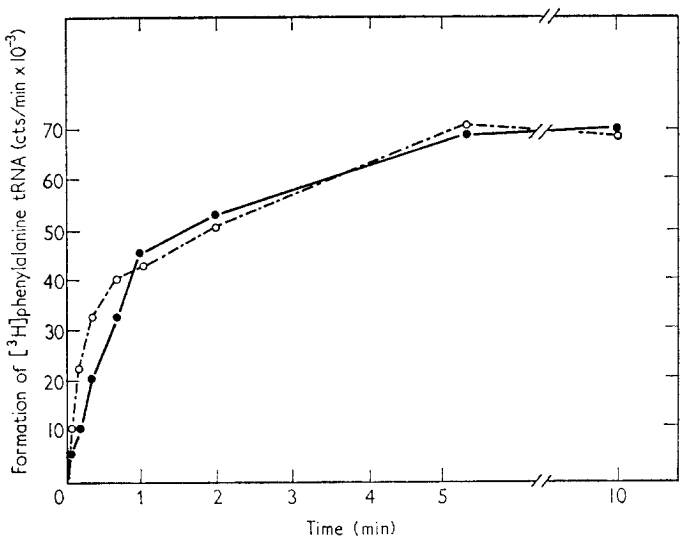


FIG. 2. Synthesis of [^{3}H]phenylalanyl-tRNA after various times of incubation. Reaction mixture as described in Materials and Methods. --○--○--, Rabbit liver RNA, rabbit kidney enzyme; —●—●—, rabbit liver RNA, rabbit liver enzyme.

this study a standard amount of different transfer RNA's (0·2 mg) was charged with [^{3}H]phenylalanine and [^{14}C]amino acid with a rabbit kidney extract. The two radioactive preparations were mixed, phenol extracted and co-precipitated three times with 95% ethanol. The samples were counted for the incorporation of ^{3}H and ^{14}C. The ratio of [^{14}C]aminoacyl-tRNA/[^{3}H]phenylalanyl-tRNA is presented in Table 1. The ratios presented are the averages of three isolated batches of tRNA. No significant differences were noted among the individually prepared lots of tRNA's. Table 1 demonstrates that large quantitative organ and species variations do not exist among those specific tRNA's examined.

TABLE 1

Ratio of [^3H]phenylalanine tRNA: [^{14}C]aminoacyl-tRNA from various sources

Organ	Amino acid					
	Alanine	Glycine	Isoleucine	Serine	Threonine	Tyrosine
Mouse liver	0·17	0·11	0·20	0·33	0·23	0·22
Mouse kidney	0·17	0·20	0·34	0·28	0·28	0·27
Mouse muscle	0·15	0·23	0·27	0·28	—	—
Rabbit liver	0·17	0·22	0·32	0·30	0·33	0·28
Rabbit kidney	0·17	0·22	0·32	0·28	0·27	0·25
Rabbit muscle	0·14	0·20	0·36	0·35	—	—
Rabbit brain	0·13	0·19	0·36	—	0·34	0·23
Bovine liver	0·15	0·23	0·36	—	0·31	0·30
Bovine kidney	0·22	0·20	0·37	0·26	0·28	0·22
Bovine muscle	0·23	—	0·33	0·35	0·31	—
Bovine brain	0·21	0·25	0·35	0·32	0·33	0·29

0·2 mg of tRNA was incubated as described in Materials and Methods with 0·1 ml. of a rabbit-kidney enzyme preparation. The reaction mixture contained 5 μc of [^{3}H]phenylalanine and 0·5 μc [^{14}C]amino acid. After 10 min incubation at 37°C the reaction was stopped by the addition of 3 ml. sodium acetate buffer, pH 5·2. The samples were prepared and counted for radioactivity as described in Materials and Methods.

A comparison of the MAK column elution profiles of alanine, leucine, lysine, phenylalanine, threonine, tyrosine, and valine aminoacyl-tRNA's isolated from various mammalian organs demonstrated the over-all similarity in the specific tRNA content of differentiated organs from the same animal. These results are summarized in Table 2. In the case of serine tRNA we have previously noted a minor species present in rabbit and mouse liver that was absent in other organs (Taylor *et al.*, 1967).

When lysine, phenylalanine and threonine tRNA's from mammalian and avian sources were compared, no detectable differences were noted. However, large quantitative and qualitative differences were observed in the elution profiles of leucyl- and tyrosyl-tRNA's from these sources.

Figure 3(a) and (b) shows typical elution profiles of chick brain leucyl-tRNA compared to rabbit brain leucyl-tRNA and mouse liver leucyl-tRNA. In both these profiles the chick leucyl-tRNA elutes earlier from the MAK column than the rabbit or mouse tRNA. A second peak of rabbit and mouse leucyl-tRNA elutes later than the major peak. That these differences are not due to modifications by the charging enzyme is demonstrated in Figure 3(c), in which the rabbit liver leucyl-tRNA is charged with chick liver enzymes. These differences have been confirmed by *in vivo* labeling of L-cells (mouse) and chick fibroblasts with [^{14}C]- and [^{3}H]leucine (Fig. 3(d)). Figure 4(a) illustrates the relative position of chick tyrosyl-tRNA in relation to mouse liver tyrosyl-tRNA, as it elutes from a MAK column. In contrast to phenylalanyl-, threonyl- and lysyl-tRNA, there is a shift in the elution profile, suggesting a structural change in the transfer RNA. However, both species of tyrosyl-tRNA are fully charged by the heterologous enzymes. Figure 4(b) and (c) compare the elution profiles of

TABLE 2

MAK chromatographic characteristics and source of aminoacylated tRNA

Aminoacyl-tRNA	Species	Organ	Characteristic (MAK column)
Alanine	rabbit	muscle liver	no detectable differences
	mouse	muscle embryo	
Glycine	rabbit	kidney liver	no detectable differences
Leucine	rabbit	brain liver muscle	no detectable differences
	mouse	liver brain fibroblasts	differences between avian and mammalian sources (Fig. 3)
Lysine	rabbit	liver muscle kidney	no detectable differences
	mouse	muscle embryo	
	human	liver	
Phenylalanine	rabbit	liver muscle kidney	no detectable differences
	mouse	liver embryo	
	chick	liver	
	bovine	muscle	
Serine	rabbit	liver kidney muscle	difference noted between kidney, muscle and liver (Taylor *et al.*, 1967)
	mouse	liver kidney muscle	
Threonine	rabbit	brain liver muscle kidney	no detectable differences
	mouse	liver	
	chick	liver	
Tyrosine	rabbit	liver	no detectable differences between mammalian organs, difference between avian and mammalian sources (Fig. 4)
	mouse	liver kidney embryo	
	human	liver	
	hamster	liver	
	chick	liver brain	
Valine	rabbit	muscle liver	no detectable differences
	mouse	liver	

0·2 mg of the respective tRNA's were incubated as described in Materials and Methods with 0·1 ml. of homologous or heterologous aminoacyl-synthetase preparation. The ^{3}H- and ^{14}C-labeled aminoacyl-tRNA's were mixed and eluted from a MAK column. Detectable differences were repeated by reverse labeling, and with different synthetase preparations.

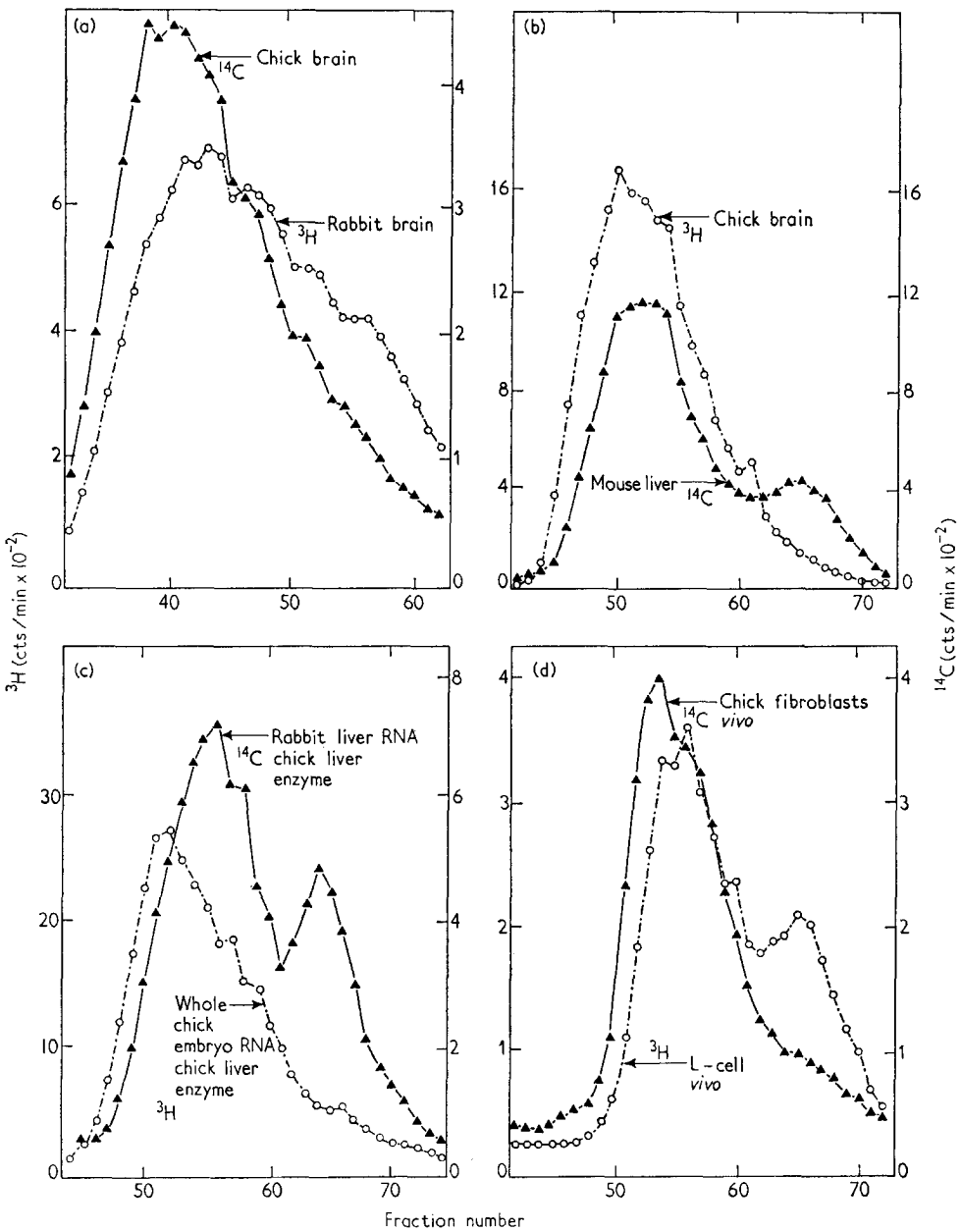


FIG. 3. MAK column elution profile of avian and mammalian leucyl-tRNA. Note the differences in elution profiles.

(a) Chick brain leucyl-tRNA and rabbit brain leucyl-tRNA—homologous enzymes.
(b) Chick brain leucyl-tRNA and mouse liver leucyl-tRNA—homologous enzymes.
(c) Chick liver leucyl-tRNA and mouse liver leucyl-tRNA—mouse liver enzyme.
(d) Chick fibroblasts (*in vivo*) and mouse L-cells (*in vivo*).

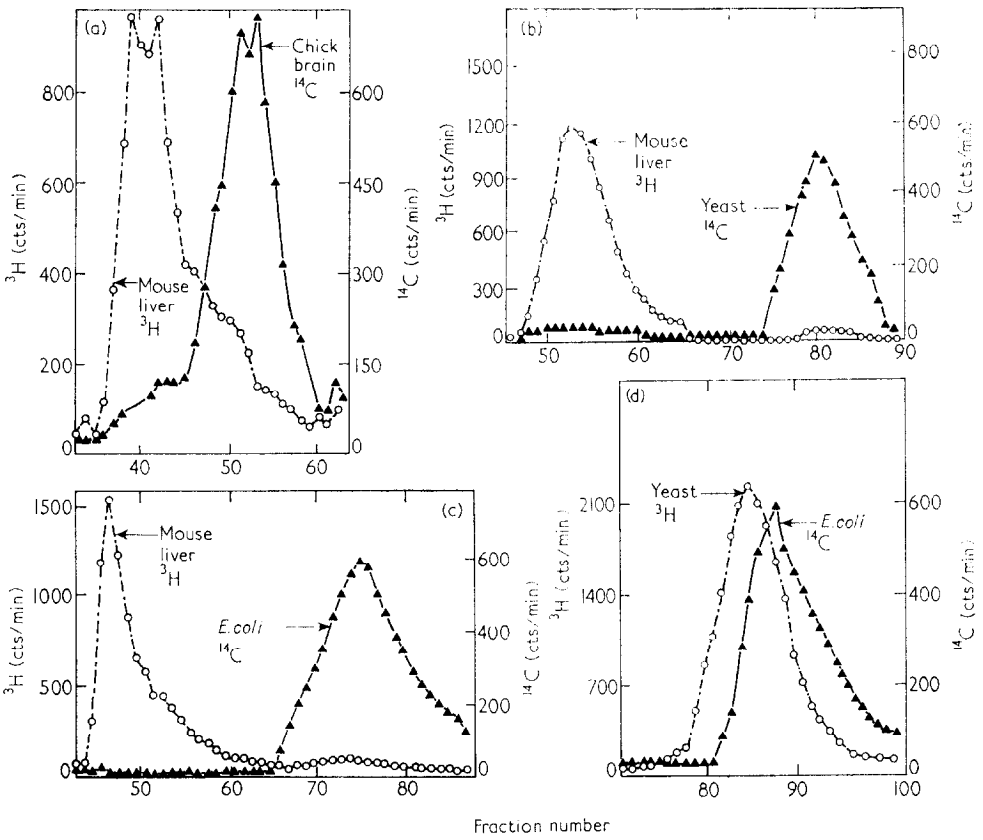


FIG. 4. MAK column elution profile of mammalian, avian, yeast and *E. coli* tyrosyl-tRNA.

yeast and *E. coli* tyrosyl-tRNA to that of a mammalian source. Note the wide chromatographic separation between the tyrosyl-tRNA's. Despite the large chromatographic difference between the mammalian and yeast tRNA, both transfer RNA's are charged equally by the heterologous enzymes (Table 3). In contrast, the *E. coli* tyrosine acyl synthetase does not acylate either yeast or mammalian tyrosine tRNA and *E. coli*

TABLE 3

Aminoacylation of tyrosine tRNA by heterologous enzymes
[^{3}H]tyrosine cts/min incorporated ($\times 10^3$)

Enzyme	Substrate		
	Rabbit liver tRNA	Yeast tRNA	*E. coli* tRNA
Rabbit liver	355	352	8·8
Yeast	64	57	4·5
E. coli	11·7	none	232

0·2 mg of each tRNA was incubated for 10 min as described in Materials and Methods, with 0·1 ml. of identical enzyme fraction. The reaction mixture contained 0·5 μc of [^{3}H]tyrosine. The above results were corrected for endogenous activity.

tRNA is not a substrate for the yeast and mammalian enzyme. These results also demonstrate that mammalian tRNA is probably richer in tyrosine tRNA than yeast tRNA. Doctor & Mudd (1963) have previously reported very little cross-charging, by heterologous enzyme, of tyrosine tRNA from yeast and *E. coli*. Figure 4(d) shows the relative elution profiles of yeast and *E. coli* tyrosyl-tRNA.

(a) *Ehrlich ascites tumor tRNA*

In contrast to the similarities of MAK elution profiles of specific tRNA's from mammalian organs, we have reported that the elution profiles of Ehrlich ascites phenylalanyl-, seryl-, glycyl- and tyrosyl-tRNA's differed from those of normal mouse organ tRNA (Taylor *et al.*, 1967). Both phenylalanyl- and seryl-tRNA isolated from Ehrlich ascites cells after *in vivo* labeling or after *in vitro* labeling show a "shift" in elution profile relative to the normal mouse tissue tRNA (Fig. 5(a) and (b)). On the other hand, an extra species of glycyl- and tyrosyl-tRNA appears to be present in Ehrlich ascites tumor tRNA that is absent, or at relatively reduced levels in normal cells (Fig. 5(c) and (d)). These tumor differences appear to be specific for only certain species of transfer RNA since the alanyl-, isoleucyl-, lysyl-, threonyl- and valyl-tRNA of Ehrlich ascites tumor shows similar elution profiles to those of normal mouse organ tRNA.

(b) *Tyrosyl-tRNA in other cells*

We have extended this study to include other tumor cells and cell lines by comparing the elution profile off MAK of tyrosyl-tRNA from these cells with that of normal organ tyrosyl-tRNA. Figure 6(a) and (b) shows that like Ehrlich ascites tumor cells, HeLa tRNA and adeno-7 virus-transformed hamster cells contain an extra peak of tyrosyl-tRNA that is absent from normal tissue. However, the tyrosyl-tRNA from human lymphatic leukemia cells is identical to that of human liver

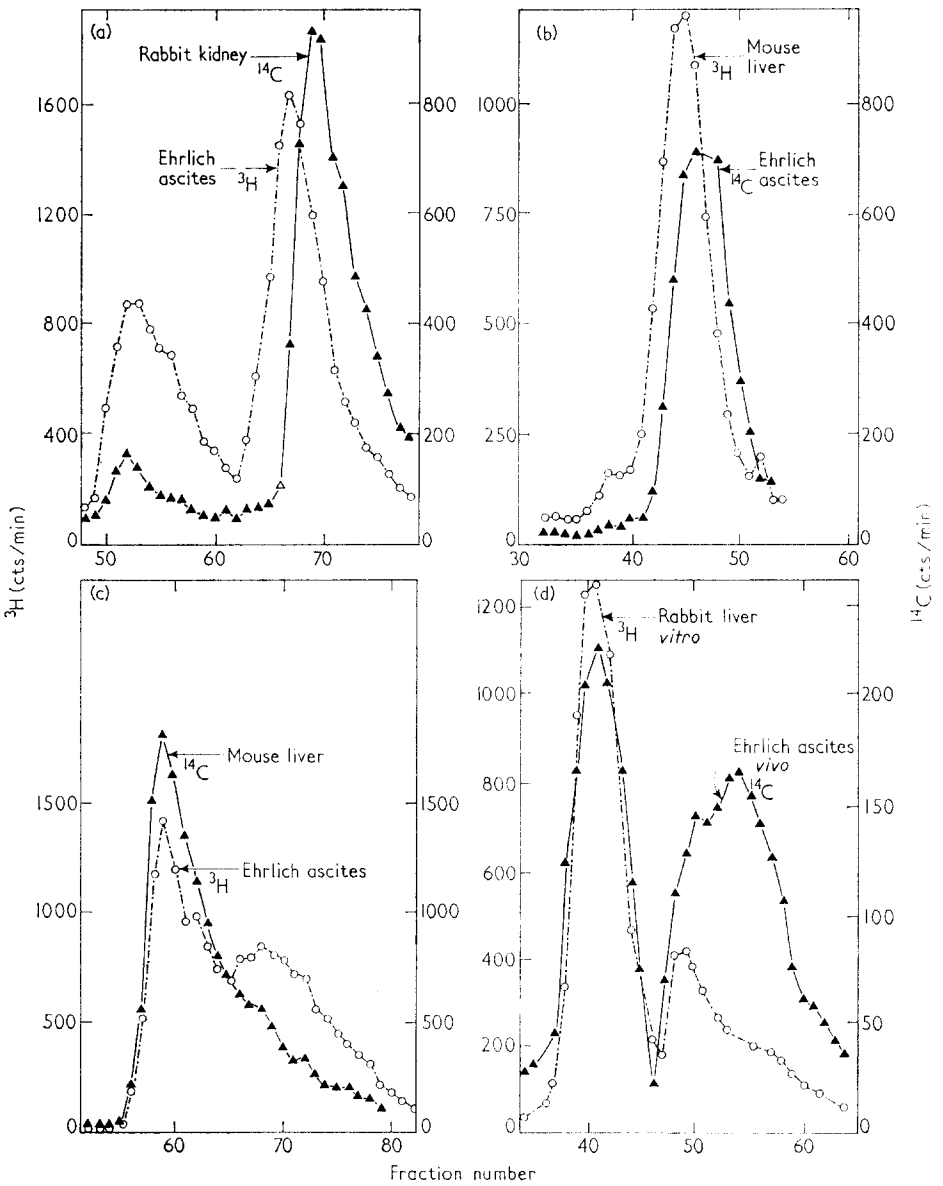


FIG. 5. MAK column elution profiles of Ehrlich ascites (a) phenylalanyl-, (b) seryl-, (c) glycyl- and (d) tyrosyl-tRNA's compared to normal mouse tissue tRNA; note altered profiles.

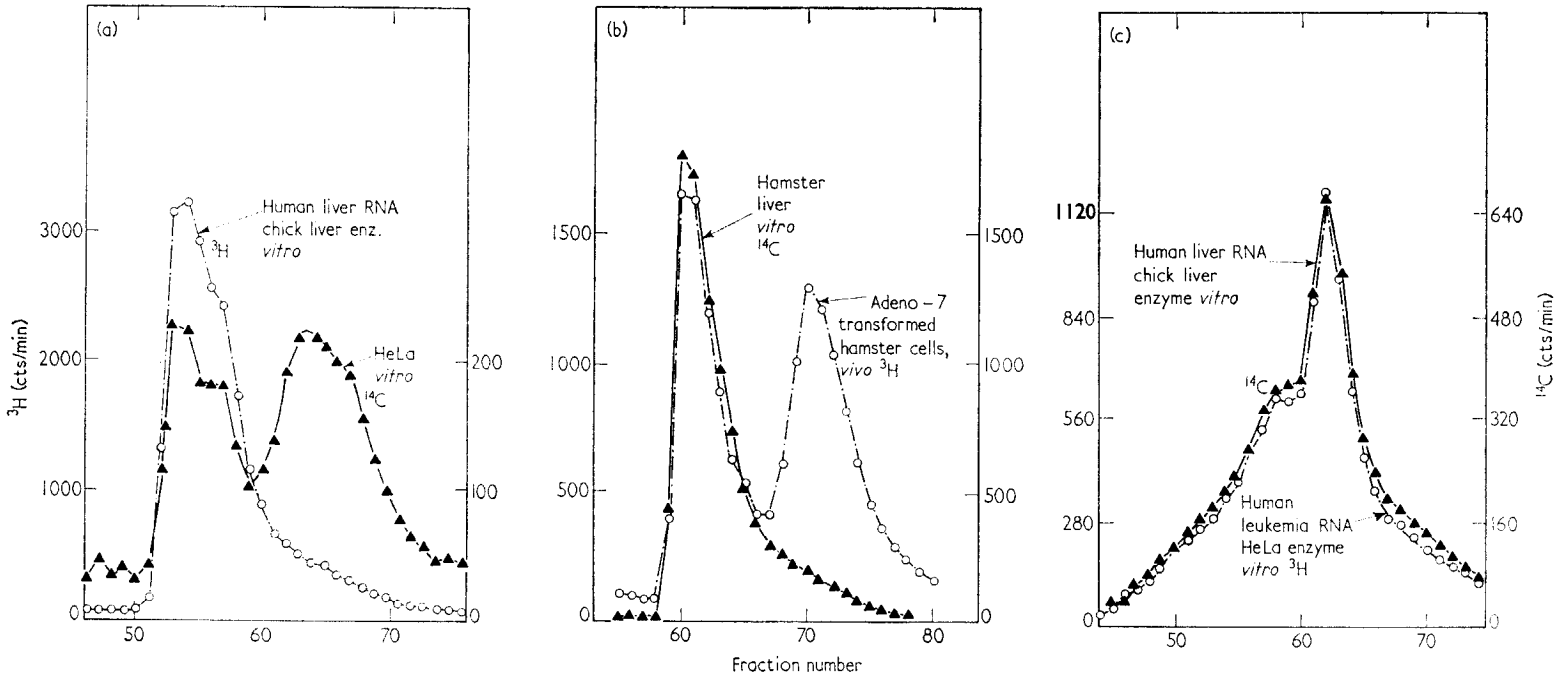
(Fig. 6). In Figure 6(d), (e) and (f) note that the tyrosyl-tRNA of mouse L-cells, Rous virus-transformed hamster cells and simian virus 40-transformed hamster cells all elute later than the normal tissue tRNA. Unlike HeLa, Ehrlich ascites and adeno-7 virus-transformed cell tyrosyl-tRNA, no front peak of labeled tRNA is present.

Except for the Ehrlich ascites tumor cells, which were grown in the peritoneum of mice, all the other tumor cell lines were grown in culture in Eagle's medium. It was possible that these differences noted in the MAK elution profiles of transfer RNA were due to the medium in which the cells were grown (Lazzarini & Santangelo, 1967) or to the phase of growth (Sueoka, Kano-Sueoka & Gartland, 1966). However, sarcoma-1, a mouse tumor line, grown in the peritoneum of mice, harvested after eight days, gave a "normal" profile of tyrosine tRNA (Fig. 7), eliminating the possible influence of the peritoneal environment. That the medium is not responsible for the changes is demonstrated in Figure 7(b), in which mouse liver tyrosyl-tRNA is compared with a tyrosyl-tRNA isolated from the Madin strain of bovine kidney cells, grown in Eagle's medium. The tyrosyl-tRNA of these bovine kidney cells was not "shifted". Figure 7(c) demonstrates that human amniotic cells labeled a few hours after delivery show the same elution profile as human liver tRNA charged *in vitro*. Figure 7(d) and (e) illustrate that human epithelial cells, isolated from the same amnion, and cultured 4 and 20 days, respectively, as monolayers on glass in Eagle's medium retain the same elution profile of tyrosyl-tRNA as a normal organ transfer RNA.

In contrast to the above results, whenever we chromatographed tyrosyl-tRNA from cultures of most "fibroblastic" cells we consistently obtained a difference in the elution profile off MAK of these tyrosyl-tRNA's when compared to tyrosyl-tRNA from epithelial cells or organs. Figure 8(a) shows the elution profile of mouse fibroblast tyrosyl-tRNA ($tRNA_F$) compared to mouse kidney tyrosyl-tRNA. Figure 8(b) shows a similar comparison of hamster embryo fibroblasts after five days of culture with hamster kidney *in vitro*. The front peak in the cultured fibroblasts may be due to the presence of some epithelial cells in the culture. When tyrosyl-tRNA from chick fibroblasts is compared with normal chick organ tRNA a shift in the elution profile is noted, in the direction opposite from that of mammalian fibroblasts. Figure 8(c) and (d) show the progressive dominance of the fibroblastic "type" of tyrosine tRNA with continuous culture. That tyrosyl-$tRNA_F$ is probably identical in chick and mammalian fibroblasts is suggested by Figure 8(e) in which both fibroblastic tyrosyl-tRNA's are superimposed. However, a shift was not noted in the tyrosyl-tRNA from the embryonic human lung fibroblastic cell line (WI-38). These results are presented in more detail elsewhere (Holland, Taylor & Buck, (1967)).

To examine whether the tyrosyl-$tRNA_F$ is unique to fibroblastic cells grown in culture or is present in the tissue of the body in its natural state, we labeled living chick embryo cells with [^{3}H]tyrosine for five minutes. These embryo cells were never grown in culture, but were obtained directly from minced nine-day chick embryo by digestion with 0·25% trypsin for 30 minutes at 37°C. These dispersed cells were filtered through gauze, washed with sterile saline, pulse-labeled and the aminoacyl-transfer RNA extracted with phenol. It can be seen in Figure 8(f) that at least half of the labeled tyrosyl-tRNA obtained in this fashion was of the shifted type.

Gartland & Sueoka (1966) have reported the interconversion of two forms of tryptophanyl-tRNA. The conversion is reversible and dependent on EDTA (Mg^{2+}) concentration, the addition of EDTA to the reaction mixture prior to phenol extraction converting type I to type II. We have performed similar experiments with


(a)
Human liver RNA
chick liver enz.
vitro
^3H
HeLa
vitro
^{14}C
^3H (cts/min)
3000
2000
1000
0
200
100
0
50
60
70
(b)
Hamster
liver
vitro
^{14}C
Adeno – 7
transformed
hamster cells,
vivo ^3H
1500
1000
500
0
60
70
80
Fraction number
(c)
Human liver RNA
chick liver
enzyme vitro
^{14}C
Human
leukemia RNA
HeLa enzyme
vitro ^3H
1120
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280
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480
320
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50
60
70
^{14}C (cts/min)

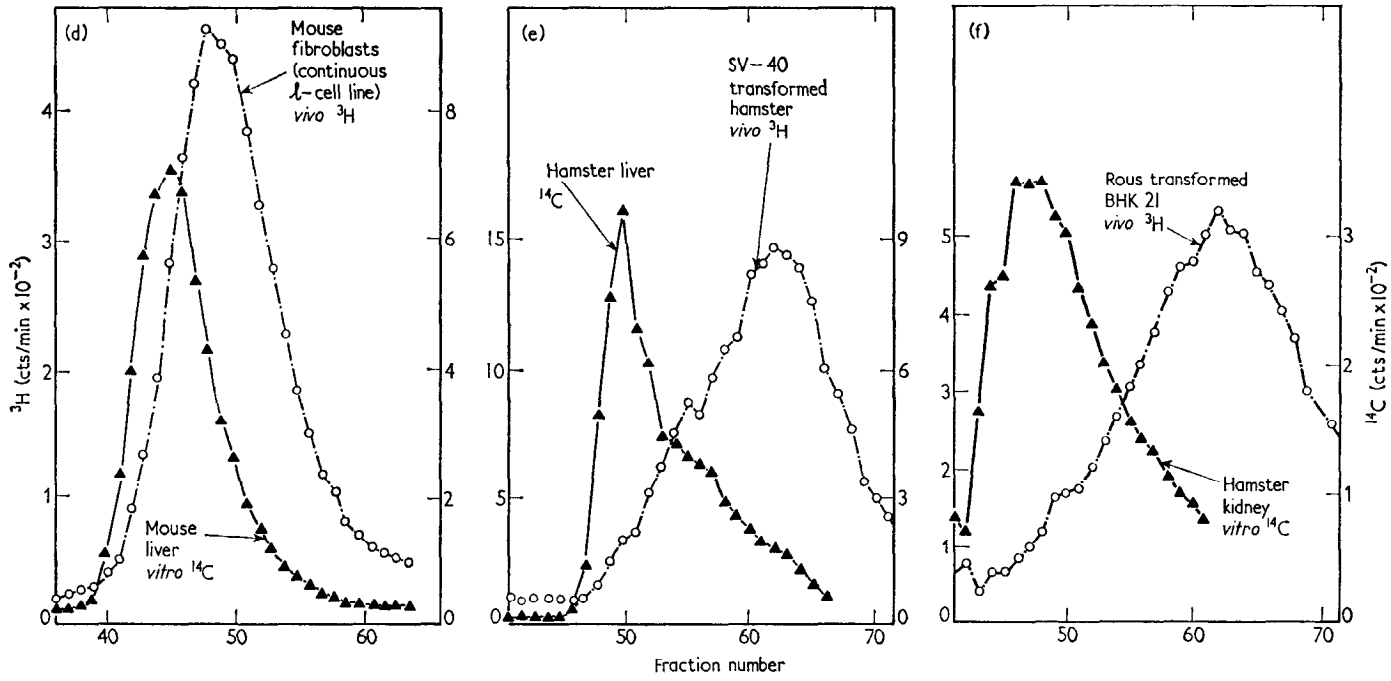
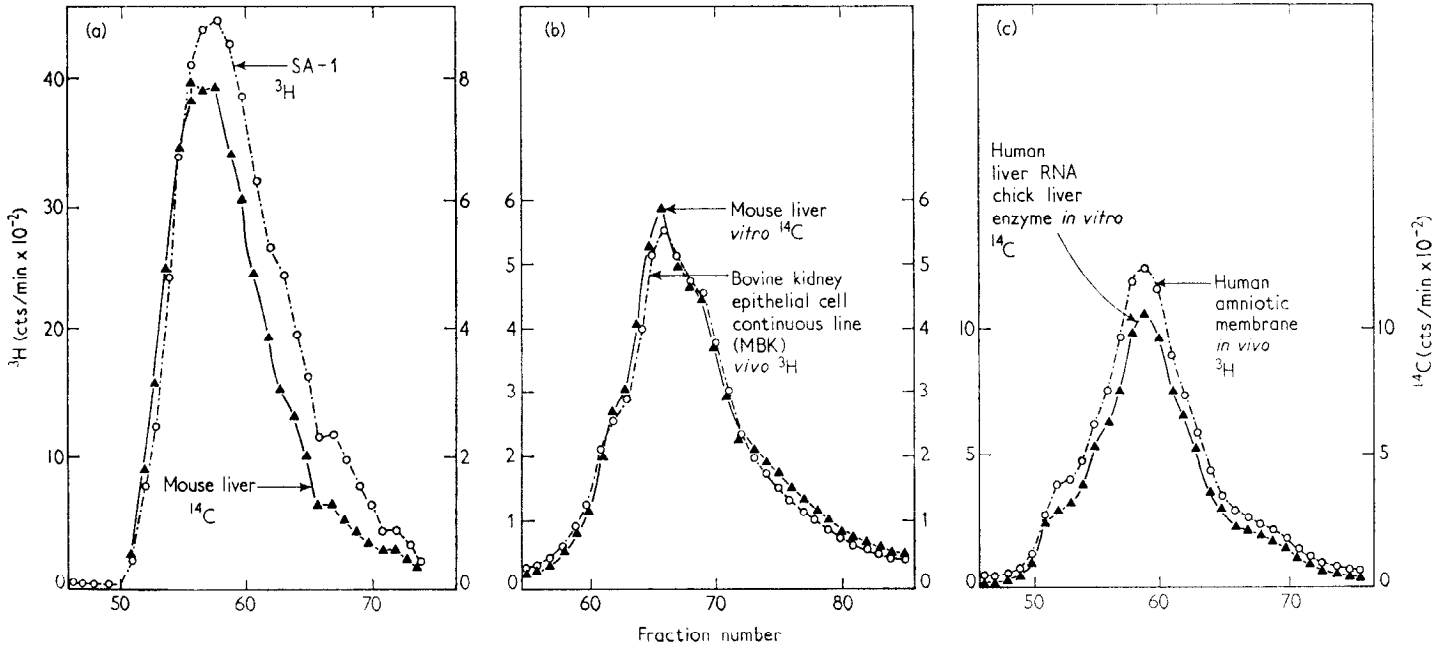


FIG. 6. MAK column elution profiles of tyrosyl-tRNA from various tumor cells.


(a)
SA-1
^{3}H
Mouse liver
^{14}C
^{3}H (cts/min x 10^{-2})
(b)
Mouse liver
vitro ^{14}C
Bovine kidney
epithelial cell
continuous line
(MBK)
vivo ^{3}H
Fraction number
(c)
Human
liver RNA
chick liver
enzyme *in vitro*
^{14}C
Human
amniotic
membrane
in vivo
^{3}H
^{14}C (cts/min x 10^{-2})

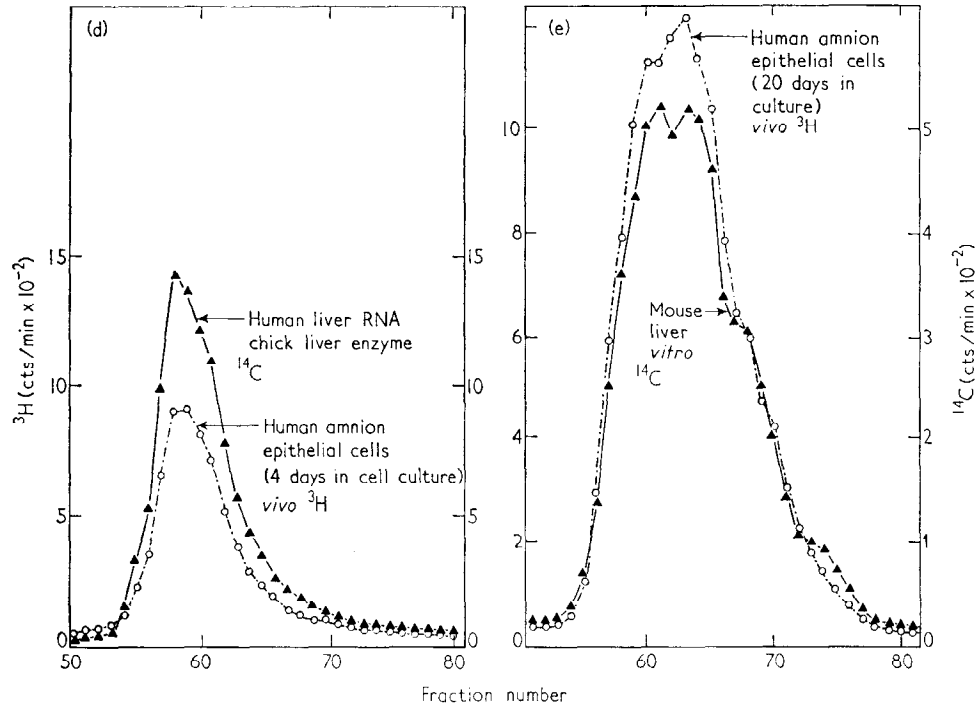
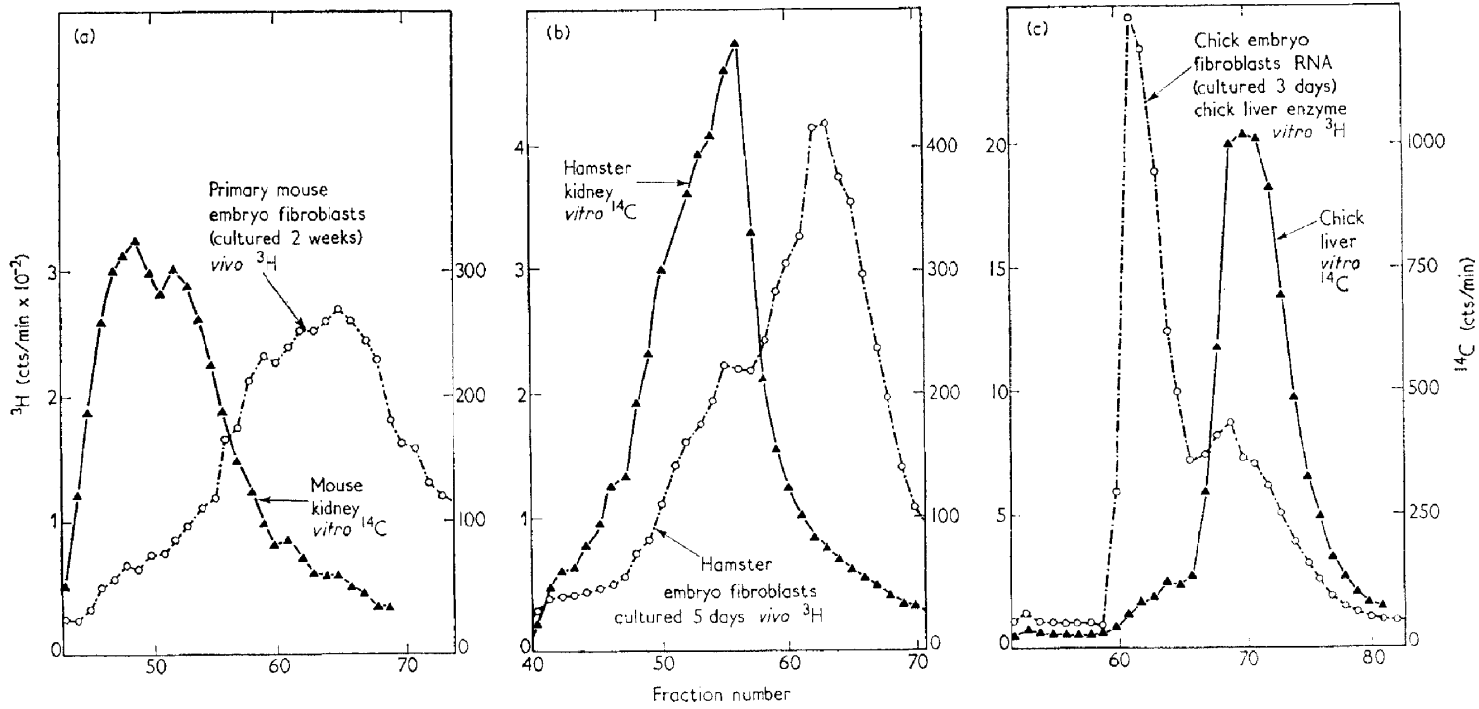


FIG. 7. MAK column elution profile of tyrosyl-tRNA from sarcoma-1 and other cell lines grown in culture, compared to organ tyrosyl-tRNA; noto identical profiles.


(a)
Primary mouse embryo fibroblasts (cultured 2 weeks) vivo ^{3}H
Mouse kidney vitro ^{14}C
^{3}H (cts/min x 10^{-2})
(b)
Hamster kidney vitro ^{14}C
Hamster embryo fibroblasts cultured 5 days vivo ^{3}H
Fraction number
(c)
Chick embryo fibroblasts RNA (cultured 3 days) chick liver enzyme vitro ^{3}H
Chick liver vitro ^{14}C
^{14}C (cts/min)

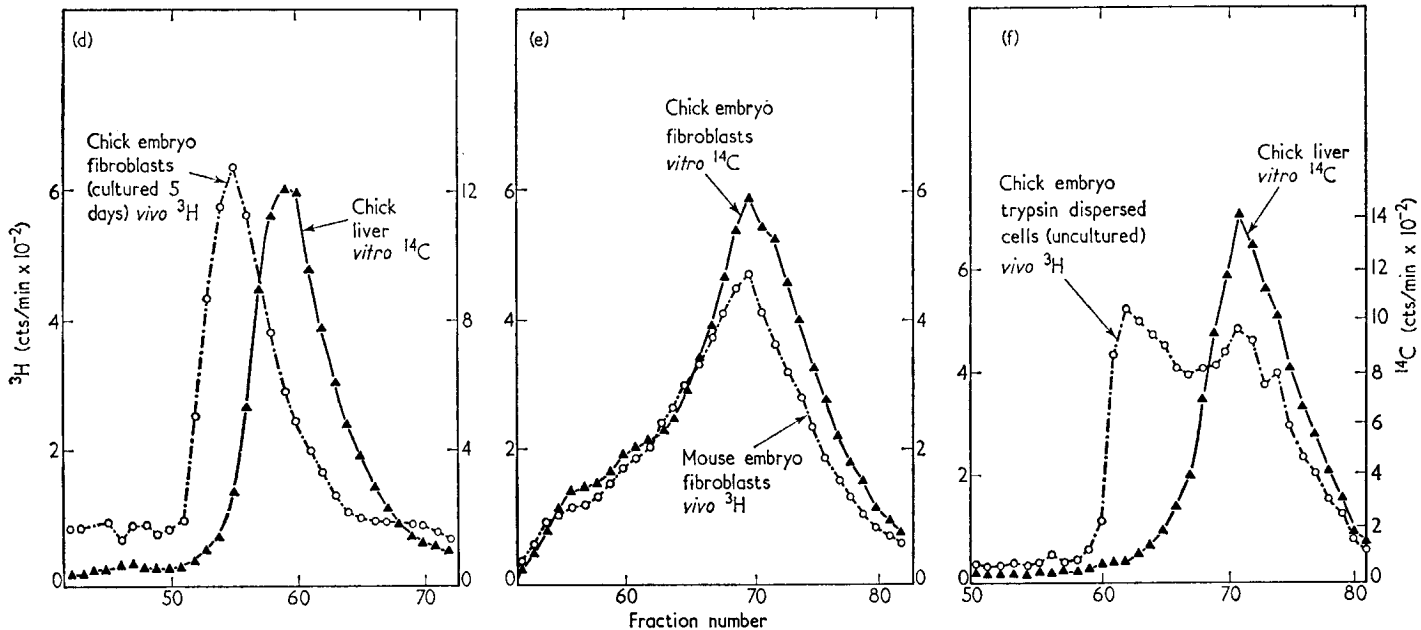


FIG. 8. Tyrosyl-tRNA from primary embryo cell (fibroblasts) propagated in culture ((a), (b), (c) and (d)). (e) Comparison of primary chick embryo fibroblasts and mouse fibroblasts tyrosyl-tRNA. (f) Trypsin released uncultured chick embryo cell; note mixed population of tyrosyl-tRNA.

HeLa tyrosine tRNA. No change in the MAK column profile of tyrosine tRNA was observed under these conditions. Furthermore, [^{3}H]tyrosine tRNA from HeLa and rat muscle was denatured and renatured according to the method of Lindahl, Adams, Geroch & Fresco (1967). Again, no alteration was noted in the MAK column profiles.

4. Discussion

The data presented in this paper demonstrate that most specific transfer RNA's in the completely differentiated mammalian organs show similar elution profiles on MAK columns. It should be noted, however, that this conclusion is based on the chromatographic results of a limited number of aminoacyl transfer RNA's and is dependent upon the resolution obtainable with a methylated albumin–kieselguhr column. Other chromatographic methods would most probably discriminate between other species of tRNA.

All comparisons that demonstrated elution differences were repeated with the radioactive label reversed, to rule out any possible effect of radioisotope labeling or contamination. All the differences in tRNA elution profiles were repeated wherever possible, by *in vivo* acylation of the tRNA and by use of heterologous enzymes. The result of *in vivo* charging and heterologous enzyme charging were consistent with the *in vitro* charging assay.

The basic similarities found in the elution profiles of a number of specific aminoacyl tRNA's from different mammalian species, and the similarities of the lysyl-, threonyl- and phenylalanyl-tRNA's of mammalian and avian origin, point to a preservation of tRNA structure during evolution. Our chromatographic data of mammalian and avian leucyl- and tyrosyl-tRNA show, however, that changes have occurred during evolution in some tRNA species. The lack of cross-acylation between yeast and mammalian tyrosyl-tRNA and charging enzymes on the one hand, and *E. coli* tyrosyl-tRNA and enzyme on the other (Table 2), would suggest at first sight that an alteration of the active site of the *E. coli* enzyme and tyrosyl-tRNA recognition site had occurred during evolution. However, Doctor, Loebel & Kellog (1966) have demonstrated that the primary structure of *E. coli* tyrosine tRNA and of yeast tyrosine tRNA is quite different. It is possible that the yeast and mammalian tyrosine tRNA's have evolved from a completely different species of tRNA than the *E. coli* tyrosyl-tRNA. However, Doctor *et al.* (1966) have found no difference in the coding properties of *E. coli* or yeast tyrosyl-tRNA.

In contrast to the near identity we have noted thus far in the elution profiles of most tRNA's isolated from various mammalian and avian organs, we have found major differences between the elution profile of specific tRNA's from Ehrlich ascites tumor cell tRNA and mouse organ tRNA. It appears that certain specific tRNA molecules in these cells have undergone modification. The relationship of these changes to tumorogenesis, is not clear.

It is clear from the results presented here that there is a reproducibly altered tyrosyl-tRNA ($tRNA_F$) in some fibroblastic type cells when compared to most epithelial or organ tyrosyl-tRNA's. It is also clear that this alteration must occur as a result of differentiation, since fibroblastic and epithelial cells derive from the same zygote. Whether the $tRNA_F$ is due to a modification of the epithelial tRNA (or *vice versa*) or is due to a different tRNA gene is not clear. Despite the difference in elution profile of chick tyrosine tRNA when compared to mammalian tyrosine tRNA, the

elution profile of chick tyrosine $tRNA_F$ is nearly identical to mammalian $tRNA_F$. One might postulate a unique species of tyrosine tRNA coded for by a particular DNA sequence that is repressed in normal epithelial or mature cells. If this shift in elution profile were the result of an enzymic alteration of the already formed tyrosine tRNA, it is unlikely that the profiles of chick fibroblast and mammalian fibroblast would be so nearly identical. However, not all fibroblastic type cells contain tyrosyl $tRNA_F$. A strain of fibroblastic cells of human origin gave the normal tyrosyl-tRNA elution profile.

Transfer RNA has long been known to contain a large number of unusual bases, including pseudouridine (Dunn, Smith & Spahr, 1960), methylated bases (Hall, 1963), and sulfur-containing bases (Lipsett, 1965) among many others. It has been postulated that these bases may have a regulatory role by altering the secondary structure of the molecule. Tsutsui, Srinivasan & Borek (1966) found elevated amounts of tRNA methylating enzymes in various tumors. Peterkofsky, Jesensky & Capra (1966) demonstrated that methyl-deficient *E. coli* leucyl-tRNA has both an altered charging enzyme affinity and codon response. Littauer, Revel & Stern (1966) have shown that methyl-deficient phenylalanine tRNA recognized codons that are not normally read by phenylalanine tRNA. However, it should be noted, that if the changes reported here result from an enzymic alteration of an already formed species of tRNA (as opposed to a DNA-directed alteration), then this alteration must be very specific for only certain species of tRNA (and therefore, for particular nucleotide sequences).

The presence of two species of tyrosine tRNA appears to be specific for certain tumor cells, but other tumor cells do not show this pattern. Whether this unusual tyrosyl-tRNA pattern is involved in transformation by certain viruses, or in certain kinds of tumorogenesis, remains to be ascertained. Work is in progress to determine the coding properties of the tyrosyl-tRNA's and their relationship, if any, to differentiation, viral transformation and viral infection.

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