*SIMILARITIES AND DIFFERENCES AMONG SPECIFIC TRNA'S IN MAMMALIAN TISSUES**

By Milton W. Taylor, G. A. Granger, C. A. Buck,† and J. J. Holland

DEPARTMENT OF MOLECULAR AND CELL BIOLOGY, UNIVERSITY OF CALIFORNIA, (IRVINE)

Communicated by Charles Yanofsky, April 3, 1967

Recent studies[1, 2] on the coding properties of synthetic polynucleotides in mammalian systems, and studies[3] on the binding of aminoacyl transfer RNA from bacteria and diverse animal sources to ribosome-oligonucleotide complexes have clearly demonstrated the general universality of the genetic code. However, we have been examining whether there might be variations in the distribution of particular transfer RNA molecules which could control the relative rates of synthesis of various proteins in animal cells. These studies were suggested as a result of our recent observations[4] that certain small RNA viruses replicate with widely varying efficiency in different animal cells. These observations might be explained by the hypothesis that the RNA of these viruses is translated with differing efficiencies according to the availability in different animal cells of certain transfer RNA molecules necessary for an accurate translation of all the codons in the viral RNA messages.

Kano-Sueoka and Sueoka[5] found a new leucyl-tRNA peak replacing a normal one following T2 phage infection of *E. coli*. Subak-Sharpe *et al.*[6, 7] have reported the formation of a new arginyl tRNA in herpes virus-infected cells. Kaneko and Doi[8] reported a change in the elution pattern of valyl-tRNA from a methylated albumin kieselguhr column during sporulation of *B. subtilis*. Kano-Sueoka and Sueoka[5] have suggested that even minor quantitative variations, or qualitative modifications, of tRNA could drastically alter the relative rates of synthesis of different classes of proteins. Many investigators have considered the possible controlling role in differentiation which could be played by variations or modifications of tRNA.

In the present study, we compared by C^{14}, H^3 double-labeling technique the chromatographic profiles on methylated albumin kieselguhr (MAK) columns of aminoacylated tRNA's from various tissues or organs of the same species, from different animal species, from cultured cells of various origins, and from tumor cells.

We found a remarkable similarity of elution profile of tRNA's from different tissues and even from widely divergent animal species. However, several differences are demonstrated, the most striking of which are the altered elution profiles of a number of tRNA's from mouse tumor cells as compared to normal tissues.

Materials and Methods.—Transfer RNA was extracted from bovine, rabbit, and mouse tissues and from various cells by phenol extraction in 0.15 *M* NaCl, 0.05 *M* Tris, pH 7.5, followed by repeated ethanol precipitation.

Ehrlich ascites tumor cells (EAT cells) and mouse sarcoma-1 cells (Sa-1 cells) were harvested from the peritoneum of mice eight days after intraperitoneal injection of the tumor inoculum. Cell cultures were propagated in Eagle's medium with 7 per cent calf serum as monolayers on glass.

The amino acid-charging enzyme fractions were freed of contaminating amino acids by passing the 30,000 *g* supernatant of a cell or tissue homogenate through

Sephadex G-50. The tRNA's were charged *in vitro* with either H^3- or C^{14}-labeled amino acids according to a modification of the procedure of Yamane and Sueoka,[9] and re-extracted with buffered phenol (pH 5.2) to free them of protein. The C^{14} and H^3 aminoacyl RNA's mixed, precipitated with ethanol three times, and loaded on MAK columns in 0.02 *M* Tris, pH 7.0.

The tRNA's were eluted from the MAK column with a linear saline gradient with an initial concentration of 0.2 *M* NaCl, 0.05 *M* Tris, pH 7.0, and a final concentration of 0.65 *M* NaCl, 0.05 *M* Tris, pH 7.0.

Radiolabeled amino acids: The source and specific activities of the labeled amino acids were as follows. New England Nuclear: C^{14}-alanine (117 mc/mM), C^{14}-glycine (116 mc/mM), C^{14}-leucine (25 mc/mM), C^{14}-serine (120 mc/mM), C^{14}-phenylalanine (393 mc/mM), C^{14}-threonine (156 mc/mM), C^{14}-tyrosine (393 mc/mM), H^3-alanine (40 c/mM), H^3-leucine (5 c/mM), C^{14}-valine (208 mc/mM), H^3-tyrosine (7.96 c/mM). Schwarz BioResearch: C^{14}-lysine (198 mc/mM), H^3-glycine (2.1 c/mM), H^3-lysine (0.48 c/mM), H^3-serine (1.2 c/mM), H^3-phenylalanine (2.5 c/mM), H^3-threonine (0.575 c/mM), H^3-valine (0.87 c/mM).

Results.—Initially, we screened the transfer RNA of a large number of different tissues, or organs from a number of animal species by comparing the relative ratio of aminoacylation of many C^{14} amino acids to the aminoacylation of H^3 phenylalanine in cell-free extracts. The ratios (not presented here) were so nearly identical as to suggest that the relative proportions of the total tRNA's for the various amino acids do not vary significantly among most animal species, nor among tissues or organs in the same animal.

tRNA from different animals and tissues: Transfer RNA's isolated from the brain, liver, kidney, and skeletal muscles of different animal species (bovine, rabbit, mouse, and chick) were compared. The specific tRNA's tested were: alanine, glycine, leucine, lysine, serine, phenylalanine, and threonine. Each tRNA was charged with the homologous enzyme, and in many cases with heterologous enzymes. Typical results of such MAK column chromatographs for each of these amino acids are shown in Figure 1 (*A–H*). It will be noted from Figure 1 that the elution profiles of specific amino acid tRNA's are quite similar irrespective of species or tissue origin. Only the elution profile of serine-tRNA (Fig. 1*G*) showed a clearly discernible difference between organs. A minor peak was present in liver tRNA, but not in kidney or skeletal muscle tRNA. This was true of both mouse and rabbit tRNA (Fig. 2*A*). When the liver tRNA was charged with the kidney aminoacylating enzyme, no front peak was noted (Fig. 2*B*). Nor was a front peak of activity found when the liver enzyme was used with kidney RNA, suggesting a lack of both the tRNA and the cognate enzyme in kidney cells (Fig. 2*C*).

Cell lines: Studies similar to those described above were carried out with tRNA and aminoacylating enzymes isolated from HeLa and MBK cell lines. Although these two cell lines are derived from different species and tissues (human cervical carcinoma and bovine kidney) and have certainly undergone extensive dedifferentiation in tissue culture, no large differences were noted among those amino acid tRNA's examined (alanine, leucine, lysine, phenylalanine, threonine, valine), either between cell lines, or when compared to whole tissues.

To examine whether *in vitro* charging reflected the true *in vivo* state of the charged tRNA, washed intact cells were pulse-labeled for ten minutes with radio-

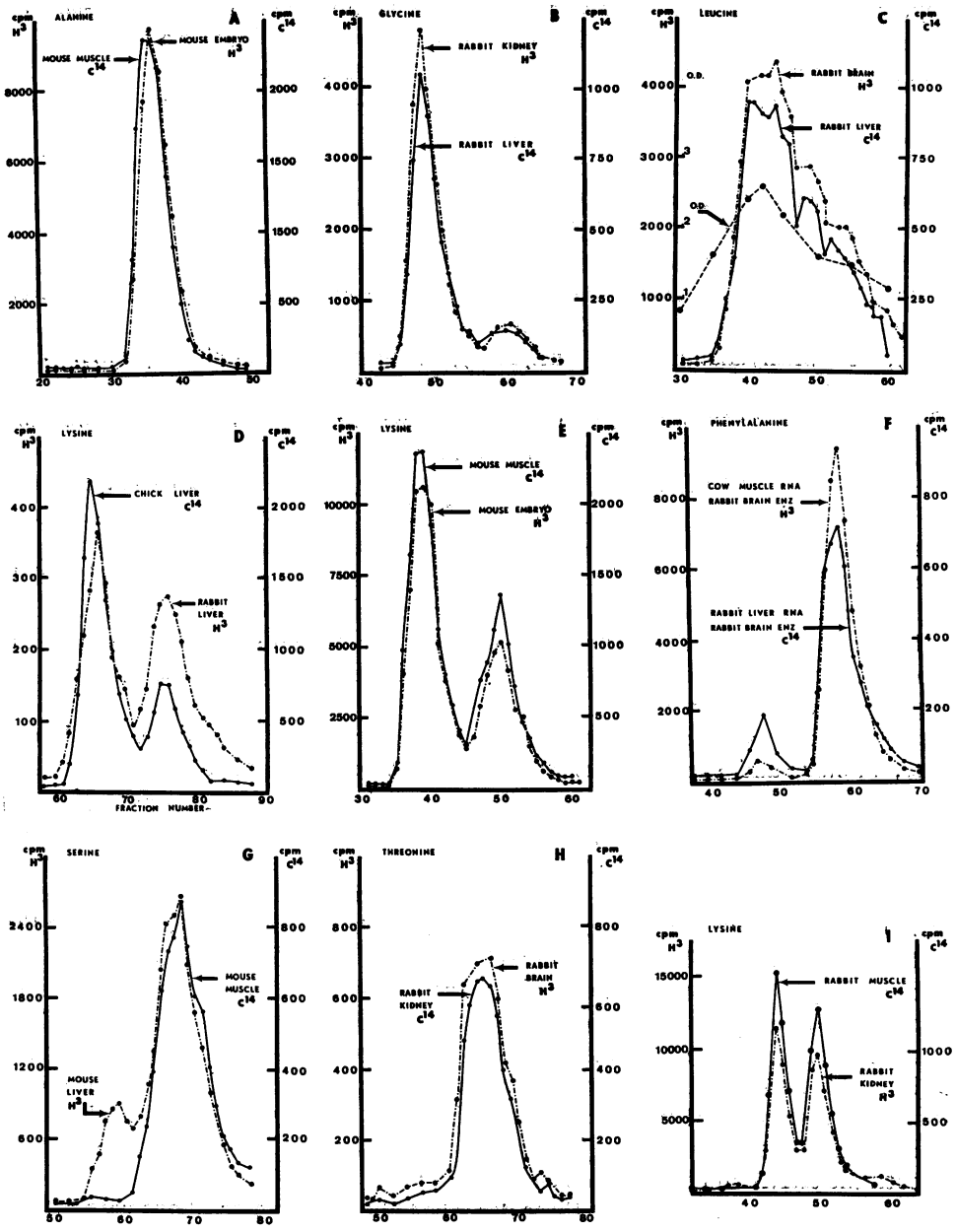

FIG. 1.—Typical elution profiles from MAK columns of amino acid-charged tRNA isolated from various mammalian tissues. Transfer RNA and charging enzyme were prepared as described in *Materials and Methods*. A typical OD profile is illustrated in (*C*). Note in (*G*) the minor front peak in mouse liver serine-tRNA.

active amino acid before phenol extraction of tRNA at pH 5.2. Such *in vivo* (H^3)-charged tRNA was compared with *in vitro* C^{14} tRNA. In all cases examined, both radioactive peaks eluted together from the MAK column (Fig. 3). Various tRNA's from other cell lines were compared: HeLa, MBK, adenovirus-3-transformed

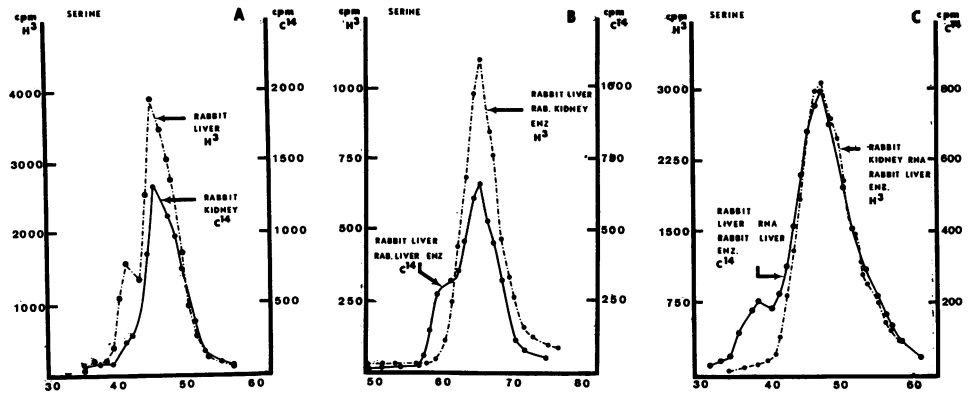

FIG. 2.—Elution profile of serine-tRNA from MAK columns. (*A*) Rabbit liver and kidney transfer RNA charged with homologous enzymes. (*B*) Rabbit liver tRNA charged with heterologous enzyme (kidney). (C) Rabbit kidney RNA charged with heterologous enzyme (liver).

hamster cells and chick-embryo fibroblasts (CE). In general, no differences were noted between the cell lines for those amino acid tRNA's tested (Fig. 3). However, a distinct quantitative difference was observed between glycine-tRNA isolated

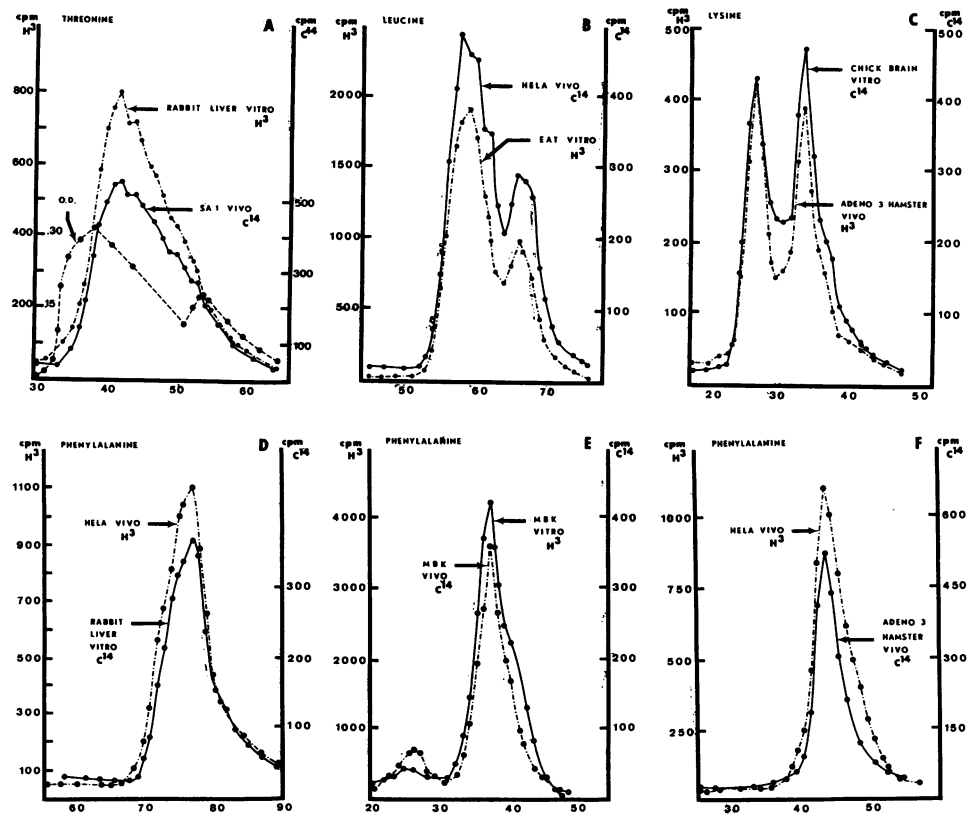

FIG. 3.—Comparison of *in vivo* and *in vitro* charging of amino acids among various cell lines.

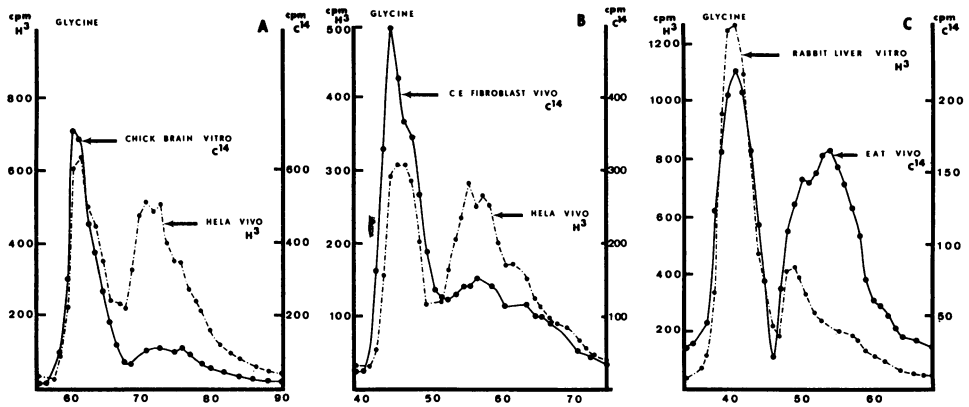

FIG. 4.—Elution profile of glycine tRNA from: (*A*) Hela cells and chick brain. (*B*) Hela cells and chick embryo (CE) fibroblasts. (*C*) Ehrlich ascites tumor (EAT) and rabbit liver.

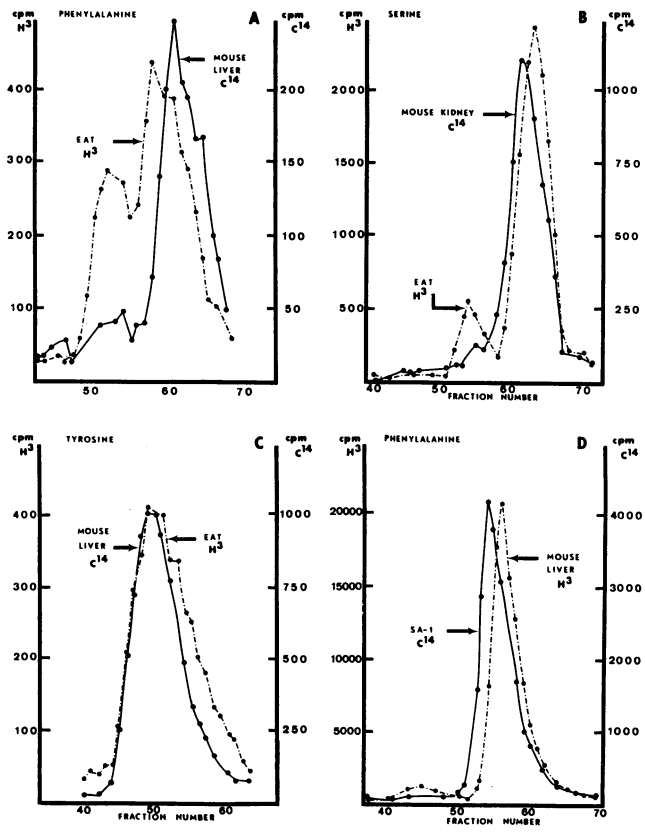

FIG. 5.—Elution profiles of mouse liver and Ehrlich ascites tumor tRNA. (*A*) Phenylalanine-tRNA, (*B*) serine-tRNA, (*C*) tyrosine-tRNA. (*D*) Elution profile of mouse liver phenylalanine tRNA and sarcoma-1 (Sa-1) phenylalanine tRNA. Note "shifts" in elution profile.

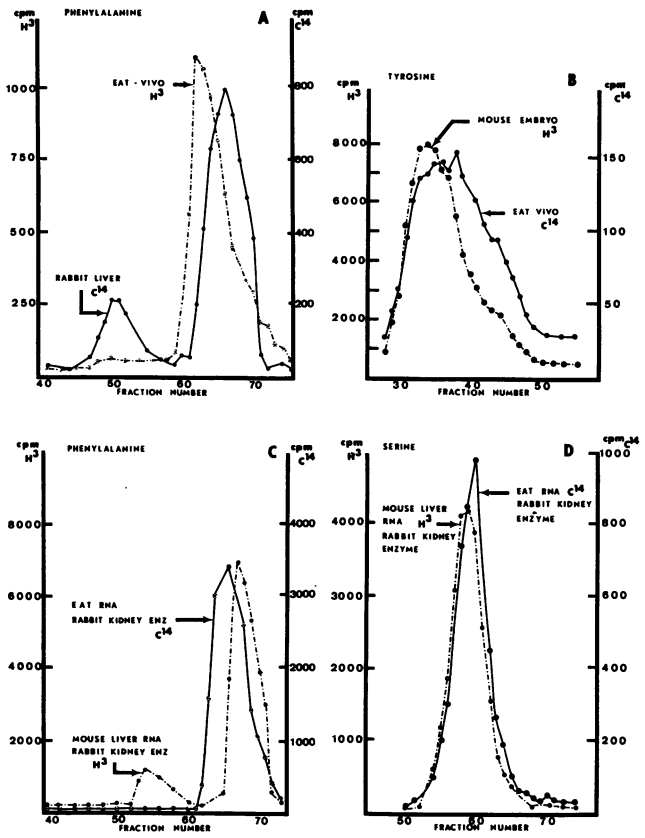

FIG. 6.—(*A*, *B*) *In vivo* labeled EAT transfer RNA compared to normal tissue tRNA. (*C*, *D*) Heterologous charging of EAT phenylalanine and serine-tRNA.

from HeLa cells and chick embryo fibroblasts. With our chromatographic technique, HeLa cells and Ehrlich tumor cells show two major peaks of glycine-tRNA; in contrast, tRNA from chick fibroblasts and from different organs (Fig. 4) exhibit a major peak and a minor one.

Tumor cell tRNA: Transfer RNA was extracted from Ehrlich ascites tumor and mouse sarcoma-1 cells and compared with tRNA from normal mammalian cells or tissues. Of those amino acids examined, the elution profiles of alanine, lysine, threonine, glycine, and leucine-tRNA were nearly identical between the tumor and mouse or rabbit tissues. However, significant differences were found in the elution profiles of phenylalanine- (Fig. 5*A*), serine- (Fig. 5*B*), and tyrosine-tRNA (Fig. 5*C*), between Ehrlich ascites tumor cells and normal tissues. In contrast, Sa-1 tumor cells differed only in phenylalanine-tRNA (Fig. 5*D*). There is a definite shift of the chromatographic positions of each of the above charged tRNA's of the tumor cell tRNA. These differences are apparently not due to a degradative enzyme in the EAT-charging enzyme, as evidenced by the fact that if the tumor cell RNA was labeled *in vivo* (Fig. 6*A*, *B*), or with a rabbit kidney synthetase (Fig. 6*C*, *D*), the shift in elution pattern still existed.

To investigate whether this shift is merely a phenomenon of rapidly dividing

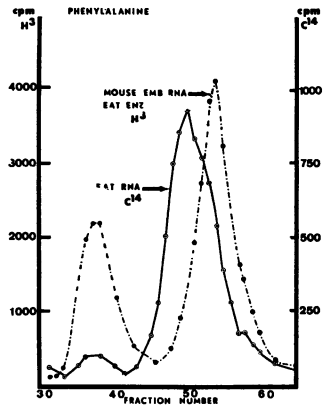

FIG. 7.—Position of primary culture of mouse embryo phenylalanine tRNA relevant to EAT RNA.

cells, primary cultures of rapidly dividing mouse embryo cell tRNA was charged with H^3-phenylalanine-utilizing EAT-activating enzyme from Ehrlich tumor cells. As is illustrated in Figure 7, the shift of EAT phenylalanine transfer RNA persists relevant to mouse embryo tRNA.

Discussion.—Our results demonstrate that at least for those particular tRNA species examined, major differences are not apparent, either at the tissue level or between animal species. This study has so far been confined only to 7 of the possible 20 amino acids, and is limited by the extent of resolution of MAK chromatography. It is possible that many of the major peaks may contain multiple species of tRNA that could be further resolved by other chromatographic methods. In fact, the elution profiles of many major peaks show shoulders which suggest multiple components.

However, our results do suggest that quantitative differences exist in some tRNA's or activating enzymes (e.g., serine-tRNA, glycine-tRNA), both between different tissues and cells from different species. That these differences are not artifacts has been demonstrated by charging specific tRNA's with heterologous enzymes, and/or by *in vivo* charging. In every case where a difference between tRNA's was found, it was confirmed by reversing the C^{14}-, H^3-labeling to the opposite tRNA's and repeating the entire experiment. This ruled out artifacts due to contamination of one of the isotopic forms of any amino acid, or artifacts due to concentration effects arising from differences in specific activity of the H^3- or C^{14}-labeled amino acid. Fractionation of *in vivo* charged tRNA confirms the techniques employed *in vitro*.

The observation that some species of tRNA from Ehrlich ascites tumor and mouse sarcoma-1 tumor are eluted at a salt concentration different from that of the same tRNA found in normal mouse tissue suggests that these tRNA's differ in structure from normal mouse tRNA. They may differ in their complement of methylated[10] or other unusual bases. Tsutsui *et al.*[11] have recently demonstrated that the specific activity of tRNA methylases isolated from a variety of tumors is much greater than the specific activity of the methylases from normal cells. Transfer RNA from such tumor cells is methylated to a much greater degree than that of normal cells. These authors have suggested that such methylation may play an important role in the carcinogenic transformation of normal cells.

Lazzarini and Peterkofsky[12] have demonstrated a "shift" in the elution pattern of leucyl-tRNA from *E. coli* off MAK column grown under methionine deprivation. Under these conditions methylation did not occur. The "shifts" that we have noted in the phenylalanine, serine, and tyrosine tRNA's of EAT origin and in phenylalanine from Sa-1 may be attributed to methylation or to the presence of other unusual bases. However, if this shift is a result of methylation, it is due to selective methylation since all tumor tRNA's do not show shifts. However, whether these alterations in various tRNA's are significant with regard to carcino-

genesis demands additional study. The molecular basis for such shifts in elution patterns and its possible relationship to tumor formation are currently being investigated in our laboratory. In addition, we are studying peptide "fingerprints" of proteins derived from RNA viruses grown in different cells from the same virus inoculum to determine if there are differences in the translation of the viral RNA in different cell lines.

Summary.—Specific transfer RNA's of mammalian origin (from organs and cultured cells) were compared by double-labeling and elution from MAK columns for changes that might have occurred during differentiation. No major differences were detected between tissues or species for alanine, leucine, lysine, phenylalanine, or threonine tRNA. However, differences were observed in the elution profiles of minor tRNA species for glycine and serine. Major differences were observed in the elution patterns of phenylalanine, serine, and tyrosine tRNA derived from Ehrlich ascites tumor cells when compared to normal mouse tissue.

We gratefully acknowledge the skilled technical assistance of Mr. James Massey.

* Supported by a grant from U.S. Public Health Service, NIH grant no. GM CA 14124-01, and by postdoctoral grants no. 1-F2-CA-30,113-01 (MWT); 1-F2-CA-25,607-01(GAG); 5-F2-A1-13,382-02(CAB).

† Present address: Department of Therapeutic Research, University of Pennsylvania, Philadelphia, Pennsylvania 19104.

[1] Maxwell, E. S., these PROCEEDINGS, **48**, 1639 (1962).

[2] Weinstein, I. B., and A. W. Schechter, these PROCEEDINGS, **48**, 1686 (1962).

[3] Marshall, R. E., C. T. Caskey, and M. Nirenberg, *Science*, **155**, 820 (1967).

[4] Buck, C. A., G. A. Granger, M. W. Taylor, and J. J. Holland, *Virology*, in press.

[5] Kano-Sueoka, T., and N. Sueoka, *J. Mol. Biol.*, **20**, 183 (1966).

[6] Subak-Sharpe, H., and J. Hay, *J. Mol. Biol.*, **12**, 924 (1965).

[7] Subak-Sharpe, H., R. Bürk, L. Crawford, J. Morrison, J. Hay, and H. Keir, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31 (1966).

[8] Kaneko, I., and R. H. Doi, these PROCEEDINGS, **55**, 565 (1966).

[9] Yamane, T., and N. Sueoka, these PROCEEDINGS, **50**, 1093 (1963).

[10] Berquist, P. L., and R. E. F. Matthews, *Biochem. J.*, **85**, 305 (1962).

[11] Tsutsue, E. P., R. Srinivasan, and E. Borek, these PROCEEDINGS, **56**, 1003 (1966).

[12] Lazzarini, R. A., and A. Peterkofsky, these PROCEEDINGS, **53**, 549 (1965).