

## DIFFERENCES IN THE TRANSFER RNA'S OF NORMAL LIVER AND NOVIKOFF HEPATOMA\*

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*Abstract.*—A comparison of the elution profiles of 18 aminoacyl-tRNA's from Novikoff hepatoma with those from normal liver on a methylated albumin-kieselguhr column revealed the occurrence of new species of tRNA for histidine, tyrosine, and asparagine in the hepatoma. In addition, the hepatoma tRNA's for arginine, isoleucine, lysine, methionine, serine, alanine, and tryptophan eluted at a higher salt concentration than the corresponding tRNA's of normal liver. The remaining eight amino acids did not show any significant differences in the elution profiles.

Several investigators have observed a marked increase in the tRNA methylases of tumor tissues.<sup>1</sup> Moreover, the tRNA's of certain tumors have been shown to contain higher levels of methylated bases than the tRNA's of the corresponding normal tissue.<sup>2</sup> These findings suggest that there may exist major differences between the tRNA population of tumor and normal cells, as a consequence of increased methylation or of other factors. The possible role of changes in the abundance and specificity of individual tRNA's in cell regulation and differentiation is now widely recognized.<sup>3</sup> Changes in the chromatographic profiles of tRNA have been demonstrated in bacteria after phage infection,<sup>4</sup> during sporulation,<sup>5</sup> and following changes in the growth media.<sup>6</sup> More recently, changes in the tRNA profiles of mammalian cells have been observed after Herpes virus infection,<sup>7</sup> in hamster cells transformed with adenovirus 7 or SV40 virus,<sup>8</sup> and during the feeding of hepatic chemical carcinogens.<sup>9, 10</sup> In an extensive study of mammalian tissues, Taylor *et al.*<sup>8</sup> found that the column elution profiles of tRNA's from different organs were usually similar. However, the elution profiles of phenylalanyl-, seryl-, glycyl-, and tyrosyl-tRNA from Ehrlich ascites tumor differed appreciably from those of the corresponding normal mouse organ tRNA's. Yang and Novelli have also described differences in the seryl-tRNA elution profiles between two mouse plasma cell tumors.<sup>11</sup>

In this communication we report comparisons of the methylated albumin-kieselguhr (MAK) column elution profiles of 18 aminoacyl-tRNA's of Novikoff hepatoma with those of normal rat liver. We have found that this hepatoma contains new tRNA peaks for histidine, tyrosine, and asparagine in addition to those present in normal rat liver. Furthermore, the hepatoma tRNA's for arginine, isoleucine, lysine, methionine, serine, and tryptophan were eluted at a higher salt concentration than the corresponding tRNA's of normal liver.

*Materials and Methods.*—Novikoff hepatoma tissue was harvested from the peritoneum of Holtzman rats 5 to 7 days after inoculation, immediately frozen in liquid nitrogen, and stored at  $-20^{\circ}$ . Transfer RNA was prepared from livers of normal Holtzman rats and from Novikoff hepatoma tissue by slight modifications of the phenol extraction procedure

described by Wevers *et al.*<sup>12</sup> The tRNA was stripped of endogenous amino acids by incubation in 0.2 M glycine buffer (pH 10) for 1 hr at 37°. It was then precipitated by the addition of 0.1 volume of 20% sodium acetate and 2 volumes of ethanol. The precipitated tRNA was dissolved in 0.01 M Tris (pH 7.4), dialyzed overnight against the same buffer, adjusted to 5–10 mg/ml, and stored at –70°.

A crude extract of aminoacyl-tRNA synthetases from normal liver or hepatoma tissue was prepared as previously described.<sup>13</sup> The reaction mixture for the preparation of aminoacyl-tRNA contained, in a total volume of 1.3 ml: 100  $\mu$ moles Tris buffer, pH 7.4; 5  $\mu$ moles  $MgCl_2$ ; 10  $\mu$ moles ATP; 10  $\mu$ c  $^{14}C$  amino acid (specific activity 52 to 394 mc/mmole) or 100  $\mu$ c  $^3H$  amino acid (specific activity 81 mc to 7.9 c/mmole); a mixture of 19 other nonradioactive amino acids, 1  $\mu$ mole each; 23  $\mu$ moles Tris (to neutralize the 19 amino acids); 1 mg tRNA; and 0.4 ml of enzyme. If  $^{14}C$  amino acid was used to charge normal tRNA, then  $^3H$  amino acid was used to charge hepatoma tRNA, or vice versa. With amino acids of very high specific activity, the corresponding unlabeled amino acid was added to raise the total amount to at least 0.03  $\mu$ mole. To enhance the charging reactions for arginine, leucine, phenylalanine, proline, and tryptophan, 5  $\mu$ moles of glutathione were included. Glutathione was also added in the case of asparagine, glutamic acid, isoleucine, and lysine, because it suppressed the background incorporation obtained in the absence of tRNA. After incubation at 37° for 15 min, the reaction mixture was deproteinized with phenol; the aminoacyl tRNA was precipitated from the aqueous layer with 3 volumes of ethanol and was kept at –20° for 1 hr.

The tRNA's of hepatoma and of normal liver labeled with either  $^3H$  or  $^{14}C$  amino acids were suspended in 1 ml of 0.2 M NaCl in 0.05 M sodium phosphate (pH 6.8) and were applied to a MAK column.<sup>9</sup> The column was then washed with 80 ml of the same buffer, followed by a linear gradient of either 0.3 M to 0.7 M NaCl or 0.35 M to 0.6 M NaCl (all in 0.05 M sodium phosphate buffer, pH 6.8). Fractions of 2 ml were collected in a refrigerated fraction collector, and their absorbance at 260  $m\mu$  was measured. The tRNA in each fraction was precipitated with trichloroacetic acid. The precipitate was washed on membrane filters and counted in a liquid scintillation spectrometer with a toluene phosphor.

Reverse-phase freon columns were prepared as described by Kelmers *et al.*<sup>14</sup> The elution scheme is given in Figure 4.

**Results.**—Of the 18 aminoacyl-tRNA's investigated, histidine, tyrosine, and asparagine manifested the most striking differences in tRNA profiles between Novikoff hepatoma and normal liver (Fig. 1). In addition to the major peaks present in normal liver, the tumor contained tRNA peaks for each of these amino acids which eluted at higher salt concentrations from the MAK column than the corresponding normal peaks. The changes observed with histidine, tyrosine, and asparagine were also apparent when the  $^{14}C$ - and  $^3H$ -labeling of the hepatoma and liver tRNA's were reversed.

The presence of novel tRNA's for these three amino acids in hepatoma suggested that altered aminoacyl-tRNA synthetases might also exist in the tumor. This was explored by comparing the MAK elution profiles of histidyl- and tyrosyl-tRNA's of normal liver and hepatoma charged with aminoacyl-tRNA synthetases prepared from the hepatoma. The results shown in Figure 1D, E exclude this possibility for these two amino acids.

In addition to the marked changes in histidyl-, tyrosyl-, and asparaginyl-tRNA's noted above, the elution profiles of seryl-, arginyl-, isoleucyl-, lysyl-, methionyl-, and tryptophanyl-tRNA's of the hepatoma were generally broader and eluted at somewhat higher salt concentration than the corresponding components in normal liver (Fig. 2). It is of interest that both of the lysyl-tRNA's

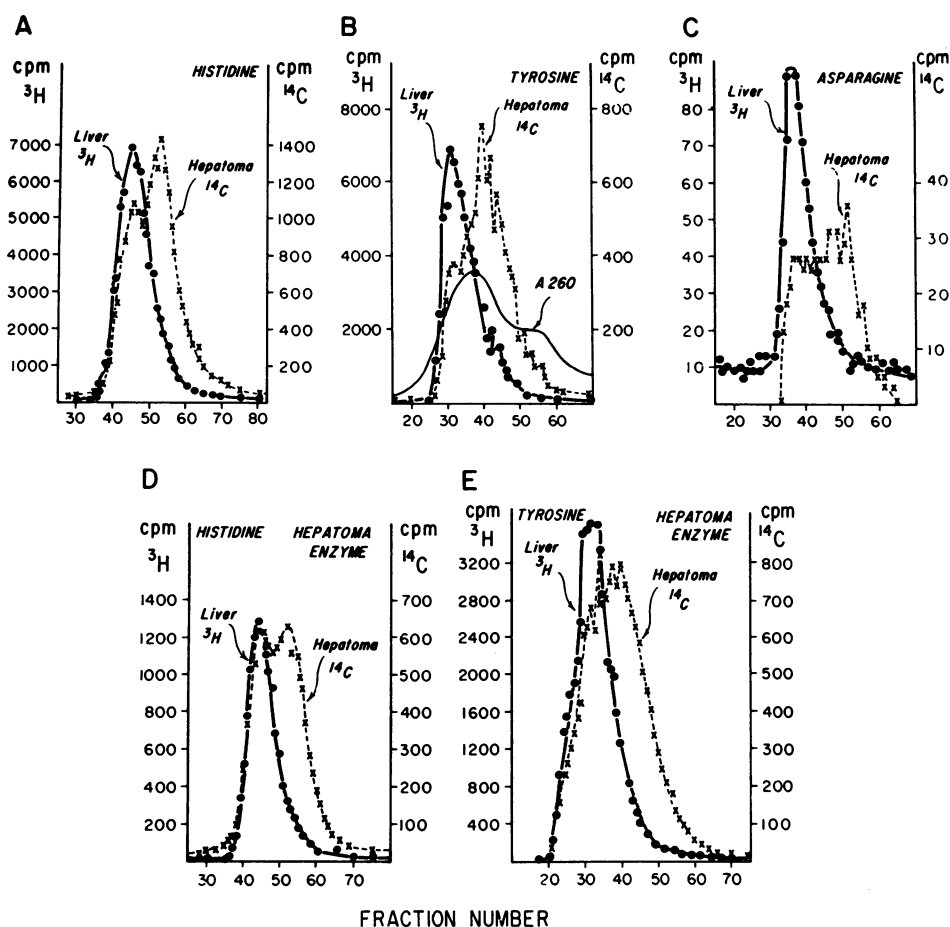


FIG. 1.—MAK-column elution profiles of aminoacyl-tRNA's of normal rat liver and Novikoff hepatoma. Hepatoma enzymes were used to charge the tRNA's in *D* and *E*, and liver enzymes were used for all other profiles. A gradient of 0.3–0.7 *M* NaCl was used in *A*, *B*, and *D*; 0.35–0.6 *M* NaCl was used in *C* and *E*. Typical optical density (A<sub>260</sub>) profile is shown in *B*. For additional details see *Materials and Methods*.

present in normal liver (Fig. 2*D* and ref. 9) showed this type of change in the hepatoma. Presumably the changes described in Figure 2 reflect secondary modifications of tRNA species present in normal liver and/or the appearance of novel tRNA species in the hepatoma (similar to those seen in Fig. 1), which are not well resolved from the normal species by the MAK column.

The remaining nine amino acids that we examined did not reveal any significant differences in the elution profiles between hepatoma and normal liver tRNA's (Fig. 3), except for a slight shift in the elution profile of hepatoma alanyl-tRNA.

Because of the limited resolution of the MAK column, it seemed important to confirm some of these changes in another chromatographic system. Figure 4 indicates that when examined on the reverse-phase freon column of Kelmers *et al.*,<sup>14</sup> tyrosyl-tRNA from the hepatoma contained a component which over-

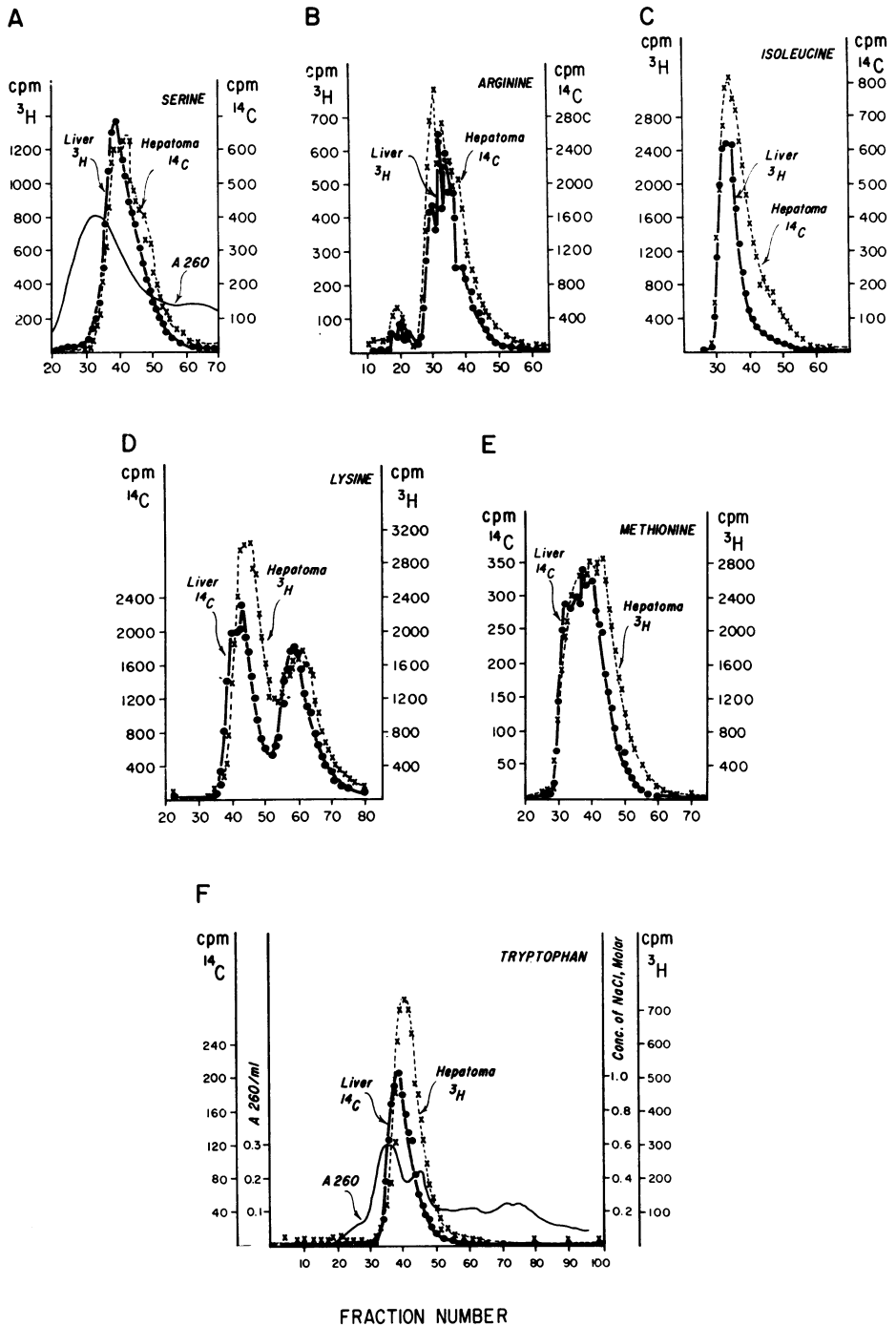


FIG. 2.—Elution profiles of serine, arginine, isoleucine, lysine, methionine, and tryptophan specific tRNA's of hepatoma and normal rat liver. Liver enzymes were used to charge all tRNA's. A gradient of 0.35–0.6 M NaCl was used, except for *F*, in which this gradient was 0.35–1.0 M NaCl.

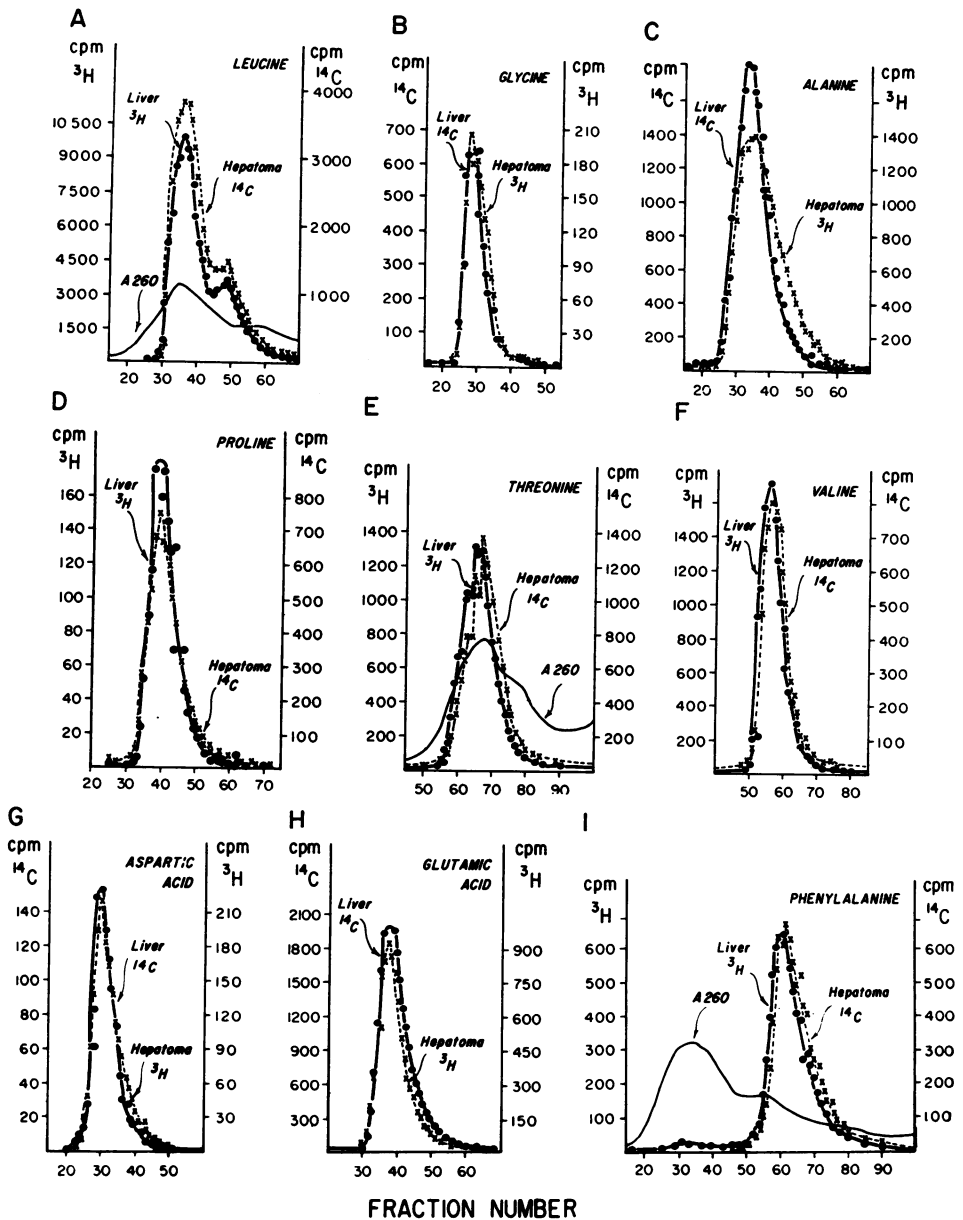


FIG. 3.—MAK-column elution profiles of other amino acid specific tRNA's of rat liver and hepatoma. A gradient of 0.35–0.6 M NaCl was used, except for E, in which the gradient was 0.2–0.7 M.

lapped the tyrosyl-tRNA of normal liver, as well as a second component which eluted distinctly later than the normal tyrosyl tRNA. This change in hepatoma tyrosyl-tRNA elution profile is qualitatively similar to that found in the MAK column. It is of interest that a qualitatively similar change in tyrosyl-tRNA has been described by Taylor *et al.*<sup>8</sup> in Ehrlich ascites tumor cells, in HeLa cells, in adeno-7 virus transformed cells, and in fibroblasts—but not in other tissues.

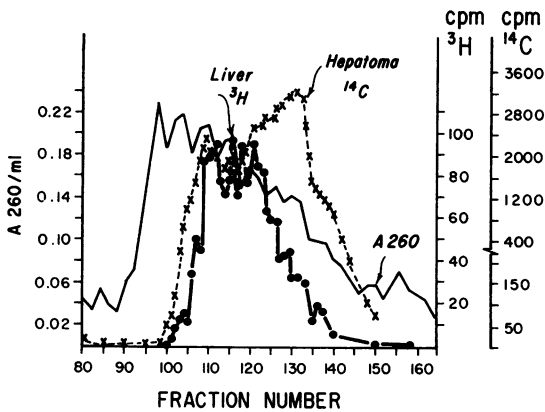


FIG. 4.—RPC-freon column elution profiles of tyrosyl-tRNA's of normal rat liver and Novikoff hepatoma. Liver enzyme was used for charging. The column ( $0.5 \times 150$  cm) was eluted at  $25^{\circ}\text{C}$  with a  $0.15$ – $0.55$   $M$   $\text{NaCl}$  gradient in  $0.05$   $M$  Sodium acetate ( $\text{pH}$  5.0),  $0.05$   $M$   $\text{MgCl}_2$ . Fraction volume was  $4.5$  ml and the flow rate was  $0.5$  ml/min.

*Discussion.*—The results presented here clearly indicate the occurrence in a hepatoma of new species of tRNA specific for histidine, tyrosine, and asparagine, and a shift in the elution profiles of six other amino acid-specific tRNA's. These changes in the tRNA population of a tumor may be due either to the synthesis of new primary sequences, or to secondary modifications of pre-existing species of tRNA's, or to a combination of these factors. Elution patterns of nucleic acids from MAK columns are a function of their size, their GC content and primary sequence, and their secondary structure.<sup>15</sup> The new species of tRNA's for histidine, tyrosine, and asparagine found in the tumor are eluted at a higher salt concentration than the corresponding tRNA's of normal rat liver; the shift in elution profiles of tRNA's for six other amino acids is also in the same direction. Since the *in vitro* tRNA methylase activity of Novikoff hepatoma is known to be about five times higher than that of normal liver,<sup>1</sup> it is possible that preferential methylation *in vivo* of specific tRNA's underlies the changes in elution profiles seen in the present study. Consistent with this explanation is the fact that methyl-deficient *E. coli* phenylalanyl-tRNA elutes from the MAK column at a lower salt concentration than the normally methylated species.<sup>16</sup> Further studies are required to determine whether increased methylation is the sole explanation for the changes seen in the present study or whether other factors are also involved.

In assessing the biological significance of these findings, it must be stressed that the Novikoff hepatoma is a relatively undifferentiated tumor which has been maintained by serial transplantation for many years. It will be of interest, therefore, to determine whether or not changes in the tRNA population also occur in "minimal deviation hepatomas." These studies are now in progress.

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