Polyribosomes in Rat Tissues

I. A Study of in Vivo Patterns in Liver and Hepatomas*

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SUMMARY

The polyribosome patterns of several hepatomas have been compared with that of liver. In contrast to liver the hepatomas are characterized by: (a) a relatively higher proportion of monomers and the lighter polyribosomes, (b) a lower and variable proportion of the heavier polyribosomes, (c) a polyribosome complement which, after release from the cell, sediments through 2.0 M sucrose without detergent treatment, and (d) a greater capacity to maintain their polyribosome pattern during fasting.

Current theory proposes that aggregates of several ribosomes, variously called polyribosomes (47), polysomes (47), or ergosomes (48), rather than single ribosomes, are the functional units of protein synthesis. Supporting evidence has come from amino acid incorporation studies in several laboratories using the following systems: (a) bacteria after infection with phage (42) or after lysis and the addition of synthetic polynucleotides (24, 44); (b) rabbit reticulocytes (4, 20, 47); (c) HeLa cells (28) and rat liver (16, 45, 48). In particular, the striking electron micrographs of polyribosomes in the lysate of reticulocytes (47) and HeLa cells (41) and the refined density gradient studies on a "heavy" ribosome fraction purified from rat liver (48) confirm the existence and emphasize the biological importance of polyribosomes in mammalian tissues.

The recent evidence concerning polyribosome structure and function has led to a modification of the original "messenger" RNA (m-RNA) concept (13). Instead of viewing the machinery for the assemblage of the polypeptide chains as a single ribosome with a single-stranded m-RNA attached, it is now proposed that the functional unit consists of several ribosomes, held in a linear array by a single strand of m-RNA. The growing peptide chain remains attached to the individual ribosomes while the m-RNA, containing the codons, moves relative to the ribosomes (5, 7, 25).

In the present study the cytoplasmic polyribosome pattern of normal rat liver has been compared with that of transplantable multiple- and minimal-deviation hepatomas. The latter, in contradistinction to the former, have been defined (37, 38) as those hepatomas which both morphologically and biochemically approximate the tissue of origin; they are highly differentiated and possess a "marker" enzyme pattern which is at least qualitatively similar to that of normal liver tissue. The minimal-deviation hepatomas can, therefore, be confidently compared with normal liver as the tissue of origin (39). Despite their similarities, the minimal-deviation hepatomas show marked differences from one another and from normal liver in their rates of enzyme induction following various metabolic adaptations (1, 2, 32, 36, 40). There is at present considerable uncertainty whether there is a common defect in the enzyme-forming systems in all the hepatomas or whether there are multiple combinations of defects in the regulatory mechanisms for individual enzymes, or even combinations of enzyme-forming system defects with structural and regulatory defects (33, 40).

One approach to the question of whether there is a common defect in the enzyme-forming system is the study of the characteristics of the polyribosomes, which have been identified as an integral part of the enzyme-forming system. The results of the present investigation demonstrate that the polyribosomes of normal and neoplastic liver differ in pattern, in the type of association or in the proportion associated with the endoplasmic reticulum, and in their response to the stress of fasting.

MATERIALS AND METHODS

The rats were housed in wire-bottomed cages in a room with controlled light from 8 A.M. to 8 P.M. and were normally maintained on a Purina chow diet with water ad libitum. Fasting animals received water only. Fasting was begun at 11:00 P.M., and the animals were sacrificed at 7-11 A.M. Normal liver tissue was obtained from 250-gm. male albino rats of the Holtzman strain (Holtzman Co., Madison). All Morris hepatomas (Hepatomas 5123, 7316, 7793, 7794, and 7795) were carried intramuscularly in Buffalo strain rats obtained from the

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National Cancer Institute. Some of the tumors were carried at the McArdle Memorial Laboratory; others were obtained through the generosity of Dr. H. P. Morris at the National Cancer Institute, who first induced this series of minimal-deviation tumors (22, 23). The Novikoff hepatoma (26) was derived from strain N1-S1 cells carried in tissue culture by Dr. P. A. Morse, Jr.; the cells were inoculated intraperitoneally into Holtzman rats.

Purified C ribosomes containing more than 90 per cent heavy ribosomes (S > 120) were prepared from normal liver, various hepatomas, and the corresponding host livers, according to the procedure of Wettstein, Staehelin, and Noll (48). In general, livers were obtained from anesthetized rats after first perfusing the liver with cold 0.25 M sucrose in TKM buffer (0.05 M Tris (hydroxymethyl)aminomethane, 0.025 M KCl, and 0.005 M MgCl₂, pH 7.5). Similarly, after removal the tumors were chilled, then dissected free of blood clots and any necrotic or fibro-fatty tissue present. All subsequent operations, including homogenization in 0.25 M sucrose, were performed near 0°C.

The C ribosomes were prepared from the post-mitochondrial supernatant (S₂₀) after the addition of 1.25 per cent deoxycholate to free the ribosomes bound to the endoplasmic reticulum (14). The S₂₀ deoxycholate was centrifuged through a double layer of sucrose (4 ml. of 0.5 M over 3 ml. of 2.0 M) in TKM buffer at 105,000 X g for 4 hours in a No. 40 rotor of a Spinco centrifuge. In the upper one-half of the tube was wiped with tissue paper. The C ribosome pellet was resuspended in a volume of TKM buffer equal to about one-half the original volume so that an evaluation could be made of the relative changes in the polyribosome pattern. It was determined (cf. "Results") that polyribosomes as small as the pentamers (those consisting of 5 ribosomal units) and possibly tetramers (4 units) were quantitatively recovered. Since the hexamer peak (6 units) was clearly discernible in all tracings, and since the individual peaks were reasonably symmetrical, the relative concentration of the other polyribosome species could be expressed approximately, as the ratio of the observed height (in O.D. units) of the peak in question to the observed height of the hexamer peak; it must be noted, however, that the observed height of the peaks is in reality an aggregate height, as a result of the partial overlap of the neighboring peaks. This lack of resolution obviated the integration of each peak by a common procedure (21).

A second standard of comparison was based on equivalent area. The tracings were replotted in terms of O.D. units (0.375 inches/O.D. unit) and ml. of eluate (0.375 inches/ml). The reference area (A₀) bounded by the curve extending from the center of the pentamer peak to the bottom of the tube was estimated to 0.01 sq. inches using a compensating polar planimeter (cf. Chart 1). From the relation n₀ = kₙ × A₀, where n₀ was the observed height in O.D. units and kₙ = a constant for a given peak 'n' and a given set of experimental conditions, an average value of kₙ was calculated from ten tracings representing normal liver from ten different rats following an 8-hour fast; this was done for a number of peaks ranging from the monomers (n = 1) near the top of the gradient to the aggregates of approximately fifteen ribosomes (n = 15) near the bottom. The experimentally observed area (Aₙ) was determined from tracings representing the polyribosome pattern of hepatoma and host liver in addition to liver under various experimental conditions, in a manner identical to that used to determine A₀. Thus, by multiplying A₀ by the various values of k determined for normal liver, the height of the corresponding peaks in a tracing for normal liver are obtained under conditions where Aₙ = A₀. A comparison of the height of the various peaks in any tracing with the height of the corresponding peak of normal liver calculated on the basis of equivalent area provides a measure of the deviation from normal.

To facilitate the presentation of experimental results, the ratio of the experimentally observed peak height n₀ (containing 'n' ribosomal units) to the experimentally observed height of the hexamer peak (6ₙ) will be expressed as n₀/6ₙ; similarly, the ratio of the height of any peak 'n' to the corresponding peak calculated for normal liver on the basis of equivalent area will be expressed as n₀/nₙ. The ratio n₀/Aₙ will be referred to as the distribution ratio.

Under the conditions employed polyribosomes consisting of more than ten ribosomal units were not separated into discernible peaks by the density gradient centrifugation routine; often seven to eight peaks was the limit. The mean positions of polyribosomes containing twelve and fifteen units was estimated by extrapolating a plot of the nominal values of 'n' from 1 to 10 vs. the mean distance (averaged from a number of tracings) each of these peaks had sedimented in the gradient. Since the sedimentation behavior of these heavy polyribosomes deviates from the more uniform behavior established by the lighter species....
(cf. 4, 41), the values obtained by extrapolation are only approximate.

RESULTS

Normal C ribosome pattern.—The C ribosomes (48) were first purified from the S₁₀ of rat liver or tumor cytoplasmic fraction, then fractionated by density gradient centrifugation. The purification procedure requires centrifuging the S₁₀ through a double layer of sucrose in which the concentration of the top and bottom layers are 0.5 M and 2.0 M, respectively. The bulk of the monomers and possibly some of the lighter polyribosome fractions do not sediment through the 2.0 M sucrose layer and are removed with the supernatant sucrose. Thus, the pattern of the C ribosomes is not entirely typical of the tissue from which they were prepared. Despite this limitation, the method offers several distinct advantages for the study of the in vivo concentration or distribution of polyribosomes in rat liver or hepatoma.

The C ribosomes are freed of a number of substances (e.g., ferritin) found in the S₁₀ of liver, which would ordinarily interfere with the ultraviolet scan of the polyribosome distribution within the sucrose gradient. Also, these purified ribosomes have a low weight ratio of protein/RNA (<1), and are concentrated into a pellet which, in contrast to that of impure preparations obtained, for example, by reducing the sucrose concentration in the bottom layer to 1.0 M, dissolves readily in buffer (48). Furthermore, should the monomers (and possibly the dimers) be quantitatively present during density gradient centrifugation, their peaks would usually be so much larger than the individual peaks of the heavier polyribosomes that they would seriously modify the polyribosome pattern in the heavier region of the spectrum. In addition, results to be presented suggest that the fraction of monomers (and dimers) recovered in the C ribosome pellet bears proportionality to the amount initially present in the S₁₀.

A typical record of the polyribosome distribution in a preparation of C ribosomes, prepared from rat liver after a 9-hour fast to reduce liver glycogen, is shown in Chart 1. A somewhat similar pattern is obtained when an exponential gradient is used (48). Only six peaks are discernible in this tracing, although up to ten have been observed under other conditions; the number 'n' assigned to each peak is nominal and refers to the number of somal units in each polyribosome. The approximate positions of the peaks containing twelve and fifteen units per polyribosome were estimated as described under "Methods."

The first peak consists of monomers (n = 1) which have been reported (48) to have a sedimentation constant of approximately 73. When the tracing is replotted in terms of O.D. units the area A, bounded by the curve from the center of peak 5 and the bottom of the gradient, is constant for any set of conditions and is used in calculating the kₐ for the various peaks (cf. "Methods"). The polyribosomes located within this region account for approximately 60-70 per cent of the ribosomes of normal rat liver (45).

Effect of centrifugation time.—Normally the C ribosomes are prepared by centrifuging the deoxycholate-treated S₁₀ through the double layer of sucrose at 105,000 X g (average) for 4 hours. The results in Table 1 demonstrate the effect of varying the time of centrifugation on the distribution ratio. The data for the 4-hour run, which are based on the averages from ten rats, are in fact the standard k values for normal liver; the data for 9-hour and 14-hour runs are from two other independent experiments. There appears to be no extensive breakdown of the polyribosome structures, even after 14 hours' centrifugation, since the distribution ratios for the heavier polyribosomes (n = 8, 12, and 15) remain essentially constant. The increase in the ratios of the lighter fractions is significant and could arise from very slight breakdown throughout the heavier region but is probably due, at least in part, to further sedimentation of the former.

Effect of sucrose concentration.—The effect of varying the sucrose concentration in the bottom layer, during the preparation of the C ribosomes from normal rat liver (11-hour fast), on the recovery of the components in peaks 1 to 5 (cf. Chart 1) was studied. The results shown in Chart 2 are presented as the ratio of the observed height of peak n to the height of peak 6 in the same tracing. Apparently only a small fraction of the monomers and dimers are recovered when the bottom layer is equal to, or greater than, 2.0 M sucrose. Lowering the sucrose concentration to 1.5 M permits a larger proportion of the monomers and dimers to pass into the pellet; however, the

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**TABLE 1**

<table>
<thead>
<tr>
<th>kₐ/Aₐ after centrifuging for:</th>
<th>4 hr.</th>
<th>9 hr.</th>
<th>14 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.23 ± 0.04</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td>2</td>
<td>0.28 ± 0.08</td>
<td>0.30</td>
<td>0.38</td>
</tr>
<tr>
<td>4</td>
<td>0.35 ± 0.06</td>
<td>0.30</td>
<td>0.42</td>
</tr>
<tr>
<td>6</td>
<td>0.50 ± 0.04</td>
<td>0.47</td>
<td>0.58</td>
</tr>
<tr>
<td>8</td>
<td>0.60 ± 0.03</td>
<td>0.58</td>
<td>0.66</td>
</tr>
<tr>
<td>12</td>
<td>0.57 ± 0.04</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>15</td>
<td>0.42 ± 0.04</td>
<td>0.47</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* Average values for k = nₐ/Aₐ from ten rats ± mean deviation.

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**CHART 1**

C ribosome pattern of normal rat liver after an 8-hour fast. Input = 0.8 mg. RNA.
TABLE 2

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Hr. fast</th>
<th>Per cent recovery (−DOC+/+DOC)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal liver</td>
<td>8</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>30</td>
</tr>
<tr>
<td>Hepatoma 5123 A</td>
<td>9</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>nil</td>
<td>86</td>
</tr>
<tr>
<td>Hepatoma 5123 C</td>
<td>nil</td>
<td>107</td>
</tr>
<tr>
<td>Hepatoma 7793</td>
<td>nil</td>
<td>112</td>
</tr>
<tr>
<td>Hepatoma 7794 A</td>
<td>nil</td>
<td>100</td>
</tr>
<tr>
<td>Hepatoma 7794 A</td>
<td>nil</td>
<td>100</td>
</tr>
<tr>
<td>Novikoff hepatoma</td>
<td>nil</td>
<td>120</td>
</tr>
</tbody>
</table>

* Recovery measured in O.D. units.

CHART 2.—Effect of varying the sucrose concentration in the bottom layer during the preparation of the C ribosomes, on the recovery of the lighter fractions. Ribosomes prepared from normal liver.

Contamination of these peaks (especially the monomer peak) by ultraviolet-absorbing impurities also increases. A considerable error is introduced into the estimation of the true heights by lowering the sucrose concentration still further. Most of the trimers \((n = 3)\) and tetrers \((n = 4)\) sediment in the C ribosome fraction; part of the apparent increase in the ratios observed in shifting from 2.0 M to 1.5 M sucrose or lower is a result of the large increase in the height of the monomer and dimer peaks. The recovery of the pentamers is essentially quantitative, since the ratio \(5\beta/6\beta\) changes very little when the sucrose concentration is reduced from 3.0 to 1.5 M.

It will be noted that between sucrose concentrations of 3.0 and 2.0 M inclusive, a large fraction of the monomers and dimers sediment independently of the sucrose concentration. It would appear that the sedimentation of these species through these higher densities of sucrose is dependent upon the geometry of the angle rotor used. For example, convection currents are known to be produced in the angle rotor which favor sedimentation along the outer wall of the cylindrical tube (31). Also, because the tube is not sector-shaped, the radial direction of sedimentation will cause the particle concentration within the main part of the fluid to decrease, with the concomitant formation of crescents of particles along the sides of the tube (3); it is highly probable that these crescents of particles would sediment as aggregates (46). A slightly smaller proportion of the monomers appeared to sediment when a swinging bucket-type rotor was substituted.

As might be expected the amount of monomers and dimers which enter the C ribosome pellet bears some relation to the concentration of these particles in the \(S_2\). In one experiment 85–90 per cent of the polyribosomes in the \(S_2\) were converted to monomers and dimers by treatment with ribonuclease. Aliquots of \(S_2 + \text{DOC}\) were diluted to 2 or 3 volumes with the homogenizing medium, and the C ribosomes were prepared in the usual manner. From a density gradient analysis of the latter it was determined that the ratio of the heights of the dimer peaks recovered from the \(S_2\) diluted 1:1, 1:2, and 1:3 was 1.00:0.48:0.34, and of the monomer peaks 1.00:0.77:0.55. This proportionality is lost if the concentration of the ribosomes is high and aggregation occurs. Similar considerations apply to the density gradient analysis. Although the SW25 rotor is of the swinging bucket type, the tubes are nonsectoral in shape. It was determined, however, that the quantity of material (in O.D. units) measured at any point within the gradient was reasonably proportional to the amount of the given C ribosome preparation which was layered on the gradient. Apparently the particles hitting the sidewalls are effectively removed by rapid sedimentation to the bottom of the tube or sediment at the same rate as those moving freely within the fluid (3).

Effect of omitting deoxycholate treatment.—Deoxycholate has become a standard reagent for the release of bound ribosomes of the microsome fraction (14, 19, 27) and is an integral step in the preparation of C ribosomes from rat liver (48). However, very little is known at present concerning the relationship between bound and free ribosomes in normal liver, or whether any profound changes occur in this relationship during the neoplastic transformation. A study has been made of the effect of omitting the deoxycholate treatment during the preparation of the C ribosomes from normal liver and hepatoma. The results, shown in Table 2, are expressed as the ratio of the number of O.D. units, recovered from a modified C ribosome preparation in which deoxycholate is omitted, to those recovered from the same volume of \(S_2\) by the standard procedure. They show that the proportion recovered from normal liver in the absence of deoxycholate varied...
from about 25 to 50 per cent, whereas the recovery from the various hepatomas studied was essentially quantitative. It is not known whether the slightly lower recovery from the Hepatoma 5123 A is significant. Recoveries in excess of 100 per cent may be the result of sedimentation of ultraviolet-absorbing contaminants ordinarily solubilized by deoxycholate treatment or to sedimentation of a larger fraction of the monomers in combination with such contaminants. Results to be presented support the former suggestion.

Thus, aside from the monomers and dimers which are not quantitatively recovered in the C ribosome fraction and which are presumed to be free in the cytoplasm, more than 50 per cent of polyribosomes in normal liver appear to be tightly bound to the endoplasmic reticulum. In contrast, few appear to be bound in such a manner in the hepatomas examined.

**C ribosome pattern of hepatomas.**—The density gradient patterns of C ribosomes prepared in the usual manner from rat liver and several hepatomas are shown in Series A, Chart 3. The patterns in Series B represent C ribosomes prepared simultaneously from the same volume of the S2 used to obtain the patterns for each tissue in Series A, but without the addition of deoxycholate. Each pattern in Series A is therefore strictly comparable to the pattern for the same tissue in Series B. The pattern of the normal liver in Series A differs from that of Chart 1 insofar as the longer (11-hr.) fast has resulted in a small increase in the lighter fractions at the expense of the heavier polyribosomes. Although the hepatomas are from nonfasted animals, it has been established that an 8- to 12-hour fast does not result in any measurable change in pattern.

The C ribosome patterns of the various hepatomas shown in Series A differ markedly from that of normal liver of the same series. Although there does not appear to be a standard tumor pattern, they have, in general, a smaller proportion of the heavier polyribosomes and a larger proportion of the lighter fractions. A significantly higher proportion of the monomers and dimers appears to be present in all tumors studied thus far, and in lieu of corrections for the nonsedimentable fraction the dimer peak is always higher than the monomer peak. With the exception of the higher monomer and dimer peaks, the pattern of the Morris 5123 A hepatoma closely approximates that of normal liver; the $n_m/n_s$ ratios reported below substantiate this observation.

The Hepatoma 7794, with its small complement of heavy polyribosomes, resembles liver least. This appears to be the true pattern of this tissue and not the result of higher nuclease activity. For example, when normal liver was homogenized in the post-microsomal supernatant (S2) of Hepatoma 7794, no significant breakdown occurred; also, when the homogenates were combined the pattern obtained appeared to be a composite of both tissues.

Although the Novikoff hepatoma may not be derived from the liver parenchyma (35), its pattern is similar in many respects to that of the minimal-deviation Hepatoma 5123 A. The polyribosome pattern of host liver (not shown) approximated that of normal liver. This was particularly true of the host liver from animals bearing Hepatomas 7794 and 7795. The host liver of animals bearing the Hepatoma 5123 usually had fewer heavy polyribosomes than normal liver, probably as a consequence of a lower food intake; also, the monomer peak was usually much higher, since this host liver contained a high concentration of ferritin.

A comparison of the patterns in Series A and Series B seems to substantiate the recovery data presented in Table 2. Apart from the discrepancy in recovery the pattern for normal liver of Series A bears some resemblance to the pattern in Series B. It is not clear, at present, why the monomer peak is higher in Series B. The tumor patterns are similar whether or not deoxycholate is used in their preparation. They do differ slightly insofar as there appears to be a higher complement of the heavy polyribosomes if deoxycholate is omitted.
### TABLE 3
A COMPARISON OF THE POLYRIBOSOME PATTERNS OF NORMAL AND NEOPLASTIC LIVER ON THE BASIS OF EQUIVALENT AREA

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>HR. PAST</th>
<th>PER CENT DEOXYCHOLATE</th>
<th>n0/nr, WHERE n =</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Normal liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.25</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>11</td>
<td>1.25</td>
<td>1.08</td>
<td>1.09</td>
</tr>
<tr>
<td>11</td>
<td>0.0</td>
<td>6.51</td>
<td>3.55</td>
</tr>
<tr>
<td>84</td>
<td>1.25</td>
<td>7.75</td>
<td>5.80</td>
</tr>
<tr>
<td>Hepatoma 5123 A</td>
<td>8</td>
<td>1.25</td>
<td>3.39</td>
</tr>
<tr>
<td>8</td>
<td>0.0</td>
<td>2.99</td>
<td>4.69</td>
</tr>
<tr>
<td>8</td>
<td>0.16</td>
<td>2.32</td>
<td>4.26</td>
</tr>
<tr>
<td>8</td>
<td>2.50</td>
<td>3.77</td>
<td>3.29</td>
</tr>
<tr>
<td>Hepatoma 5123 D</td>
<td>8</td>
<td>1.25</td>
<td>8.87</td>
</tr>
<tr>
<td>8</td>
<td>1.25</td>
<td>7.17</td>
<td>8.62</td>
</tr>
<tr>
<td>84</td>
<td>1.25</td>
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</tr>
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<td>Hepatoma 5123 TC</td>
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<td>7.10</td>
</tr>
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<td>1.25</td>
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<td>5.79</td>
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<td>5.69</td>
<td>6.79</td>
<td>2.25</td>
</tr>
<tr>
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<td>14.00</td>
<td>14.11</td>
<td>4.06</td>
</tr>
<tr>
<td>Hepatoma 7794 A</td>
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<td>1.25</td>
<td>27.90</td>
</tr>
<tr>
<td>0.0</td>
<td>14.00</td>
<td>14.11</td>
<td>4.06</td>
</tr>
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<td>6.57</td>
</tr>
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<td>0.0</td>
<td>5.45</td>
<td>7.90</td>
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<tr>
<td>Hepatoma 7316*</td>
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<td>7.30</td>
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<td>2.96</td>
<td>8.22</td>
<td>1.85</td>
</tr>
<tr>
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<td>4.85</td>
</tr>
<tr>
<td>0.0</td>
<td>2.96</td>
<td>8.22</td>
<td>1.85</td>
</tr>
</tbody>
</table>

* Homogenized in 0.44 M sucrose instead of 0.25 M sucrose.

**Analysis on the basis of equivalent area.**—The data tabulated in Table 3 confirm and extend previous data and give in quantitative terms an estimate of the deviation from the normal polyribosome pattern of the tissues listed. The $n_0/n_r$ ratio for $n = 1$ and $n = 2$ peaks is increased significantly if C ribosomes are prepared from normal liver in the absence of deoxycholate, from liver of animals fasted for 88 hours, or from hepatomas of fed or fasted animals. It is possible that in normal liver most of the monomers and dimers are free, whereas most of the polyribosomes are bound and require deoxycholate for their release. An 88-hour fast caused a marked re-distribution of the polyribosome sizes, the lighter fractions increasing at the expense of the heavier polyribosomes. In the tumors the ratio for the tetramers ($n = 4$) is somewhat higher, whereas the ratios for the hexamers ($n = 6$) and octamers ($n = 8$) are closer to those for normal liver. The ratios for polyribosomes consisting of approximately twelve and fifteen ribosomal units vary with the hepatoma in question, being approximately normal in the Hepatoma 5123 A and exceptionally low in the Hepatoma 7794. In contrast to rat liver there was no breakdown of the polyribosomes during an 84-hour fast in Hepatomas 5123 TC or 5123 D.

The effect on the polyribosome pattern of isolating the C ribosomes from the tumors with 2.5, 1.25, 0.16 per cent of no deoxycholate requires special comment. As was noted in Chart 3, more polyribosomes appear to be present when deoxycholate is omitted. However, in the case of the Hepatoma 5123A, increasing the deoxycholate concentration from 0.16 per cent to 2.50 per cent does not cause any further breakdown of polyribosomes over that caused by 0.16 per cent. This suggests that the deoxycholate is not disrupting the polyribosomes, but rather is solubilizing ultraviolet-absorbing contaminants. Previous investigators found (19, 48) that the ratio of deoxycholate to microsomal protein is more important than the final concentration of deoxycholate, in order that the ribosomal preparations show optimal amino acid-incorporating activity. In view of this it is important to note that all the tumor data listed in Table 3 were obtained under conditions where the yield of ribosomes per ml. of 52 was as high or higher than that for normal liver. It is therefore unlikely that the change in the C ribosome pattern is due to polyribosome breakdown by excess deoxycholate.

The difference in the $n_0/n_r$ ratios for the three sublines of the Morris hepatoma 5123 remain to be explained. These ratios for the heavier polyribosomes in Hepatomas...
The polyribosome pattern has been found sensitive to nuclease and is stabilized by low temperature (48) and optimum concentrations of magnesium ions (42, 43, 48). Nonspecific ribosomal aggregation is inhibited by increasing the ionic strength through the addition of potassium chloride (8). The conditions used in the present study to isolate the polyribosomes from the cytoplasm of rat liver and hepatomas as C ribosomes are known to provide relatively pure preparations which are highly active in amino acid-incorporating systems (14, 48) and presumably protein synthesis. The administration to rats of actinomycin D, an inhibitor of nuclear DNA-dependent RNA synthesis (6, 12), causes a breakdown of the liver polyribosomes and loss of their capacity for amino acid incorporation (16, 45). Similar breakdown of the polyribosomes to monomers and dimers has been observed in the Morris 5123 hepatoma, 13 hours after the administration of actinomycin D to a host rat which had a portacaval shunt; furthermore, it has been possible to pulse label RNA attached to polyribosomes in the cytoplasmic fraction of tumor cells.1 These results provide strong evidence in favor of the theory that the m-RNA is an integral part of the polyribosome of the cytoplasm of liver and of hepatomas. Thus, the polyribosome complement of the cytoplasm should provide a gross measure both of the general status of the protein-synthesizing machinery and of the expression of the genes concerned with this region of the cell.

Normal liver consistently gives the identical C ribosome pattern when the ribosomes are isolated under identical conditions. Any change in this pattern which is not attributable to the isolation procedure must be explained in terms of nutritional, hormonal, or actual genetic change. It is for this reason that it was of interest to compare the polyribosomes from the same primary tumor, the Hepatoma 5123 A (G-39). Although the three sublines were derived from the same primary tumor, the Hepatoma 5123 A appeared to have less necrosis than 5123 D and 5123 TC and killed the animals within 1½ months after bilateral intramuscular inoculation. In contrast, the original tumor is described (23) as slowly growing, reaching a diameter of only 2.0 cm. in about 2 months, with rats apparently surviving up to 6 months after intraperitoneal inoculation. It is not certain whether the observed differences are due to an actual genetic change in a subline of the tumor.

DISCUSSION

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There is additional circumstantial evidence for aberrant control mechanisms, since some of the hepatomas lack, in relative terms, a full complement of the heavy polyribosomes—Hepatoma 7794 being an extreme example. It might be expected that the latter would have a reduced capacity for the synthesis of certain proteins; it does, in fact, appear to have a significantly lower complement of many of the catabolic enzymes of amino acid metabolism than do other minimal-deviation hepatomas (34). It is not clear whether the differences observed are due to the loss of hormonal control or to an actual genetic change, or to a combination of changes. Growth hormone apparently exerts some control on m-RNA production in normal liver (15).

In addition to the possibilities already outlined, atypical polyribosome patterns might arise from other modifications of control at the transcriptional and translational level. Modifications in the control of m-RNA production probably occur at the transcriptional level in the differentiating reticulocyte. The polyribosome pattern and protein synthesis in the mature reticulocyte appears to be regulated almost exclusively by a relatively stable mRNA which directs the synthesis of the peptide chains of hemoglobin; both the polyribosome pattern and the type of protein synthesized can be changed by adding synthetic polynucleotide (messengers) to the isolated system (9). Alternatively, a modification of the control of protein synthesis at the translational level without marked changes in the stability of m-RNA might lead to the excessive production of certain proteins, which through negative feedback might inhibit m-RNA production; as a consequence, a certain fraction of the ribosomes might be released from the task of protein synthesis.

The patterns of liver and hepatoma also seem to differ insofar as the heavy polyribosomes of the former but not of Hepatoma 5123A markedly decreased relative to the lighter fractions during prolonged fasting. The capacity of the hepatoma to maintain its original pattern under the stress of nitrogen depletion may be related to a capacity to compete strongly for host nitrogen (cf. 18). It should be emphasized that the polyribosome pattern of hepatoma may respond to longer periods of fasting; also, it has been found1 that the fasting pattern of normal liver is not a simple increase of the smaller polyribosomes with a corresponding gradual decrease of the larger varieties, but has superimposed a periodic redistribution of the polyribosome species.

Although a considerable portion of the protein-synthesizing machinery of liver must be involved in the production of plasma proteins, the synthesis of the individual species has not been identified to date with any particular polyribosome fraction of liver. The situation is complicated by the possibility that polycistronic m-RNA may code for several different types of proteins (41). The Novikoff hepatoma, which may not be derived from liver parenchyma (35) and therefore may not synthesize many of the liver proteins, offers no clue in this regard, since its pattern is not characteristically different from the minimal-deviation hepatomas.

The recovery data on the purified C ribosomes prepared in the presence and absence of deoxycholate have several

1 Unpublished data.
important implications. Deoxycholate releases (19, 27) the bound ribosomes of the microsomal fraction, presumably through solubilization of the lipid fraction of the endoplasmic reticulum. Recent evidence (10) suggests that deoxycholate acts by displacing an equilibrium reaction between the ribosomes and the membrane. The present data suggest that at least 50 per cent of the polyribosomes in normal liver are tightly bound; conversely, it appears that there is a complete recovery of polyribosomes from hepatoma cells without prior deoxycholate treatment. It would appear either that most of the polyribosomes are free in the tumor cells or that they are so loosely bound as to be released during homogenization and centrifugation. These observations may be relevant to the suggestion (33) that faulty interaction between polyribosomes and the endoplasmic reticulum could be the cause of changes in the stability of the m-RNA in the minimal-deviation hepatomas. Even very low concentrations of deoxycholate cause slight modifications in the pattern of C ribosomes prepared from Hepatoma 5123A in the absence of deoxycholate, suggesting that small fragments of the endoplasmic reticulum or other extraneous protein may be bound to some of the polyribosomes. Earlier studies (17, 29, 30), concerned with the isolation of ribosomes chiefly as monomers from normal liver and hepatomas without detergent treatment, support this view. Such fragments must be very small, since the sucrose in the bottom layer used to prepare the C ribosomes is 2.0 M or 68.5 per cent (w/v). Liver catalase, known to be synthesized by and associated with, the rough endoplasmic reticulum is not detected in the 105,000 X g pellet when the microsome fraction is layered over 60 per cent sucrose (w/v) and centrifuged for 16 hours (11).

Although similar aberrations have been found in both multiple- and minimal-deviation hepatomas, there is as yet no evidence that they bear any relation to change(s) essential to the neoplastic process.

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