Metabolism of Nucleolar Ribonucleic Acid after Partial Hepatectomy

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When a large part of the liver of a mammal is removed, there follows, after a lag period, a dramatic increase in the number of hepatic cells making DNA. Partial hepatectomy has, for this reason, been commonly used to investigate the mechanisms that normally govern entry of a cell into the period of DNA formation. The liver appears to be informed immediately about the partial hepatectomy, and several hepatic changes, including an increase in the rate of RNA metabolism, begin at once after the operation (1). The early changes in liver RNA synthesis that follow partial hepatectomy have become of particular interest in view of the results obtained in similar studies with cultured kidney cells (2-4). With the kidney cells, control of DNA replication has been shown to depend largely on the control of RNA metabolism.

Stowell (5) made volumetric measurements of liver nucleoli 24 hours after partial hepatectomy and found a 4-fold increase in their size. With microphotometric measurements of a bound basic dye, Kleinfeld (6) and Swift, Rebhun, Rasch, and Woodard (7) then showed that the enlargement of the nucleolus is accompanied by an increase in nucleolar RNA. Although their first measurements were made 14 hours after the operation, the rise appeared to begin immediately postoperatively. Later, using radioglucose, Schneider and Potter (8) found an increase, 6 hours postoperatively, in the rate of incorporation of label into hepatic RNA.

More recently (1), a linear rise in the rate of incorporation of 14C- orotate into liver RNA was shown to begin immediately after partial hepatectomy, reaching a maximum about twice the initial rate by about 6 hours. Enhancement of the synthetic rate was equally reflected in each of the subcellular fractions examined: nuclear, mitochondrial, microsomal, and soluble. Of interest are the observations that were made with two inhibitors, actinomycin D and p-fluorophenylalanine. In amounts that completely and reversibly suppressed the rate increase that followed partial hepatectomy.

Especially in view of the work of Swift et al. (7), it was of interest to compare the metabolic changes following soon after partial hepatectomy in liver nucleolar RNA with those of the over-all hepatic RNA. Such studies, it was hoped, might throw some light on the role of the nucleoli in the period preceding DNA synthesis. To this end, estimations of nucleolar RNA were made with azure B (7, 9-11), and its rate of synthesis and that of the total liver RNA were measured with 14C- orotate. The purpose of this report is to present the results of studies of nucleolar RNA metabolism in the livers of partially hepatectomized rats and to compare them with the changes that occur in the total RNA metabolism of the liver.

EXPERIMENTAL PROCEDURE

Materials—Male albino rats, obtained locally, received food and water ad libitum at all times, and were used when they weighed about 80 g. Twice crystallized beef pancreatic deoxyribonuclease was from the Sigma Chemical Company, azure B was a product of the Dajac Laboratories, and dL-p-fluorophenylalanine was obtained from the Nutritional Biochemicals Corporation. Orotic acid-14C (5.4 μc per pmole) was from the New England Nuclear Corporation. Actinomycin D was kindly supplied by Dr. Elmer Alpert, Merck Sharp and Dohme Research Laboratories, and puromycin by Dr. Stanton M. Hardy, Lederle Laboratories.

Methods—Surgery was performed under ether anesthesia. Partial hepatectomy refers to the removal of 67% of the liver (median and left lateral lobes) (12). The sham operation was performed in the same way except that no liver was excised. Injections were made into the tail vein.

For the photometric estimation of nucleolar RNA, thin slices of liver (1 to 2 mm thick) were fixed in a freshly prepared mixture of glacial acetic acid-absolute ethanol, 1:3 (v/v), for 1 hour and paraffin sections about 6 μ thick were prepared. After removal of the paraffin, the sections were treated with DNase and stained with azure B (9), and the dye bound to nucleolar RNA was estimated with visible light (10, 11). Only cells with a single nucleolus were used, and the values shown represent the average result from 10 cells. These measurements were made in the laboratory of Dr. Hewson Swift, Department of Zoology, University of Chicago, and the authors gratefully acknowledge their indebtedness for the use of his instrument and for his invaluable instruction and guidance.

To obtain nucleoli for radioactivity measurements, liver homogenates were prepared in 9 volumes of ice-cold 0.25 M sucrose-0.0033 M CaCl2 with a glass homogenizer fitted with a motor-driven plastic pestle. Nuclei were purified from the homogenates according to the method of Busch, Staubke, and Davis (13) except that 2.2 instead of 2 M sucrose was used and centrifugation was at a higher speed and for a longer time, 105,000 × g for 1 hour. The procedure of Muramatsu, Suetana, and Busch (14) was then followed for the purification of nucleoli.
 except that sonication was carried out with smaller volumes (4 to 6 ml) of nuclear suspensions and for a longer time (4 to 5 minutes). The purified preparations had ratios of protein to RNA and RNA to DNA identical with those of Muramatsu et al. (14).

The rate of RNA synthesis was measured as previously described (1). The rats were treated with injections of 0.5 μg of ^14C orotate, and they were killed by decapitation 7 minutes later. Liver homogenates were immediately used for the preparation of nucleoli. Isotope incorporation was assayed in a procedure involving washes with trichloroacetic acid, ethanol, and ether, and radioassay was in a Packard Tri-Carb liquid scintillation spectrometer.

DNA was estimated essentially by the method of Burton (15), RNA pentose according to Miehaim (16), and protein by the procedure of Lowry, Rosebrough, Farr, and Randall (17).

**Results**

**Increase in Nucleolar RNA and Its Rate of Synthesis after Partial Hepatectomy**—The amount of nucleolar RNA and the rate of its synthesis were compared, as a function of time after partial hepatectomy, with the rate of formation of total liver RNA (Table I). As shown in the table, the postoperative increases were similar. With both the nucleoli and the homogenates (total RNA), the increases appeared to begin immediately after the operation, and by 6 hours about a doubling in amount or rate had been achieved. On the other hand, whereas the turnover rates reached a maximum after about 6 hours, and no further change occurred by 10 hours, Swift et al. (7) have shown that the nucleolar RNA increases until about 20 hours postoperatively.

In view of the large quantities of DNA in the nucleolar preparations, the possibility was considered that the radioactivity incorporated in them was in chromatin-associated rather than in nucleolar RNA. Although it is not certain that hydrolysis of the DNA would liberate chromatin-associated RNA, it seemed worthwhile to determine the effect of DNase on RNA-labeled nucleoli (Table II). As can be seen from the table, the removal of DNA was not accompanied by a significant decrease in nucleolar (easily sedimentable) radioactivity or pentose.

**Amount and Rate of Synthesis of Nucleolar RNA after Removal of Different Quantities of Liver**—On the basis of both estimations of dye binding and rates of incorporation, a relationship similar to the one previously shown for total hepatic RNA (1) was found between the quantity of liver removed and the early postoperative increase in nucleolar RNA and in the rate of its synthesis (Table III). Extirpation of 10% of the liver (caudate lobe) caused only a small change in the amount and synthetic rate of nucleolar RNA, and successive increases were observed with removal of 37% (left lateral lobe) and 67% (left lateral and median lobes). For comparison, also shown are the results obtained with homogenates from the same livers.

**Comparison of Effects of Actinomycin D and p-Fluorophenylalanine on Nucleolar and Total RNA**—Multiple injections of actinomycin D and p-fluorophenylalanine in amounts that had little or no effect on the rates of RNA synthesis in untreated or sham-operated animals, completely suppressed the nucleolar increases that ordinarily follow partial hepatectomy (Tables IV and V). The tables also compare the effects of levels of the inhibitors that did not completely block the increases. As shown, at all the in-

**Table I**

<table>
<thead>
<tr>
<th>Operative treatment</th>
<th>Postoperative time</th>
<th>Azur B bound</th>
<th>Radioactivity incorporated in 7 min</th>
<th>c.p.m./g wet liver</th>
<th>c.p.m./mg protein*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham hepatectomy</td>
<td>10</td>
<td>100</td>
<td>690</td>
<td>6,550</td>
<td>33</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>2</td>
<td>134</td>
<td>766</td>
<td>8,830</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>100</td>
<td>1,000</td>
<td>9,160</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>175</td>
<td>1,510</td>
<td>13,800</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>1,700</td>
<td>14,250</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1,720</td>
<td>14,450</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The specific activities of the labeled nucleoli calculated from their RNA content were about 10 times greater than the values based on protein, and with the homogenates, the differences were about 20-fold. The relationships among the various samples, however, remained the same regardless of the basis of the specific activity calculation.

**Table II**

<table>
<thead>
<tr>
<th>Initial</th>
<th>Sedimentable RNA</th>
<th>Sedimentable DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleolar preparation</td>
<td>3770</td>
<td>3200</td>
</tr>
<tr>
<td>Nucleolar preparation + DNase</td>
<td>3420</td>
<td>210</td>
</tr>
</tbody>
</table>

* The specific activities of the labeled nucleoli calculated from their RNA content were about 10 times greater than the values based on protein, and with the homogenates, the differences were about 20-fold. The relationships among the various samples, however, remained the same regardless of the basis of the specific activity calculation.
Nucleolar RNA and its rate of synthesis after removal of 10, 37, and 67% of liver

The rats were given 0.5 μC of 14C-ornithine 6 hours postoperatively. They were decapitated 7 minutes later, and the liver samples were prepared for radioassay and for the photometric estimation of nucleolar RNA (bound azure B) as described in “Methods.”

**Table III**

<table>
<thead>
<tr>
<th>Liver removed</th>
<th>Nucleolar RNA</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lobs</td>
<td>Total liver</td>
<td>Azuro B bound</td>
<td>Radio-activity*</td>
<td>Total RNA*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>incorporated</td>
<td></td>
</tr>
<tr>
<td>None removed</td>
<td></td>
<td>O†</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Caudate</td>
<td>10</td>
<td>111</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Left lateral</td>
<td>37</td>
<td>155</td>
<td>159</td>
<td>143</td>
</tr>
<tr>
<td>Left lateral + median</td>
<td>97</td>
<td>182</td>
<td>281</td>
<td>223</td>
</tr>
</tbody>
</table>

* The results are based on calculations of specific activities (counts per minute per mg of protein).
† Sham-hepatectomized.

**Table IV**

Comparison of effects of actinomycin D on nucleolar and total RNA

Actinomycin D was injected intravenously at 0, 2, and 4 hours postoperatively. RNA and its rate of synthesis were estimated after 6 hours as described in “Methods.”

**Table V**

Comparison of effects of p-fluorophenylalanine on nucleolar and total RNA

p-Fluorophenylalanine injections were made intravenously at 0, 2, and 4 hours postoperatively as indicated. RNA and its rate of synthesis were estimated after 6 hours as described in “Methods.”

**Table VI**

Reversibility of inhibitory action of actinomycin D and p-fluorophenylalanine

Actinomycin D (2 μC per dose) was injected intravenously at 0, 2, and 4 hours postoperatively; with p-fluorophenylalanine, 2.8 μoles were injected at zero time and 2.8 μoles at each of the two later times. RNA and its rate of synthesis were estimated at the indicated postoperative times as described in “Methods.”

* The results are based on calculations of specific activities (counts per minute per mg of protein).
after nine daily injections of thioacetamide.

On the other hand, they tested their animals only during their purification (14), the nucleolar contribution would contribute a relatively large share to the increased rate of liver RNA synthesis. Nevertheless, their implications are not inconsistent with the high metabolic activity of the nucleolus (18, 20-22) or with its increased size in rapidly growing cells.

Table VII

<table>
<thead>
<tr>
<th>Operative treatment</th>
<th>Inhibitor</th>
<th>Nucleolar RNA</th>
<th>Total RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puromycin</td>
<td>Thioacetamide</td>
<td>% sham-operated controls*</td>
</tr>
<tr>
<td>Sham hepatectomy</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>84</td>
<td>86</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>0</td>
<td>229</td>
<td>239</td>
</tr>
<tr>
<td></td>
<td>1.1</td>
<td>206</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>142</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>4.4</td>
<td>131</td>
<td>118</td>
</tr>
<tr>
<td>Sham hepatectomy</td>
<td>200</td>
<td>98</td>
<td>85</td>
</tr>
<tr>
<td>Partial hepatectomy</td>
<td>67</td>
<td>153</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>132</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>101</td>
<td>112</td>
</tr>
</tbody>
</table>

* The results are based on calculations of specific activities (counts per minute per mg of protein).

The results with thioacetamide are in apparent disagreement with those of Busch et al. (18), who found a greater inhibition of extranucleolar than nucleolar RNA synthesis. This discrepancy may be related to the difference in the amounts of inhibitor used. In the experiment described in Table VII, even the lowest quantity used per rat is about 4 times greater than the daily dose of Busch et al. On the other hand, they tested their animals only after nine daily injections of thioacetamide.

In the rate of synthesis of nucleolar or extranucleolar hepatic RNA (Table VII). As the table shows, these agents also failed to show a differential effect.

The results with thioacetamide are in apparent disagreement with those of Busch et al. (18), who found a greater inhibition of extranucleolar than nucleolar RNA synthesis. This discrepancy may be related to the difference in the amounts of inhibitor used. In the experiment described in Table VII, even the lowest quantity used per rat is about 4 times greater than the daily dose of Busch et al. On the other hand, they tested their animals only after nine daily injections of thioacetamide.

DISCUSSION

As previously shown (1), partial hepatectomy leads to a gradual rise in the rate of turnover of liver RNA that begins at once and reaches a maximum about 6 hours postoperatively. Increases in the rate of synthesis of liver nucleolar RNA and in the amount of RNA per nucleolus also begin immediately after the operation. Not only are the initial kinetics similar for all three series and because the purified preparations were contaminated with RNA from perinucleolar (19) and extranucleolar (see Footnote 2) chromatin. Nevertheless, their implications are not inconsistent with the high metabolic activity of the nucleolus (18, 20-22) or with its increased size in rapidly growing cells.

Nucleolar enlargement in tumor cells (23, 24) has long been recognized by the pathologist.

SUMMARY

Changes in liver nucleolar RNA after partial hepatectomy of the rat were followed microphotometrically and by measuring the rate of nucleolar RNA synthesis with 14C- orotate. Just as with the over-all RNA metabolism of the liver, the increases in nucleolar RNA and in its rate of labeling begin immediately after the operation and are related to the quantity of liver removed. Removal of only 10% of the liver results in little change during the time period studied. Whereas the increases in the nucleolar and over-all rates of RNA synthesis (measured with 14C- orotate) reach a maximum of about twice the initial rate by 6 hours after the operation, the amount of RNA per nucleolus, as shown by others (7), becomes maximal only after about 20 hours, and more than a 2-fold increase is achieved.

Actinomycin D and p-fluorophenylalanine, in amounts that have no effect on the normal rate of RNA synthesis, completely but reversibly suppress both the nucleolar and extranucleolar rises. The minimal amount of inhibitor required to block totally each of the three rises is the same. In addition, with smaller amounts, amounts that only partially prevent the rises, the same degrees of inhibition are produced.

REFERENCES

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