Effects of Hydroxyurea and 6-Mercaptopurine on Growth and Some Aspects of Carbohydrate Metabolism in Regenerating and Neoplastic Liver

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SUMMARY

Previous observations have shown changes in several pathways of carbohydrate metabolism in a variety of hepatomas. Glycogen levels and the activities of the glycolytic enzymes causing the phosphorylation of glucose and fructose 6-phosphate were chosen as important examples of such changes, and a study was made to observe how closely the alterations were related to changes in growth rate. Growth in regenerating and neoplastic liver of rats was measured by the increase in tissue weight and by DNA synthesis. Daily administration of hydroxyurea (250 or 1000 mg/kg) and 6-mercaptopurine (10 or 45 mg/kg) had a greater effect on the inhibition of DNA synthesis than on liver weight or liver/body weight ratios for regenerating liver. The depletion of glycogen in this tissue either was not affected or was increased by administration of the two compounds. The high dose level of 6-mercaptopurine blocked the increase in the activity of low-K_m hexokinase in regenerating liver. Hydroxyurea was a very effective inhibitor of thymidine-^3H incorporation into DNA in four hepatoma lines (7800, 5123-D, 7777, and 3924-A). Time-course studies showed a rapid recovery from the inhibition of DNA synthesis. The administration of six injections of hydroxyurea (1000 mg/kg) at 5-hr intervals was found to cause a 30% reduction in DNA concentration and a 27% decrease in phosphofructokinase activity in hepatoma 7777. A significant reduction in low-K_m hexokinase activity was also observed. Under the conditions used, no tendency was observed for a restoration of high-K_m glucokinase activity in hepatomas after a decrease in growth rate.

INTRODUCTION

Work by a number of investigators has shown that there are correlations between a variety of metabolic parameters and growth rates of hepatomas (18). It appears appropriate to identify the most crucial of the changes related to growth and the extent to which they are reversible. The regenerating liver provides a tissue in which factors controlling normal rapid growth in nonneoplastic tissue may be identified. The metabolic pattern of regenerating liver may be compared with that of hepatomas in an attempt to identify features characteristic of neoplasia. The present investigation was designed to study metabolic parameters during altered growth in regenerating and neoplastic liver for the purpose of determining the degree of correlation between metabolic parameters and growth rate. 6MP^2 and hydroxyurea were used as agents that inhibit cell division with different sites of action (3, 12). Changes in glycogen synthesis and the activities of glycolytic enzymes observed in hepatomas may also occur to some degree in regenerating liver (6). Parameters of this type were compared under conditions of altered growth. The action of hydroxyurea was considered of particular interest, as this compound appears to inhibit DNA synthesis with little immediate effect on RNA formation. It offers the opportunity, therefore, to study the expression of genetic information under conditions of altered cellular proliferation.

MATERIALS AND METHODS

Animals. The animals were kept in separate cages, which were illuminated from 6 a.m. to 7 p.m. Water and food (Wayne Lab-Blox) were available ad libitum. Male Sprague-Dawley rats were used for experiments on regenerating liver. Animals in the weight range of 175 to 250 g were partially hepatectomized by removal of approximately 66% of the liver (5). The operations were performed between 11 a.m. and 12 noon. The tumors used included hepatoma 7800, Generation 42, hepatoma 5123-D, Generation 77, and hepatoma 7777, Generations 35, 50, and 52. The tumors were all transplanted bilaterally s.c. in male Buffalo strain rats; hepatoma 3924-A, generations 266 and 267, were transplanted into male ACI strain rats. The biology, histology, and growth properties of the tumors were described previously (9, 10).

Reagents. Fructose 6-phosphate, ATP, NADP, aldolase (EC 4.1.2.13), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), hydroxyurea, and 6MP hydrate were obtained from Sigma Chemical Company (St. Louis, Mo.). Methylthymidine-^3H was purchased from New England Nuclear Corporation (Boston, Mass.).

Preparation of Tissues and Assay Methods. The methods used for preparation of tissue homogenates and supernatant fractions have been described (6). The procedures for the assay of hexokinase (EC 2.7.1.1), glucokinase (EC 2.7.1.2), and phosphofructokinase (EC 2.7.1.11) were presented previously.
Regulation of Liver and Hepatoma Growth

Table 1

Liver/body weight ratio of regenerating liver

The liver weight is expressed as a percentage of the rat body weight. All values are means ± S.E.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of dose (mg/kg)</th>
<th>Liver/body weight ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before operation</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>4.45 ± 0.21 (5)°</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>250</td>
<td>1.83 ± 0.05 (6)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>2.25 ± 0.08 (5)</td>
</tr>
<tr>
<td>6 MP</td>
<td>10</td>
<td>1.82 ± 0.10 (5)</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>1.64 ± 0.13 (5)</td>
</tr>
</tbody>
</table>

° Figures in parentheses indicate number of experiments.

Chart 1. Effect of hydroxyurea and 6MP on thymidine-3H incorporation into DNA of regenerating rat liver. The animals received a daily administration of hydroxyurea (HU) or 6MP at the specified dose levels. Control animals received 0.15 M sodium chloride injections. Means and standard errors are given for 4 to 14 animals.

Expression and Evaluation of Results. Data are presented as means of a stated number of experiments ± S.E. Each experiment represents duplicate determinations on a single animal. The results were subjected to statistical evaluation by the t test for small samples. Differences between means giving a probability of less than 5% were considered to be significant.

RESULTS

Three measures of growth were used to follow cellular proliferation in the regenerating liver. These criteria were the liver/body weight ratio, the incorporation of thymidine-3H into DNA, and the total DNA content of the liver. Hydroxyurea (250 or 1000 mg/kg) and 6MP (10 or 45 mg/kg) were administered by i.p. injection in 0.15 M sodium chloride. The injections were given 1 hr before partial hepatectomy and at daily intervals thereafter. On the day the animals were killed, thymidine-3H was injected 1 hr after administration of hydroxyurea or 6MP and the rats were sacrificed 1 hr later. Control animals received 0.15 M sodium chloride injections instead of hydroxyurea or 6MP.

In control animals, the liver/body weight ratio was decreased to 41% of intact rats at 24 hr after partial hepatectomy and by 96 hr was restored to 75% of initial values (Table 1). The only significant reduction of liver/body weight ratio in comparison with control rats was observed with 45 mg 6MP/kg 48 hr after the operation (p < 0.001). For all groups, the mean body weight 24 hr after partial hepatectomy was 87 to 89% of that prior to the operation. In the 0.15 M sodium chloride-treated controls, the mean body weight increased to 93% of the preoperative weight at 96 hr after partial hepatectomy. The corresponding values for animals treated with 250 and 1000 mg hydroxyurea/kg were 89 and 85%, and with 10 and 45 mg 6MP the values were 92 and 83%, respectively. Due to the differences in body weight after treatment with the drugs, the mean liver weights showed somewhat greater changes than the liver/body weight ratios. In the animals treated with 45 mg 6MP/kg, there were significant reductions (p < 0.001) in total liver weight at both 48 and 96 hr after partial hepatectomy to 75 and 80% of control values, respectively.

The incorporation of thymidine-3H into DNA of control rats was increased more than 20-fold at 24 hr after partial hepatectomy (Chart 1). At 48 and 96 hr, the values were increased more than 7-fold and 4-fold, respectively. The administration of hydroxyurea and 6MP caused a large reduction of thymidine-3H incorporation into DNA with both dose levels and at all times examined, except with 10 mg 6MP/kg at 48 hr.

In control rats, the total DNA content of regenerating liver was decreased at 24 hr to 40% of that in unoperated animals and at 48 and 96 hr was 69 and 97%, respectively (Table 2). It appeared that the DNA was restored more rapidly than the liver/body weight ratio. The high dose of hydroxyurea (p < 0.02) at 96 hr and both doses of 6MP (p < 0.001) at 48 and 96 hr caused a significant reduction in total DNA. However, the changes were less marked than those for thymidine-3H incorporation into DNA.
Table 2

DNA content of regenerating liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of dose (mg/kg)</th>
<th>Before operation</th>
<th>24 hr</th>
<th>48 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td>19.8 ± 1.4 (4)²</td>
<td>7.9 ± 0.8 (6)</td>
<td>13.7 ± 0.5 (14)</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>250</td>
<td></td>
<td>7.4 ± 0.3 (4)</td>
<td>12.5 ± 1.4 (6)</td>
<td>19.1 ± 0.6 (5)</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>1000</td>
<td></td>
<td>8.4 ± 0.8 (5)</td>
<td>12.8 ± 1.1 (5)</td>
<td>15.8 ± 0.6 (5)</td>
</tr>
<tr>
<td>6 MP</td>
<td>10</td>
<td></td>
<td>8.8 ± 1.1 (5)</td>
<td>9.5 ± 0.8 (5)</td>
<td>13.2 ± 1.0 (4)</td>
</tr>
<tr>
<td>6 MP</td>
<td>45</td>
<td></td>
<td>6.1 ± 1.2 (5)</td>
<td>7.1 ± 0.5 (5)</td>
<td>9.7 ± 0.8 (6)</td>
</tr>
</tbody>
</table>

² Figures in parentheses indicate number of experiments.

Table 3

Glycogen content of regenerating liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of dose (mg/kg)</th>
<th>Before operation</th>
<th>24 hr</th>
<th>48 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>53.8 ± 3.5 (5)²</td>
<td>9.6 ± 2.4 (9)</td>
<td>19.4 ± 2.2 (14)</td>
<td>21.5 ± 2.9 (11)</td>
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<tr>
<td>Hydroxyurea</td>
<td>250</td>
<td>3.7 ± 0.8 (6)</td>
<td>11.7 ± 4.0 (6)</td>
<td>20.7 ± 3.2 (5)</td>
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</tr>
<tr>
<td>Hydroxyurea</td>
<td>1000</td>
<td>8.5 ± 1.9 (5)</td>
<td>4.7 ± 1.8 (5)</td>
<td>23.7 ± 4.9 (5)</td>
<td></td>
</tr>
<tr>
<td>6 MP</td>
<td>10</td>
<td>2.9 ± 2.2 (5)</td>
<td>12.6 ± 5.0 (5)</td>
<td>26.3 ± 3.2 (4)</td>
<td></td>
</tr>
<tr>
<td>6 MP</td>
<td>45</td>
<td>1.2 ± 0.4 (5)</td>
<td>4.5 ± 2.8 (5)</td>
<td>2.1 ± 0.7 (6)</td>
<td></td>
</tr>
</tbody>
</table>

² Figures in parentheses indicate number of experiments.

Table 4

Glucokinase activity of regenerating liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of dose (mg/kg)</th>
<th>Before operation</th>
<th>24 hr</th>
<th>48 hr</th>
<th>96 hr</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td></td>
<td>1.50 ± 0.10 (5)²</td>
<td>0.67 ± 0.11 (9)</td>
<td>0.37 ± 0.06 (4)</td>
<td>0.55 ± 0.06 (11)</td>
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<tr>
<td>Hydroxyurea</td>
<td>250</td>
<td>0.73 ± 0.12 (6)</td>
<td>0.37 ± 0.12 (6)</td>
<td>0.52 ± 0.07 (5)</td>
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<tr>
<td>Hydroxyurea</td>
<td>1000</td>
<td>0.87 ± 0.06 (5)</td>
<td>0.23 ± 0.16 (5)</td>
<td>0.55 ± 0.06 (5)</td>
<td></td>
</tr>
<tr>
<td>6 MP</td>
<td>10</td>
<td>0.84 ± 0.06 (5)</td>
<td>0.41 ± 0.11 (5)</td>
<td>0.89 ± 0.10 (4)</td>
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</tr>
<tr>
<td>6 MP</td>
<td>45</td>
<td>0.85 ± 0.08 (5)</td>
<td>0.37 ± 0.23 (5)</td>
<td>0.52 ± 0.09 (6)</td>
<td></td>
</tr>
</tbody>
</table>

² Figures in parentheses indicate number of experiments.

Table 5

Hexokinase activity of regenerating liver

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amount of dose (mg/kg)</th>
<th>Before operation</th>
<th>24 hr</th>
<th>48 hr</th>
<th>96 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.58 ± 0.09 (5)²</td>
<td>0.65 ± 0.13 (9)</td>
<td>0.87 ± 0.09 (14)</td>
<td>0.80 ± 0.12 (11)</td>
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<tr>
<td>Hydroxyurea</td>
<td>250</td>
<td>0.33 ± 0.02 (6)</td>
<td>0.61 ± 0.16 (6)</td>
<td>0.65 ± 0.02 (5)</td>
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</tr>
<tr>
<td>Hydroxyurea</td>
<td>1000</td>
<td>0.47 ± 0.06 (5)</td>
<td>0.60 ± 0.19 (5)</td>
<td>0.93 ± 0.11 (5)</td>
<td></td>
</tr>
<tr>
<td>6 MP</td>
<td>10</td>
<td>0.66 ± 0.06 (5)</td>
<td>0.80 ± 0.11 (5)</td>
<td>0.93 ± 0.05 (4)</td>
<td></td>
</tr>
<tr>
<td>6 MP</td>
<td>45</td>
<td>0.53 ± 0.08 (5)</td>
<td>0.41 ± 0.09 (5)</td>
<td>0.40 ± 0.04 (6)</td>
<td></td>
</tr>
</tbody>
</table>

² Figures in parentheses indicate number of experiments.

In the control animals, glycogen levels were greatly depleted 24 hr after partial hepatectomy and after 96 hr were restored to about 50% of values before operation (Table 3). Hydroxyurea and 6MP administration either did not affect or caused a significant reduction in the glycogen content of regenerating liver. Lower values than controls were seen with 250 mg hydroxyurea/kg at 24 hr (p < 0.05) and with 1000 mg hydroxyurea at 48 hr (p < 0.001). The high dose of 6MP...
6MP, 45

contrast to glycogen content, where the greatest depletion was
caused a large reduction of glycogen content at 24, 48, and 96

The pattern shown by the low-Km hexokinase activity (Table
hi.

seen at 24 hi. The administration of hydroxyurea and 6MP
indicate that a dose of 22.5 mg 6MP/kg/day caused significant

activities in control rats were observed 48 hr after partial hepa-

was injected 60 min after the last administration of 6MP and

control values. The tumor weights represent the sum of 2
tumors/rat. These studies were performed with Generation 35
of hepatoma 7777. As judged by tumor weight, there has been
an increase of growth rate in more recent generations of this
tumor. The incorporation of thymidine into DNA was not
significantly changed at the 5% probability level by the 2 dose
levels of 6MP studied. An examination was made of the effect
of 45 mg 6MP/kg/day on hepatoma 7800. However, the toxic
action of the drug on the host over the prolonged periods
necessary for treatment of this more slowly growing tumor
made it impossible to conduct satisfactory studies on the
action on hepatoma growth.

The administration of a single injection of hydroxyurea at a
dose level of 1000 mg/kg was found to have a marked inhibi-
tory effect on thymidine-3H incorporation into hepatoma
DNA when the nucleic acid precursor was injected 60 min
after the hydroxyurea (Table 7). With hepatoma 7800, the
incorporation was reduced to about 5% of control values, and
with hepatomas 5123-D and 7777 the reduction was to about
2 and 1% of control values, respectively. The action of
hydroxyurea on hepatoma 3924-A was almost as effective as it
was on the other tumors examined, with incorporation values
which were 6 to 7% of those of controls.

It was apparent from time-course studies of the action of
hydroxyurea that there was a rapid recovery from the inhibi-
tory effect on DNA synthesis (Chart 2). Two dose levels of
hydroxyurea (250 and 1000 mg/kg) caused almost complete
inhibition of thymidine-3H incorporation into DNA of hepato-
toma 7777, but 5 to 6 hr after administration of the hydroxy-
urea the values were restored to near the normal range. From
this study it was concluded that frequent administration of
hydroxyurea would be necessary for a prolonged inhibition of
growth rate.

The effect of multiple injections of hydroxyurea was
examined with 2 hepatomas (Table 8). Measurements were
made of DNA synthesis and the activities of phosphofructo-
kinase and hexokinase. The DNA concentration was signifi-
cantly reduced by the 6 injections of hydroxyurea to 70% of
control values in hepatoma 7777 (p < 0.001) and to 84% of
control values in hepatoma 3924-A (p < 0.01). The incorpo-
ration of thymidine-3H into DNA 5 hr after the last injection
of hydroxyurea was not significantly different from control

The lowest activities of the high-Km glucokinase were
observed 48 hr after partial hepatectomy (Table 4). This is in
counter to glycogen content, where the greatest depletion was
seen at 24 hr. The administration of hydroxyurea and 6MP
had little effect on glucokinase activity in regenerating liver.
The pattern shown by the low-Km hexokinase activity (Table
5) differed from that of glucokinase. The greatest hexokinase
activities in control rats were observed 48 hr after partial hepa-
tectomy, when the activity was 150% of that in unoperated
animals (p < 0.05). This increase was blocked by 45 mg
6MP/kg, but not by 10 mg/kg of this compound, and was not
significantly affected by the 2 dose levels of hydroxyurea.

In a study of the action of 6MP on tumor growth, this
compound was given by injection daily for 10 days, starting 7
days after transplantation of hepatoma 7777. Thymidine-3H
was injected 60 min after the last administration of 6MP and
the rats were killed 1 hr later. The data presented in Table 6
indicate that a dose of 22.5 mg 6MP/kg/day caused significant
reductions (p < 0.001) in DNA concentration and tumor
weight to 82 and 60% of control values, respectively. With a
dose level of 45 mg 6MP/kg/day, these parameters were
reduced to 66 (p < 0.001) and 31% (p < 0.005) of respective
control values. The tumor weights represent the sum of 2
tumors/rat. These studies were performed with Generation 35
of hepatoma 7777. As judged by tumor weight, there has been
an increase of growth rate in more recent generations of this

<table>
<thead>
<tr>
<th>Treatment</th>
<th>DNA (mg/g tissue)</th>
<th>Tumor weight (g)</th>
<th>cpm/g tissue</th>
<th>cpm/mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6)a</td>
<td>5.09 ± 0.10</td>
<td>1.58 ± 0.15</td>
<td>5584 ± 751</td>
<td>1086 ± 132</td>
</tr>
<tr>
<td>6MP, 22.5 mg/kg/day (6)</td>
<td>4.15 ± 0.09</td>
<td>0.95 ± 0.12</td>
<td>4764 ± 480</td>
<td>1176 ± 146</td>
</tr>
<tr>
<td>6MP, 45 mg/kg/day (4)</td>
<td>3.37 ± 0.16</td>
<td>0.49 ± 0.18</td>
<td>3720 ± 897</td>
<td>1100 ± 247</td>
</tr>
</tbody>
</table>

a Figures in parentheses indicate number of experiments.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>cpm/g tissue</th>
<th>cpm/mg DNA</th>
<th>cpm/g tissue</th>
<th>cpm/mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7800</td>
<td>674 ± 191 (7)a</td>
<td>318 ± 91</td>
<td>35 ± 5 (4)</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>5123-D</td>
<td>2835 ± 464 (7)</td>
<td>1125 ± 199</td>
<td>57 ± 22 (3)</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>7777</td>
<td>6623 ± 434 (7)</td>
<td>1283 ± 73</td>
<td>91 ± 17 (8)</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>3924-A</td>
<td>3837 ± 713 (7)</td>
<td>654 ± 107</td>
<td>242 ± 49 (4)</td>
<td>43 ± 7</td>
</tr>
</tbody>
</table>

a Figures in parentheses indicate number of experiments.

Table 6
Effect of 6MP on growth of hepatoma 7777
All values are means ± S.E.

Table 7
Effect of hydroxyurea of thymidine-3H incorporation into DNA
All values are means ± S.E. Hydroxyurea was given as a single i.p.
injection 60 min before administration of thymidine-3H.

Table 8
Effect of multiple injections of hydroxyurea on thymidine-3H incorporation into DNA

<table>
<thead>
<tr>
<th>Tumor</th>
<th>cpm/g tissue</th>
<th>cpm/mg DNA</th>
<th>cpm/g tissue</th>
<th>cpm/mg DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>7800</td>
<td>674 ± 191 (7)a</td>
<td>318 ± 91</td>
<td>35 ± 5 (4)</td>
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</tr>
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<td>5123-D</td>
<td>2835 ± 464 (7)</td>
<td>1125 ± 199</td>
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<td>91 ± 17 (8)</td>
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</tr>
<tr>
<td>3924-A</td>
<td>3837 ± 713 (7)</td>
<td>654 ± 107</td>
<td>242 ± 49 (4)</td>
<td>43 ± 7</td>
</tr>
</tbody>
</table>
values in the 2 tumors. The protein concentration of the 3924-A hepatomas was measured to see if the decrease in DNA concentration was a feature of other tissue constituents. In control rats, the tumor homogenates contained $110 \pm 6$ mg protein/g tissue, and in hydroxyurea-treated animals the concentration was $109 \pm 4$ mg protein/g tissue. There was a similar lack of significant change in the protein concentration of the supernatant fractions used in enzyme assays. The concentration was $57 \pm 3$ and $58 \pm 2$ mg/g tissue in control and hydroxyurea-treated animals after 1 more hr.

**DISCUSSION**

Several parameters may be used for the measurement of growth. Changes in tissue weight may reflect alterations in the synthesis of individual cellular constituents. Perhaps a more useful criterion is the increase of DNA synthesis, assuming there is little change in the DNA content per cell (2). The rapid metabolism of thymidine and its specific incorporation into DNA provide a measure of growth over a narrow time interval. On the other hand, changes in total DNA content of a tissue offer a measure of growth for 1 day or more. The present studies provided evidence that hydroxyurea and 6MP, at the dose levels studied, caused a marked inhibition of DNA synthesis in regenerating liver 1 hr after their administration. The values for DNA content and liver weight suggested that the effects on the synthesis of DNA and other cellular constituents were less marked over 24-hr periods. This was particularly true for hydroxyurea. Gershbein and Pedroso (4) found no effect on regenerating liver weight when $60 \text{mg}$ hydroxyurea/day were administered to rats for 7 days after partial hepatectomy. Other workers, in agreement with the present study, found a marked inhibition of thymidine incorporation into DNA with similar dose levels of hydroxyurea (13, 19). It is possible that the rapid metabolism and excretion of hydroxyurea prevents a prolonged effect with a daily administration schedule (1). 6MP was found to be more effective at lower dose levels than hydroxyurea in inhibiting the restoration of DNA levels in the regenerating liver.

Differences in the susceptibility of regenerating liver and hepatomas to the action of drugs could be due to the greater blood flow through the liver, to differences in permeability, and to possible variation in the sensitivity of enzymes in the different tissues. The greater synchronization of cell division in the regenerating liver could also make the timing of drug administration more critical than in hepatomas. One or more of these factors may have resulted in the greater effect of 6MP on thymidine incorporation into DNA in regenerating liver than in hepatoma 7777. As there were differences in the relative effect of the drug on the short-term measurement of thymidine incorporation into DNA and the longer-term effect on total DNA content in the 2 tissues, it may be suggested that the duration of action of 6MP differed in the regenerating and neoplastic tissues.

One objective in studies of growth and changes in metabolism is to distinguish between cause and effect. The depletion of glycogen in the regenerating liver could provide glycolytic substrates and thereby yield energy for synthetic reactions. Alternatively, the response may be enforced by a requirement to maintain blood glucose levels with a reduced carbohydrate reserve in the liver remnant. As liver glycogen levels were decreased when growth was inhibited, the 2nd alternative appeared more probable. The aim of this experiment was to see whether an inverse correlation existed between glycogen concentration and the growth of the regenerating liver, and it was concluded that such a correlation need not occur. The nature of the changes in enzyme activity which underlie the alteration of glycogen concentration in the regenerating liver remains to be established.

There is a tendency for rapidly growing hepatic tissues to show a decrease in the activity of the high-$K_m$ glucokinase and an increase in low-$K_m$ hexokinase activity. This is true for
fetal (17), regenerating, and neoplastic liver (14) in comparison to normal adult liver. The correlation with growth rate is not, however, an exact one. For example, in the regenerating liver the change in activities of hexokinase isoenzymes is greatest 48 hr after partial hepatectomy, whereas growth rate is greatest in the 24-hr regenerating liver. Although 45 mg 6MP/kg/day prevented the increase in low-$K_m$ hexokinase activity, there was no significant effect on high-$K_m$ glucokinase activity. Previous work indicated that the changes in hexokinase isoenzymes after partial hepatectomy are related to removal of a portion of the liver and are not observed after sham operations (6). The higher doses of hydroxyurea and 6MP inhibited the restoration of body weight after partial hepatectomy. It is possible that it was accompanied by a decrease in food intake which may have influenced the results. The high-$K_m$ glucokinase assayed by the kinetic procedure can usually be equated with the type IV hexokinase seen on separation of hexokinase isoenzymes by electrophoresis. However, Shatton et al. (14) concluded from their electrophoresis experiments that type II hexokinase probably contributes to apparent glucokinase activity in hepatoma 3924-A. Whatever the contributory factors to high-$K_m$ activity, an increase in activity should be detected by the kinetic assay procedure. It was concluded, therefore, that an inhibition of growth in regenerating and neoplastic liver need not be accompanied by a trend towards restoration of normal high-$K_m$ glucokinase activity.

Hydroxyurea was observed to inhibit DNA synthesis in a variety of hepatomas. These included the well-differentiated and slowly growing 7800 tumor and, in contrast, the poorly differentiated and rapidly growing hepatoma 3924-A. Smith et al. (16) found there was a rapid recovery of DNA synthetic ability after inhibition by hydroxyurea in the mouse liver. The present results emphasize this feature in 2 Morris hepatomas, 7777 and 3924-A. Such data give confidence in the use of hydroxyurea as an agent modifying cellular proliferation without producing a moribund tissue in short-term experiments. The results, however, do indicate the necessity for frequent administration of the compound to give a prolonged inhibition of growth. Multiple injections of hydroxyurea caused a reduction of DNA concentration in the 2 tumors examined, and a similar decline in phosphofructokinase activity was observed. In preliminary studies, little change was observed in phosphofructokinase activity after partial hepatectomy, and an investigation of relationship to growth rate was not pursued. A positive correlation between the activity of this enzyme and the growth rates of a spectrum of hepatomas was noted by Shonk et al. (15). It appears that the increase in phosphofructokinase activity in hepatomas may be reversed. A similar situation was observed for low-$K_m$ hexokinase activity in hepatoma 7777. The lack of significant change in total protein concentration suggests some selectivity in changes of enzyme activity. Hepatoma 7777 was somewhat more responsive than 3924-A to the action of hydroxyurea and may provide a more useful model in further studies on relationships between metabolic parameters and growth rates.

**REFERENCES**


Effects of Hydroxyurea and 6-Mercaptopurine on Growth and Some Aspects of Carbohydrate Metabolism in Regenerating and Neoplastic Liver

M. A. Lea, D. Sasovetz, A. Musella, et al.