In previous work (3, 4), we described the purine utilization patterns of hamsters and rats bearing human neoplasms. These studies showed that, insofar as the synthesis of the purine moiety of their nucleic acids is concerned, these tumors possess distinctive characteristics as compared with the host tissues. In the synthesis of the nucleic acids, the neoplastic tissue used guanine and hypoxanthine inefficiently as compared with the host, but used adenine more extensively and glycine to an even greater degree. Other workers (5, 13) have reported findings consistent with the observation that tumors preferentially synthesize their nucleic acid purines de novo, even when the option of using preformed purines is available. In agreement with this concept, it has been shown that guanylic and adenylic acids are very poorly utilized as precursors of the nucleic acids of tumors relative to those of the host’s tissues.1

Knowledge about nucleic acid synthesis in another rapidly growing tissue should permit a somewhat more lucid interpretation of the unusual pattern of purine incorporation manifested by tumors. Cell division in the intestinal mucosa occurs at a rate comparable to that of the tumor (18), but cells are constantly being sloughed. A tissue growing at a rate greater than normal should be a more suitable basis for comparison. Such a system is regenerating liver. The cells formed by the hyperplastic liver are retained, thus obviating the objection to the use of intestinal mucosa as a reference rapidly growing tissue.

Since the presence of rapidly multiplying cells does influence the metabolism of other tissues of an animal (e.g., the nucleic acid metabolism is altered by the presence of a tumor or a fetus [14, 15]), it was of interest to determine whether or not there was any effect on tumor tissue attributable to the rapidly growing liver. To this end, the ability of various tissues of animals with and without tumors to utilize various purine precursors was determined, and the extents of incorporation observed were compared with the results obtained in similar experiments in which the animals had been previously partially hepatectomized. The results of these investigations are presented here, and they reveal an influence on tumor metabolism due, directly or indirectly, to partial hepatectomy. The ability of the tumor to utilize adenine as a nucleic acid precursor has been increased, and the apparent rate of synthesis de novo of nucleic acid purines has been decreased.

MATERIALS AND METHODS

Five precursors were studied. Adenine-8-C14 (7) and guanine-8-C14 (1) were synthesized in these laboratories. Hypoxanthine-8-C14 and 2,6-diaminopurine-2-C14 were obtained from the Southern Research Institute, Birmingham, Alabama, and glycine-1-C14 from Isotopes Specialties Company, Burbank, California.

Hamsters carrying implants of human sarcoma #1 (H.S. #1) or human epidermoid carcinoma #8 (H.Ep. #8) were obtained from Dr. H. W. Toolan (19). These animals (60-gm. weanlings) had received cortisone at time of implantation and were used when the implants were 12 days old. Normal hamsters were obtained from the same source as were those used by Dr. Toolan (Lakeview Hamster Colony). Each experiment was carried out on a group of six to ten animals.

The subtotal hepatectomies were performed in a manner similar to that of Higgins and Anderson (11). The animals were anesthetized under ether, and the right lateral and two central lobes were resected about ½ inch distal to the ligature. The gall bladder was removed with the central lobes.1

The animals were injected intraperitoneally with the precursor under study 24 hours postoperatively, and the controls received the precursors at the corresponding time. The purines were administered at a level of 0.1 mM/kg; glycine was given at a level of 0.117 mM/kg. The animals were maintained for 24 hours on a diet of Purina chow, to which they were accustomed. At the end of this time they were sacrificed under ether. The intestine, spleen, liver, kidneys, and tumors were

1 In a control experiment, removal of the gall bladder has no effect on adenine utilization.
removed and immediately frozen on dry ice. All the samples of each tissue were pooled. The tissues were each minced in a high-speed blender with cold trichloroacetic acid. The nucleic acids were removed by salt extraction (16) and the pentose nucleic acid (PNA) separated from the deoxypentosenucleic acid (DNA) following alkaline hydrolysis (17) by acidification. The PNA was further hydrolyzed with acid, and the purines precipitated as silver salts. The purines were regenerated and separated by paper chromatography. The PNA adenine and guanine were prepared for the determination of radioactivity as has previously been described (2). The activities were determined in an internal Geiger-Müller flow counter (Radiation Counter Laboratories, mark 12, model 1, helium isobutane gas). The radioactivities were presented as relative specific activities (RSA) where

\[
RSA = \frac{\text{counts/minute/e isolated compound}}{\text{counts/minute/e injected compound}} \times 100.
\]

In those cases where the RSA's are about 0.3 or more, the activities on individual planchets have been determined to a standard error of 5 per cent or less. In all other cases the standard error of the determination was 10 per cent or less, except for the glycine experiment, in which errors were 5 per cent for RSA's of more than 0.08 (18). On the basis of results of previous work (4), variations between experiments of less than 80 per cent have not been considered as significant.

**RESULTS**

The values given for the incorporation into normal hamsters (Experiments 1, 2, 3, 4, and 5) and into the hamsters with H.S. #1 (Experiments 41, 42, 43, 44, and 45) are values previously reported (4) and are included here to permit direct comparison of the effects of partial hepatectomy (Tables 1–5). The results for Experiments 11, 12, 13, and 15 show the effects of partial hepatectomy and/or the resulting regeneration of the liver on the utilization of various purine precursors by each of the several

### TABLE 1

**EFFECTS OF REGENERATING LIVER ON INCORPORATION OF ADENINE-8-C14 INTO PNA ADENINE (A) AND GUANINE (G)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Exp. 1</th>
<th>Exp. 11</th>
<th>Exp. 41</th>
<th>Exp. 45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>1.72</td>
<td>0.25</td>
<td>2.22</td>
<td>1.73</td>
</tr>
<tr>
<td>Liver</td>
<td>0.85</td>
<td>0.14</td>
<td>5.53</td>
<td>4.27</td>
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<tr>
<td>Kidney</td>
<td>0.50</td>
<td>0.20</td>
<td>2.83</td>
<td>2.13</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.04</td>
<td>0.14</td>
<td>0.47</td>
<td>0.92</td>
</tr>
<tr>
<td>Tumor</td>
<td>0.45</td>
<td>0.07</td>
<td>1.47</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Activity of precursor was 940 cpm/µg.

Exp. 1: Normal hamsters.

Exp. 11: Normal hamsters, hepatectomised, two independent experiments.

Exp. 41: Hamsters carrying HS #1.

Exp. 45: Hamsters carrying HS #1, hepatectomised, two independent experiments.

### TABLE 2

**EFFECTS OF REGENERATING LIVER ON INCORPORATION OF 5,6-DIAMINOPURINE-8-C14 INTO PNA GUANINE**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Exp. 8</th>
<th>Exp. 18</th>
<th>Exp. 48</th>
<th>Exp. 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>0.43</td>
<td>0.63</td>
<td>0.53</td>
<td>0.59</td>
</tr>
<tr>
<td>Liver</td>
<td>0.56</td>
<td>0.82</td>
<td>0.88</td>
<td>1.04</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.25</td>
<td>0.37</td>
<td>0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.27</td>
<td>0.37</td>
<td>0.27</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Activity of precursor was 1,500 cpm/µg.

Exp. 8: Normal hamsters.

Exp. 18: Normal hamsters, hepatectomised.

Exp. 48: Hamsters carrying HS #1.

Exp. 58: Hamsters carrying HS #1, hepatectomised.

### TABLE 3

**EFFECTS OF REGENERATING LIVER ON INCORPORATION OF GUANINE-8-C14 INTO PNA GUANINE**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Exp. 8</th>
<th>Exp. 18</th>
<th>Exp. 45</th>
<th>Exp. 58</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine</td>
<td>0.22</td>
<td>0.07</td>
<td>0.19</td>
<td>0.10</td>
</tr>
<tr>
<td>Liver</td>
<td>0.30</td>
<td>0.45</td>
<td>0.24</td>
<td>0.59</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.22</td>
<td>0.42</td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.15</td>
<td>0.08</td>
<td>0.09</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Activity of precursor was 1,000 cpm/µg.

Exp. 8: Normal hamsters.

Exp. 18: Normal hamsters, hepatectomised, average of two values.

Exp. 45: Hamsters carrying HS #1.

Exp. 58: Hamsters carrying HS #1, hepatectomised, average of two values.
tissues studied.

There was a decrease in the incorporation of guanine and, perhaps, also of hypoxanthine into the intestinal PNA of the hepatectomized animals, but, in general, there was an increase in the synthesis of nucleic acid purines from the precursors studied. This increase was less pronounced in the intestines and not observed with guanine as a precursor. With adenine, 2,6-diaminopurine, and glycine as precursors, the results obtained in the hepatectomized animals indicated that a shift in uptake had occurred which was similar to that observed in the tumor-bearing animals. This was especially pronounced in the glycine experiments. When tumor-bearing animals were hepatectomized (Experiments 51, 52, 53, 54, and 55) there were no striking or systematic changes noted in the host's tissues. It is noteworthy, however, that the tumors responded differently to the hepatectomy with each precursor. When diaminopurine, guanine, or hypoxanthine were used, there was no ap-
normal liver. It is apparent that there are both similarities and dissimilarities between rapidly growing noncancerous tissues (e.g., regenerating liver and intestine) and cancers.

As a result of the partial hepatectomy certain changes in the biogenesis of the purines of nucleic acids have been noted. It is not possible a priori to determine whether or not these influences on metabolism are due to the hyperplastic liver or to the reduced quantity of functioning liver. One approach to this problem lies in the use of totally hepatectomized animals.

The uptake of labeled purine precursors by the PNA of regenerating liver has been determined in the rat in a series of experiments in which the precursors (adenine and glycine) were administered over a period of 5 days (8, 9). No effect of adenine utilization was noted, and the glycine uptake was more than doubled in the regenerating liver. The changes in uptake observed in the data presented here are not necessarily contradictory, since these effects were found during the 24-48-hour period following hepatectomy. It is quite possible that, though pronounced changes do occur during this period, by the end of 5 days the later metabolism dilutes the changes so that the resultant values are not so large. An alternate explanation may be that the earlier work was done with adult rats, and these experiments with weanling hamsters.

The stimulation of liver growth following partial hepatectomy has been ascribed to the presence or absence of humoral factors (6, 10). It is not possible at the present time to determine the relationship between liver hyperplasia and the altered tumor metabolism reported herein. It must be emphasized that the observations reported were made with one tumor in one host, and there is no justification for assuming that they would of necessity be found with all tumors in all hosts.4

There does exist a strong possibility that the observed alterations in metabolism are reflections of an alteration in the balance of growth-regulatory substances. These effects might be manifestations of response of other tissues to the changed amounts of such substances.

SUMMARY

The utilization of five precursors of nucleic acid purine (adenine, 2,6-diaminopurine, guanine, hypoxanthine, and glycine) have been determined in partially hepatectomized hamsters. There was a greatly increased uptake of all precursors except guanine into the tissues of the hamsters with re-

4 Preliminary data from this laboratory with other tumors and animals indicate that these effects are not always found.

References


Studies on the Metabolism of Human Tumors: III. Influence of Regenerating Liver on Purine Metabolism

M. Earl Balis, Dina Van Praag and George Bosworth Brown


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