The Mechanism of Action of 6-Mercaptopurine

II. Basis for Specificity

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SUMMARY

The conversion of IMP to AMP and XMP by tumors and liver was examined as part of the study of the basis for the anti-tumor specificity of 6-MP. Liver preparations convert IMP to AMP more efficiently than those from tumors. In incubations with tumor preparations, but not with liver, an accumulation of succinoadenylate was observed. A 6-MP resistant S-180 was examined and found to resemble liver in its capacity to form adenylate. The succinoadenylate formed was less than half that found with sensitive S-180. These findings, which indicate that a lack of adenylsuccinase may be a major cause of deficiency in adenyl synthesis, were confirmed by direct assays for this activity. In line with the observations that the same enzyme catalyzes the cleavage of both succinoadenylate and succinoaminoimidazolecarboxamide ribonucleotide (SAICAR) in other species, the ability of S-180 to cleave these 2 substrates, compared to liver, was reduced to the same extent. Similar studies of the conversion of IMP to XMP indicate that the tumor also had less capacity to synthesize XMP than did the host liver.

Tissues from mice which had received prior treatment with 6-MP were somewhat less active in these conversions than those from controls. The reduction in the capacity to synthesize AMP appears to be greater in both tissues. The relative effect appears to be greater in the tumors than in the livers.

These data suggest that the basis for the tissue specificity of 6-MP may be attributable in part to reduced enzymatic capacity of certain tissues to synthesize AMP and XMP from IMP. Since this capacity seems to be less in tumors, inhibition by 6-thio IMP of these metabolic steps might be more critical in tumors.

MATERIALS AND METHODS

IMP-8-14C was obtained from Schwanz BioResearch, Inc.; ATP (disodium salt), IMP (sodium salt), GTP (tri-
sodium salt), NAD (β-DPN), L-glutamine, fructose-1,6-diphosphate (sodium salt), α-ketoglutarate, reduced glutathione (crystalline), and pyridoxyl phosphate were obtained from Sigma Chemical Company.

Xanthine oxidase was purchased from Worthington Biochemical Corporation. Yeast adenylosuccinase was prepared from Fleischmann's active dry yeast (Standard Brands, Inc.) according to the method of Carter and Cohen (6).

SAICAR was prepared from a Biogen culture of Escherichia coli strain B974 according to the method of Gots and Gollub (9). Succinoadenylate was prepared by the method of Carter and Cohen (6).

HA/ICR Swiss mice (approximately 20 gm each) with a 3- or 7-day-old s.c. implant of S-180 were kindly supplied by Dr. H. Schwarts and Dr. C. Reilly of this institute. Mice with 11-day-old s.c. implants of a resistant variant of S-180 were supplied by Dr. C. Clarke. In all instances, tumors with perceptible necrosis were discarded. C57 black mice (approximately 20 gm each) with 9- and 18-day-old s.c. implants of Adenocarcinoma E 0771 were supplied by Dr. K. Sugura of this institute.

Mice with 3-day-old implants of S-180 were given i.p. injections of 6-MP (50 mg/kg body weight) for 4 successive days. Control animals were given CMC-saline injections. The animals were sacrificed on the 7th day and the tumor and liver removed.

The preparation and use of acetone powders of the 2 tissues have been discussed elsewhere (23).

**Preparation of Cell-Free Extracts**

A. Extracts converting inosinate to adenylate.—Acetone powder of frozen tissue was homogenized in a medium containing 0.16 M KCl and 0.05 M Tris HCl buffer, pH 7.4 (17) (4 gm wet weight/ml) in a Virtis “45” homogenizer for 3 min. To partially fractionate the active system(s) were centrifuged at 15,000 X g for 30 min, and at 34,800 X g for 60 min. All the operations were carried out at 4°C.

B. Extracts of adenylsuccinase assay.—Fresh or frozen tissue was homogenized in a Virtis “45” homogenizer for 2 min in 0.025 M phosphate buffer, pH 7.2 (4 gm wet weight/10 ml). The preparation was cleared of cellular debris by centrifuging at 10,000 X g for 30 min. All preparations were carried out at 4°C.

C. Extracts converting inosinate to guanylate.—Acetone powder or frozen tissue was homogenized in a Virtis “45” homogenizer with 0.05 M phosphate buffer, pH 7.4 (2 gm wet weight/10 ml). Dialyzed 20,000 X g supernatants were prepared according to the method used by Lagerkvist for pigeon liver extracts (14).

**Assay Methods**

A. Conversion of inosinate to adenylate.—Unless otherwise designated, each 10 ml of incubation mixture contained the extract from 2 gm wet weight of the tissue (or the equivalent amount of acetone powder); 2.5 mmole ATP; 2.5 mmole L-aspartate; 5 mmole fructose-1,6-diphosphate; 0.5 mmole MgCl₂; 5 mmole sodium phosphate buffer, pH 7.4; 2.5 mmole α-ketoglutarate; 1 mmole NAD; and “x” μmoles IMP-8-14C (specific activity, 6.31 X 10⁴ cpm/μmol). In studies comparing the energy source in this conversion, 2.5 mmole of GTP were used in place of ATP, NAD, fructose-1,6-diphosphate, and α-ketoglutarate. The tissue extracts were added to the substrate solution after a 30-min equilibration period, and the incubations were carried out either at 37.5°C for 2 hr (unless otherwise specified) or at 20°C for 4 hr in a Dubnoff metabolic shaker. The lower temperature was chosen for some of the experiments since the incorporation studies of Newton and Perry (17) have shown that succinoadenylate accumulated in skeletal muscle preparations incubated at this temperature.

At the end of the incubation, the reaction was stopped by immersion of the vessels in an acetone Dry Ice bath. Sufficient cold 60% TCA was added to the reaction mixture to give a final TCA concentration of 7%. The precipitated protein was removed by centrifugation in the cold and resuspended and washed twice with cold 5% TCA. The combined supernatant solutions were extracted 10 times with ether to remove the TCA. The clear solution was either lyophilized to dryness or neutralized and stored at −20°C.

The dry samples were taken up in freshly prepared 1 N HCl and hydrolyzed for 24 hr at 100°C. The free purines were isolated by chromatography on Dowex-50 (hydrogen form, AGW, X8, 200–400 mesh) columns with HCl (1).

The components of neutralized and deproteinized mixtures, on the other hand, were separated on Dowex-1 (chloride form, X10, 200–400 mesh) columns by a modification of the method of Deutch and Nilsson (7). This consisted of stepwise elution with (a) 0.003 N HCl, (b) 0.005 N HCl, (c) 0.01 N HCl-0.02 M NaCl, (d) 0.01 N HCl-0.05 M NaCl, (e) 0.01 N HCl-0.2 M NaCl, (f) 0.01 N HCl, and (g) 1.0 N HCl. The identities of the various fractions were checked spectrally and by paper chromatography. The identity of succinoadenylate was further confirmed by incubating it with yeast adenylsuccinate (6).

The radioactivities of the various compounds were determined on infinitely thin films on aluminum planchets in a Geiger-Müller internal flow counter.

B. Adenylosuccinase activity.—The cleavage of succinoadenylate was followed spectrally, i.e., the decrease in OD at 260 nm (6), and by paper chromatography. Each mixture containing 449 μmoles of substrate in 0.4 ml phosphate buffer (pH 7.2) and 2.0 ml tissue extract was incubated at 37.5°C. The reaction was stopped by heating for 3 min at 100°C, cooling rapidly, and centrifuging. For spectral determinations, the precipitate was resuspended and washed with buffer. The combined supernatant solutions were made up to a final volume of 5 ml. To isolate the adenylate formed, replicate samples were similarly deproteinized, the precipitate washed with water, and the supernatant solution concentrated to a minimal volume for paper chromatography (1-AmOH—K₂HPO₄) (6).

The cleavage of SAICAR was followed by using a modified Bratton-Marshall procedure in which coupling was delayed 8 min after diazotization (9). Each incubation mixture consisted of 2.0 ml tissue extract and 0.25 ml buffer (pH 7.2) containing 640 μmoles of substrate.
C. Conversion of inosinate to guanylate.—The initial incubations were carried out in the complex medium described by Lagerkvist (14). Each incubation mixture contained the dialyzed extract from 2 gm wet weight of tissue or the equivalent amount of acetone powder; 1.41 μmoles IMP-8-14C (specific activity, 5.26 × 10^4 cpm/μmole); 4 μmoles ATP; 100 μmoles fructose-1,6-diphosphate; 20 μmoles NAD; 120 μmoles nicotinamide; 120 μmoles L-aspartate; 120 μmoles L-glutamate; 120 μmoles L-glutamine; 120 μmoles MgSO4; 150 μg pyridoxal phosphate; and 1000 μmoles sodium phosphate buffer, pH 7.4 (20 ml total volume). This will be referred to as “complex medium” in the text and tables.

Since the conversion to xanthylate is the step inhibited by 6-thio IMP (Chart 5), subsequent incubations (20 ml) consisted of dialyzed extracts from 2 gm wet weight, 20 μmoles NAD, 120 μmoles of phosphate buffer, pH 7.4, 40 μmoles of glutathione, and “x” μmoles of IMP-8-14C (referred to as “simple medium”).

The tissue extracts were added to the substrate after a 20-min equilibration period and the incubations were carried out for 90 min (unless otherwise specified) at 37.5°C in a Dubnoff metabolic shaker. The reaction was stopped by immersing each vessel for 3 min in a boiling water bath, cooled to 37.5°C, and further incubated for 30 min with 2500 units of xanthine oxidase to remove any xanthine that might have arisen from degradation of IMP. At the end of this incubation, the reaction was stopped by freezing, and the acid-soluble fraction was prepared in the manner previously described in the assay of the conversion of inosinate to adenylate.

The components of the acid-soluble fraction (adjusted to pH 9 with concentrated NH4OH) were separated on Dowex-1 (bromide form, X10, 200–400 mesh) columns into 3 fractions, namely, (a) feed water wash, containing nicotinamide and nucleosides, (b) 0.0005 N HBr, containing free purines, and (c) 0.18–1.0 N HBr, containing combined nucleotides.

All 3 fractions were concentrated by lyophilization for subsequent column chromatography. The nucleotide fraction was adjusted to 1 N with respect to HCl and hydrolyzed for 24 hr at 100°C. The purines were isolated from these fractions by chromatography on Dowex-50 (hydrogen form, AGW X8, 200–400 mesh) by either stepwise (1) or gradient elution with HCl. With a 16 × 1 cm resin-bed column, a suitable linear gradient (18) was obtained by feeding 1920 ml of 5 N HCl into a mixer chamber containing 400 ml of 1 N HCl; in turn, this was fed into a second mixer chamber containing 400 ml of 0.1 N HCl. The rate of flow of the eluent was regulated to about 0.5 ml/min.

The nicotinamide-nucleoside fraction was chromatographed on paper after an initial fractionation with HCl gradient. The identities of the various components were checked spectrally and by paper chromatography and the radioactivities were determined.

RESULTS AND DISCUSSION

The conversion of inosinate to adenine ribonucleotides by various preparations of liver and S-180 is summarized in Table 1. In the liver, the activity of the adenine-nucleotide fraction was consistently about 3 times that found in S-180. The latter, however, accumulated one third of its total adenine derivatives in the form of the intermediate, succinoadenylate, which was not found in the various liver preparations under these experimental conditions. The total adenine derivatives formed by S-180 were about one-half that of the liver. Doubling of the substrate concentration essentially doubled the conversion without any effect on the relative activity of the two tissues.

The most active of the preparations was the 15,000 × g supernatant solution; this has been used for all subsequent studies. The 34,800 × g supernatant solution was about as active as the whole homogenate. These results suggest that at 15,000 × g some of the degradative enzyme system(s) were removed. At 34,800 × g some of the enzyme systems involved in the regeneration of ATP (and perhaps, subsequently GTP) were probably also removed.

A number of workers (16, 17, 25, 26) have shown that in mammalian tissues ATP and an ATP-regenerating system could substitute for the GTP required (15) in the conversion of inosinate to adenylate. Studies with liver and S-180 are in agreement with these observations (Table 2). The endogenous guanine nucleotides, 0.92 and 0.75 μmoles/gm wet weight of liver and S-180, respectively (23), supplemented by added ATP and substrates for ATP regeneration, seem to be as efficient as added GTP in these relatively crude extracts. Consequently ATP was used in the studies presented here. A 3-fold increase in the ATP and substrates required for its regeneration did not affect the conversion.

The results of varying substrate concentrations (Table 3) show that the formation of adenylate was proportional to substrate concentration at and below 5.16 μmoles/10 ml incubation. Higher concentrations had no effect on S-180 and caused only a 20% increase in liver. At the higher concentrations, the difference between the two tissues be

<table>
<thead>
<tr>
<th>TISSUE PREPARATION</th>
<th>LIVER (μmole/gm wet wt./hr)</th>
<th>S-180 (μmole/gm wet wt./hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMP, ADP, ATP</td>
<td>Succin-AMP</td>
</tr>
<tr>
<td>Whole homogenate</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>Supernatant</td>
<td>115</td>
<td>0</td>
</tr>
<tr>
<td>15,000 × g</td>
<td>303</td>
<td>0</td>
</tr>
<tr>
<td>34,800 × g</td>
<td>80</td>
<td>0</td>
</tr>
</tbody>
</table>

* See “Assay Methods.” All these incubations were carried out at 20°C for 4 hr with an IMP concentration of 2.88 μmoles/incubation.

* Abbreviations used are: AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

* This incubation was carried out under the same conditions but with 5.16 μmoles of IMP/incubation.

* For the purpose of this study, this represents the sum of the mono-, di-, and triphosphates.

* There was less inosine in the incubations with either supernatant fraction than in those with whole homogenates.
TABLE 2

Comparison of Energy Sources in the Conversion of Inosinate to Adenylate

<table>
<thead>
<tr>
<th>ATP</th>
<th>GTP</th>
<th>Total Adenine Derivatives (mmol/mg wet wt./hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver                                 S-180</td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>&lt;2                                     &lt;2</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>185                                    91</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>198                                    95</td>
</tr>
<tr>
<td>+ +</td>
<td>−</td>
<td>201                                    99</td>
</tr>
<tr>
<td>+ + +</td>
<td>−</td>
<td>204                                    104</td>
</tr>
</tbody>
</table>

*See “Assay Method.” These incubations were carried out at 37.5°C for 2 hr with 5.16 µmole IMP/10 ml incubation. The 15,000 x g supernatant of the tissue preparation was used.

b Adenosine triphosphate. The energy source consisted of 2.5 mmoles ATP, 5 mmoles fructose-1,6-diphosphate, 2.5 mmoles α-ketoglutarate, and 1 mmole nicotinamide adenine dinucleotide/10 ml incubation, designated as (+). Three times this amount was used in the experiment indicated as +++. Guanosine triphosphate. 2.5 mmoles GTP were substituted for the aforementioned ATP system (footnote b).

c All these samples were hydrolyzed to free purines before chromatography. Neither succinoadenine nor its riboside was detectable in these samples. The conversion was measured as labeled adenine isolated.

d These are average values from 4 hr incubations at 20°C; succinoadenosine monophosphate is included in the S-180 values.

These average values from 4 hr incubations at 20°C; succinoadenosine monophosphate is included in the S-180 values.

comes magnified, i.e., the capacity in S-180 is only about 40% that in the liver. The results obtained with the two different incubation conditions were essentially the same. No succinoadenine derivatives, however, were found on fractionation of the mixtures incubated at 37.5°C.

Under the experimental conditions used in these studies, the conversion to adenylate was proportional to the amount of tissue added, (Table 4) and proceeded linearly for more than 3 hr (Chart 1).

In the resistant variant of S-180 studied (Table 5), the conversion to adenine ribonucleotides was about 80% that of the corresponding host liver; less than 10% (in contrast to the 30% observed in sensitive S-180) accumulated in the form of succinoadenylate. The enzymatic activity of a preparation of sensitive tumor studied at the same time was about half that of the resistant variant. The liver of
the host, however, exhibited the same enzymatic capacity regardless of which tumor was carried.

Treatment of the host animals with a therapeutic dose of 6-MP resulted in a slight decrease in the enzymatic activity under consideration. The ability of the liver and tumor preparations of these animals to form adenylate was found to be 80% and 65%, respectively, of the control values (Table 6). The total over-all conversion by S-180 was about 35% that of the corresponding liver. Just as in the control animals, succinoadenylate was found only in the tumor; about 30% of the total adenine nucleotides were in this form.

**TABLE 6**

**EFFECT OF 6-MERCAPTOPURINE (6-MP) THERAPY ON THE CONVERSION OF INOSINATE TO ADENINE RIBONUCLEOTIDES**

<table>
<thead>
<tr>
<th></th>
<th>AMP, ADP, ATP (mumoles/gm wet wt./hr)</th>
<th>Succino-AMP (mumoles/gm wet wt./hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (uninjected)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>303</td>
<td>0</td>
</tr>
<tr>
<td>S-180</td>
<td>77</td>
<td>27</td>
</tr>
<tr>
<td>Control (CMC-saline)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>212</td>
<td>0</td>
</tr>
<tr>
<td>S-180</td>
<td>74</td>
<td>32</td>
</tr>
<tr>
<td>Treated (6-MP, 50 mg/kg body wt.)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>173</td>
<td>0</td>
</tr>
<tr>
<td>S-180</td>
<td>52</td>
<td>18</td>
</tr>
</tbody>
</table>

* See "Materials and Methods," and "Assay Methods." Incubations were carried out at 20°C for 4 hr with 5.16 mumoles inosine monophosphate and 15,000 × g supernatant.

Abbreviations used are: AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; CMC, carboxymethyl cellulose.

Mice with 3-day-old s.c. implants of S-180 were injected i.p. or 4 successive days.

The accumulation of succinoadenylate in extracts of S-180 suggests that there may be a lower level of adenylosuccinate activity in this tissue. This activity has been assayed with both succinoadenylate (Chart 2) and SAICAR (Chart 3) as substrate. The activity was determined by following the spectral change. In the case of succinoadenylate, the product in replicate samples was isolated by paper chromatography; the two values obtained corresponded closely. In both instances, the enzymatic activity in S-180 was 35-40% that in the liver preparations.

Table 7 summarizes the data on the conversion of inosinate to xanthylate. This study has been extended to include the tissues of C57 black mice bearing the tumor E 0771. The conversion to xanthylate in extracts of these

**TABLE 7**

**CONVERSION OF INOSINATE TO XANTHYLATE BY VARIOUS TISSUE PREPARATIONS**

<table>
<thead>
<tr>
<th>Tissue preparation</th>
<th>Inosine monophosphate (mumoles/incubation)</th>
<th>Xanthylate (mumoles/gm wet wt/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA/ICR Swiss mice bearing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-day-old S-180 implant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.41</td>
<td>31</td>
</tr>
<tr>
<td>S-180</td>
<td>1.41</td>
<td>11</td>
</tr>
<tr>
<td>Liver</td>
<td>2.82</td>
<td>80</td>
</tr>
<tr>
<td>S-180</td>
<td>2.82</td>
<td>20</td>
</tr>
<tr>
<td>C57 black mice bearing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E 0771 implant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9-day implant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>2.82</td>
<td>232</td>
</tr>
<tr>
<td>E 0771</td>
<td>2.82</td>
<td>33</td>
</tr>
<tr>
<td>18-day implant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>1.41</td>
<td>170</td>
</tr>
</tbody>
</table>

* See "Assay Methods." Incubations were carried out with the complex incubation medium. In all instances, the amount of guanylate formed was negligible.

[Charts 2 and 3 are not included in the text.]
tumors is much lower than that in the corresponding liver preparations, i.e., about 25—30% in S-180 and 12% and 30% in E 0771. In the relatively slow growing tumor, E 0771, two different implants have been examined to determine if age of the tumor can be correlated with enzymatic capacity. In spite of the lower substrate concentration used in the particular assay, the extracts of 18-day-old implants were actually found to be more efficient in this conversion than those from 9-day-old implants. With the same set of assay conditions, the liver preparations from the animals with the older implants seemed to be less efficient. These observations may bear some relationship to the lesser response of older, more established tumors to therapy.

The conversion of inosinate to xanthylate by extracts of liver and S-180 was studied at several different concentrations of IMP (Table 8). The relative conversion of the 2 tissues was the same at all the concentrations studied; furthermore, the saturation concentration seemed to be the same for both tissues, i.e., 7.13 μmoles. The higher values observed here compared to Table 7 could probably be attributed to the protective effect of the glutathione (12) in the “simple medium” (see “Assay Methods”) used in these studies.

The ability of extracts to catalyze the formation of xanthylate with time was studied. It can be seen that with both tissues, the reaction was linear for about 3 hr of incubation by column chromatography. (XMP, xanthosine monophosphate; GMP, guanosine monophosphate; IMP, inosine monophosphate; XMP, guanosine monophosphate; NAD, nicotinamide adenine dinucleotide.

**TABLE 8**

<table>
<thead>
<tr>
<th>Inosine Monophosphate (μ mole/incubation)</th>
<th>Xanthylate (μ mole/gm wet wt/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>0.713</td>
<td>25</td>
</tr>
<tr>
<td>1.426</td>
<td>55</td>
</tr>
<tr>
<td>2.852</td>
<td>117</td>
</tr>
<tr>
<td>7.130</td>
<td>184</td>
</tr>
<tr>
<td>12.834</td>
<td>193</td>
</tr>
</tbody>
</table>

* See “Assay Methods.” Incubations were carried out in “simple medium.”

**CHART 4.—Formation of xanthylate from inosinate by tissue extracts.** Incubations were carried out for 45—270 min with 7.13 μmoles inosine monophosphate in the “simple medium” described in “Assay Methods.” The product was isolated after various times of incubation by column chromatography. (XMP, xanthosine monophosphate.)

In studying the mechanism of action of a drug, it is evident that even though the site of action may be common to many living systems, there is considerable variation in biologic response. Qualitatively different responses could result from quantitative reduction in the enzymatic capacities of a system. Numerous examples can be cited from studies with bacteria, e.g., (a) a 50-fold increase in a mutant’s resistance to 6-MP is accompanied by a 30% reduction in its ability (compared to the parent) to convert this compound in its active form, 6-thio IMP (13, 24); (b) a total loss of ability of a mutant to grow on 2,6-diaminopurine as a purine source occurs concomitantly with a 30% reduction in its ability to incorporate this substrate (3). It is conceivable that the selective susceptibility of tumors to various chemotherapeutic agents is due, in part, to such a quantitative reduction in certain enzymatic capacities.

The lower endogenous inosinate concentration in S-180, in spite of its greater capacity for purine synthesis de novo and its slightly greater capacity to synthesize the active compound, 6-thio IMP (23), cannot explain the degree of specificity seen in vivo. It seems more likely to lie, at

**TABLE 9**

<table>
<thead>
<tr>
<th></th>
<th>Xanthylate (μ mole/gm wet wt/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Uninjected</td>
<td>80</td>
</tr>
<tr>
<td>Carboxymethyl cellulose saline</td>
<td>84</td>
</tr>
<tr>
<td>Treated (50 mg/kg body wt.)</td>
<td>70</td>
</tr>
</tbody>
</table>

* See “Assay Methods.” Incubations were in “complex medium” with 2.82 μmole inosine monophosphate.

* Mice with 3-day-old s.c. implants of S-180 were injected i.p. for 4 successive days.

**CHART 5.—Inhibition of nucleotide interconversions by 6-thioinosinate.** Dotted lines indicate sites of inhibition. GTP, guanosine triphosphate; ATP, adenosine triphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; XMP, xanthosine monophosphate; GMP, guanosine monophosphate; NAD, nicotinamide adenine dinucleotide.

Conclusions

In studying the mechanism of action of a drug, it is evident that even though the site of action may be common to many living systems, there is considerable variation in biologic response. Qualitatively different responses could result from quantitative reduction in the enzymatic capacities of a system. Numerous examples can be cited from studies with bacteria, e.g., (a) a 50-fold increase in a mutant’s resistance to 6-MP is accompanied by a 30% reduction in its ability (compared to the parent) to convert this compound in its active form, 6-thio IMP (13, 24); (b) a total loss of ability of a mutant to grow on 2,6-diaminopurine as a purine source occurs concomitantly with a 30% reduction in its ability to incorporate this substrate (3). It is conceivable that the selective susceptibility of tumors to various chemotherapeutic agents is due, in part, to such a quantitative reduction in certain enzymatic capacities.

The lower endogenous inosinate concentration in S-180, in spite of its greater capacity for purine synthesis de novo and its slightly greater capacity to synthesize the active compound, 6-thio IMP (23), cannot explain the degree of specificity seen in vivo. It seems more likely to lie, at
least in part, in differences in the nucleotide conversions (Chart 5) shown to be inhibited by 6-thio IMP (24).

From the data presented, the over-all conversions of inosinate to adenine and xanthine derivatives in S-180 are about 50% and 25–30%, respectively, of those found in the liver. Consequently, inhibition by 6-thio IMP of the further conversion of inosinate could result in reduction of the tumor's inherently lower enzymatic capacity below the threshold required for growth. A similar reduction of the process in liver might not cause a serious deficiency. The relative reduction would be less in the liver, which has a "reserve" capacity, as reflected by its higher endogenous nucleotide concentrations (23).

The lower adenylosuccinase activity (about 35–45% of that in the liver) in S-180 is consistent with the observed accumulation of this intermediate (about 30% of the total adenine derivatives) in the incubation mixtures of the tumor. This lowered enzymatic capacity has been further substantiated by the accumulation of SAICAR in S-180 (compared to liver) in purine synthesis de novo with aspartate-14C as precursor. The similarity in the enzymatic cleavages of the 2 substrates (succinoadenylate and SAICAR) suggests that in these 2 tissues, as in microbial systems (9), the same enzyme is involved. Although all the parameters measured indicate qualitative identity of the enzyme in liver and in S-180, the possibility of an "altered" enzyme in S-180 has to be considered.

The usefulness of any anti-cancer agent stems from the fact that host tissues are inherently more resistant to the action of the agent than the tumor. Such host resistance may be because the enzymatic makeup of the host cells is qualitatively and/or quantitatively different from the tumor cell. The more efficient the agent is, the greater the difference between the two. Consequently, resistance in tumors can, in some cases, be considered as a shift in certain enzymatic capacities to those found in the host.

The foregoing data indicate that the host tissue studied is relatively resistant by virtue of an excess of enzymes which are involved in the sequence of metabolic steps inhibited by 6-thio IMP (Chart 5). In the resistant variant studied, the tumor seems to have undergone a change, making it resemble the host liver. Its resistance could be attributed partially to this shift in enzymatic capacity.

It is possible that anti-purines of chemotherapeutic value (e.g., 6-MP, by virtue of its structural similarity), could affect purine interconversions through enzyme repression. The results obtained from treated animals show only a small decrease in enzymatic activity of both liver and S-180. Nevertheless, this reduction, however small, could increase the differential between the 2 tissues, i.e., enhancing the inherently lower enzymatic capacity of the tumor. It is possible, however, that these observed reductions are not specific for the particular enzyme systems studied but is merely a secondary manifestation of the toxicity of the drug. Similar lowering of enzymatic conversions have been observed with other purine analogs (4, 22).

It is too early to extrapolate to tumors in general even though in the conversion to xanthylate, E 0771 parallels the results obtained with S-180. The available data have been obtained from cell-free preparations of liver (the only host tissue studied) and tumor tissues, and do not necessarily reflect the over-all picture of in vivo systems. They do lend support to the concept that quantitative and/or qualitative biochemical differences between tissues could serve as a basis for effective anti-tumor chemotherapy. Ultimately, other host tissues will have to be studied in order to obtain a more complete understanding of the response in the intact animal.

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The Mechanism of Action of 6-Mercaptopurine: II. Basis for Specificity

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