Short Communication

A Method for Testing the Specificity of Inhibitors of Deoxyribonucleic Acid Synthesis in Growth Studies

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Inhibitors of DNA synthesis are often used to infer the role of DNA synthesis in growth regulation (1, 11). The usefulness of such an inhibitor depends upon the extent to which it is both effective and specific. The requirement of effectiveness demands that the inhibitor truly prevent subsequent DNA synthesis. The requirement of specificity demands that essentially all the growth-inhibition result from inhibition of DNA synthesis; in other words, there should be an absence of "nonspecific growth-inhibition," which we here define as a reduction in growth that is not caused directly or indirectly by the inhibition of DNA synthesis. Whereas the requirement of effectiveness is often tested, the requirement of specificity is seldom if ever checked, probably owing to the lack of a suitable method. We here describe a method for testing the specificity of inhibitors of DNA synthesis. We also show how this test of specificity, when coupled with an independent test of effectiveness, permits conclusions concerning usefulness of chemicals as specific inhibitors of DNA synthesis in studies of growth.

We test the specificity of inhibitors by examining their actions in two systems that do not require DNA synthesis. These systems are the germination of heavily irradiated lettuce seeds, which occur without detectable DNA synthesis (8), and the growth of wheat "gamma-plantlet" seedlings, which does not require DNA synthesis (5). Since both systems gave similar results, we present only the findings for wheat. Despite the heavy seed irradiation and because of the absence of many of the typical cytogenetic effects of ionizing radiations (6), gamma-plantlet growth is remarkably normal in many respects, including sensitivity to growth regulators (5, 7, 8, 12). Since in gamma-plantlets there is no DNA synthesis to inhibit, any growth-inhibition that is caused by an inhibitor must be a nonspecific growth-inhibition. The inhibitors tested were FdUrd\(^8\) (4, 10, 11), hydroxyurea (1, 13), and phenethyl alcohol (2, 11).

The wheat, *Triticum vulgare* Vill. (*Triticum aestivum* L.) var. Lemhi had a moisture content of about 10% when irradiated with 500 krad of \(^60\)Co gamma rays at 4.5 krad/min. Germination and growth conditions are described elsewhere (9). The pH of all solutions was 5.7. In some experiments the grains were sown directly in solutions of the inhibitors. In other experiments seedlings were treated with inhibitors after germination and selected for uniformity when the coleoptile length was 3 to 4 mm. DNA per group of 40 seedlings, including scutella and excluding endosperm, was extracted by the method of Smillie and Krotkov (14) and determined by the diphenylamine method (3).

The capacity of the inhibitors to produce nonspecific growth-inhibition can be seen in Table I as a decreased growth resulting from their application to gamma-plantlets. The inhibitors are more effective in producing nonspecific growth inhibition when applied from the beginning of imbibition than when applied a few days later, during early germination. The inefficacy of FdUrd on growth of gamma-plantlet seedlings relative to unirradiated seedlings cannot be attributed to differences in uptake, because the uptake of 2-\(^3\)H-FdUrd was the same into gamma-plantlet and unirradiated control seedlings of the same size (unpublished). In Figure 1 (stippled bars) we plot the concentration ranges for which gamma-plantlet growth is not significantly affected by application of inhibitors to the seedlings. Thus, the stippled bars represent the concentration ranges for which the requirement of specificity seems valid.

For a specific inhibitor of DNA synthesis to be useful, concentration ranges for specificity and effectiveness must overlap. We therefore determined the DNA content per unirradiated seedling so as to determine the concentration range necessary for effectiveness for each inhibitor. The DNA content of the 2-day-old seedlings just before treatment with the inhibitors was approximately 3 \(\mu\)g per axis. Within the next 5 days there was an approximately 4-fold increase in DNA in controls not exposed to any inhibitor. (These values may be compared with the initial value, at the time of sowing, of approximately 2 \(\mu\)g DNA per embryo, which value was maintained during growth of gamma-plantlets). The concentrations of inhibitors necessary to prevent detectable increases in DNA content, when applied to unirradiated seedlings, are represented in Figure 1 by the solid bars. The high concentrations necessary for apparent inhibition of DNA synthesis are partly a reflection of the method of application to the whole seedlings and partly a reflection of our requirement that the inhibition of increase in DNA per seedling be complete rather than partial.

Absence of a detectable increase in DNA content would not
preclude DNA turnover or small amounts of synthesis. Thus, the concentrations for fulfilling completely the requirement of effectiveness might be somewhat higher than represented by the solid bars in Figure 1. Also, since there may be nonspecific effects of the inhibitors that might not show up on growth in the gamma-plantlet test system, the concentrations for absolute biochemical specificity might be somewhat lower than the range indicated by the stippled bars. Consequently, in comparing the concentration ranges for complete effectiveness and absolute specificity, an overlap may be smaller, and a gap may be larger, than indicated. A usable inhibitory treatment (e.g., FdUrd given to seedlings) also produces a plateau in the curve relating unirradiated seedling growth to concentration (Table I). This plateau corresponds to the overlap in concentration ranges for effectiveness and specificity.

We conclude that there are no concentrations of hydroxyurea or phenethyl alcohol that satisfy both the requirements of effectiveness and specificity. By contrast, FdUrd seems useful as a specific inhibitor of DNA synthesis in studies of wheat seedling growth.

**LITERATURE CITED**


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**Table I. Effects of Inhibitors on Leaf Elongation in Wheat Gamma-Plantlets and Unirradiated Controls**

<table>
<thead>
<tr>
<th>Inhibitor and Concentration</th>
<th>FdUrd</th>
<th>Hydroxyurea</th>
<th>Phenethyl alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorodeoxyuridine, 10^-4 M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-3 M</td>
<td>10.4 ± 3.2</td>
<td>14.0 ± 0.9</td>
<td>79.8 ± 9.5</td>
</tr>
<tr>
<td>10^-2 M</td>
<td>5.0 ± 0.8</td>
<td>9.5 ± 1.0</td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td>10^-1 M</td>
<td>3.5 ± 0.3</td>
<td>8.6 ± 1.5</td>
<td>4.0 ± 1.3</td>
</tr>
</tbody>
</table>

*These seedlings were in contact with the inhibitor from the time of sowing until the time of measuring.*

*At this time, the coleoptile was 3 to 4 mm long and enclosed the first foliage leaf, which was approximately 1.5 mm long. These seedlings were exposed to the inhibitor from this time until the time of measurement.*

*Measurements, given for first foliage leaf, refer to mean and 95% confidence limits. Gamma-plantlets and unirradiated controls were 11 and 7 days old, respectively, when measured.*

*Germination did not occur.*

*No significant elongation after treatment with 10^-1 M phenethyl alcohol.*

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**Fig. 1. Comparison of the concentration ranges for effectiveness (solid bars) and specificity (stippled bars) after treatment of germinating wheat seedlings with inhibitors of DNA synthesis.**


