Studies of the terephthalanilide derivatives have shown that they have a high degree of inhibitory activity against a broad spectrum of transplanted mouse leukemias (3, 6—11, 13) and against leukemia P815Y (12) in tissue culture (2), and that there seems to be reasonably good correlation between their effects in vitro and in vivo.

As has been noted previously (1, 4), the anti-leukemic effects of certain of the terephthalanilide derivatives can be antagonized by certain sulfonic analogs in the mouse. Simultaneous studies have been done on these and other compounds in tissue culture, and the results will be reported here.

METHODS

For these studies, the leukemic cells were grown according to the Fischer technic in a liquid medium (5). The cell line used was mouse leukemia P815Y adapted to tissue culture by Schindler et al. (12). The medium was supplemented with 10% horse serum.

Cells for experiments were taken from stock cultures and diluted with the medium to give a concentration of $10^5$ cells/ml; 0.5 ml aliquots of this suspension were added to screw-cap culture tubes containing 4.5 ml of the medium, resulting in a final count of $10^6$ cells/ml.

The compounds were dissolved in sterile distilled water and diluted to a concentration 50 times greater than the experimental level desired, so that 0.1 ml of the compound solution could be added to 5 ml of growth medium. In each experiment 2 tubes were inoculated at 37°C and counted after 72 hr incubation. Fresh medium was not added unless expressly noted. All cell counts were done by diluting the culture 1:20 and then counting with an electronic cell counter (Coulter counter, model A). The concentration of cells in the control tubes after 72 hr was approximately 700,000/ml.

In the experiment shown in Chart 2, NSC-53306 was added to all tubes at 0 hr to give final concentrations of 0.3, 1.0, and 3.0 pig/ml and then, as indicated, NSC-55156 at 60 pig/ml was added at 0, 2, 4, 8, or 24 hr after NSC-53306. In these studies, the cells were counted 72 hr after the addition of NSC-53306.

RESULTS

The ability of the 2 sulfonic analogs, NSC-55156 and NSC-60349, and the phosphoric acid analog, HR-2504, (Chart 1) to prevent the inhibitory effects of 5 terephthalanilide derivatives of different chemical structure is shown in Table 1. It can be seen that with 4',4''-bis(2-imidazolin - 2 - yl)2 - chloroterephthalanilide (NSC-38280) blocking of the inhibitory effect could be obtained with all 3 antagonists, whereas with 4',4''-bis(2-imidazolin-2-yl-amino)terephthalanilide (NSC-53306) there was no blocking with NSC-55156. On the other hand, with the quinolinium derivative, HR-2289, prevention occurred with the naphthalene sulfonic (NSC-60349) and the phosphoric (HR-2504) antagonists, but not with NSC-55156. With $N,N''$ - bis[p - ($N'$ - methylamidino)phenyl]terephthalamidine (NSC-57155), however, there was no blocking of inhibition by any of the 3 antagonists.

That this effect was rather specific can also be seen in Table 1, which shows that the inhibitory effects of 3 µg/ml...
of NSC-53306 could be blocked by 6 μg/ml of NSC-55156; whereas 120 μg/ml of NSC-60349 could not prevent the inhibitory effect of 0.1 μg/ml of NSC-53306. Thus NSC-55156 blocked at a ratio of 2:1, whereas NSC-60349 was inactive even at a ratio of 1200:1.

With the quinolinium derivative HR-2289, however, the situation was reversed, and NSC-60349 was active at a ratio of 6:1, whereas NSC-55156 was inactive at a ratio of 300:1. The inhibitory effect of both NSC-53306 and HR-2289, could be blocked by HR-2504 at a ratio of 10:1. With the terephthalanilide derivative NSC-57155, however, no blocking was seen with any of the antagonists at a ratio of 360:1. In contrast to NSC-57155, a tetrahydro-oxazolopyrimidine analog, HR-2365, could be blocked by both NSC-55156 and NSC-60349 at ratios of 6:1 or 3:1.

### Chart 1
Sulfonic and phosphoric analogs of the terephthalanilides.

R-N-C=O—N=C—R

R= _K@jS03Na NSC 55156
R= _f@-P0@Na HR 2504

### Chart 2
The effect of time between additions of the terephthalanilide, NSC-53306, and its sulfonic analog, NSC-55156, on the blocking of the inhibition by NSC-53306 of P815Y cells in tissue culture.

All readings were taken at 72 hr. Average control growth was 659,000 cells/ml; the range was 517,000—796,000 cells/ml.

### Table 1
Blocking of Phthalanilide Inhibition of P815Y Cells in Tissue Culture by Various Sulfonic and Phosphoric Analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (μg/ml)</th>
<th>Percent Inhibition of Cell Growth at 72 hra</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 30 60 120</td>
<td>NSC-55156 NSC-60349 HR-2504</td>
</tr>
<tr>
<td>NSC-38280</td>
<td>3.0 97 39</td>
<td>32 97 77</td>
</tr>
<tr>
<td></td>
<td>1.0 96 87</td>
<td>37 4 68 12 77 29</td>
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<tr>
<td></td>
<td>0.3 95 80</td>
<td>19 58 5 95 23 32</td>
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<tr>
<td></td>
<td>0.1 87 31</td>
<td>8 0 20</td>
</tr>
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<td>NSC-53306</td>
<td>3.0 95 13</td>
<td>6 11 7 49 0 6</td>
</tr>
<tr>
<td></td>
<td>1.0 92 15</td>
<td>7 9 5 20 3 6</td>
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<td>0 8 92 92 91 8</td>
</tr>
<tr>
<td></td>
<td>0.1 78 3</td>
<td>6 1 81 77 77 79 5</td>
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<tr>
<td>HR-2289</td>
<td>10.0 97</td>
<td>87 95 48 12 21 98 95 77</td>
</tr>
<tr>
<td></td>
<td>3.0 96</td>
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<tr>
<td></td>
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<td>63 74 22 14</td>
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<td>10.0 96</td>
<td>94 91 30 0 0 80 0 0 0 0</td>
</tr>
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<td>93 92 2 0 0 0 0 0 0 0 0</td>
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<tr>
<td></td>
<td>1.0 86</td>
<td>0 0 0 0 0 0</td>
</tr>
<tr>
<td>NSC-57155</td>
<td>3.0 88</td>
<td>76 93 92 93</td>
</tr>
<tr>
<td></td>
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<td>66 89 90 89 87 91 91 85</td>
</tr>
<tr>
<td></td>
<td>0.3 66</td>
<td>51 64 75 74 48 71 74 53</td>
</tr>
</tbody>
</table>

a Average Control, 785,000 cells/ml; range, 600,000—1,000,000 cells/ml.

b Dosage in μg/ml.
The effect of the time interval between addition of NSC-53306 and its antagonist, NSC-55156, in tissue culture has been shown in Chart 2. It can be seen that even when the antagonist is added to the culture tube 4 hr after NSC-53306, there was still considerable blocking of the inhibitory effect of NSC-53306 except at the highest level (3 \( \mu g/ml \)), but that this effect was essentially lost at all levels, if the antagonist was added 8 or 24 hr after NSC-53306.

DISCUSSION

The blocking of the inhibitory effects of various terephthalanilide derivatives by sulfonic and phosphoric acid antagonists appears to be rather specific. Kensler (7) has suggested that the formation of complexes between the terephthalanilide derivatives and ribonucleic acid and deoxyribonucleic acid may be on the basis of ionic rather than covalent binding; Yesair et al. (14) have suggested a similar situation between the complexes and lipids. The specificity reported here suggests that this type of binding is involved and that there may be specific structural relationships between the antagonistic pairs. The lack of effect shown in Chart 2, when the antagonist NSC-55156 is added 8 hr or more after NSC-53306, suggests that the blocking takes place either in the medium or at the cell surface, and that no blocking of the inhibitory activity can be achieved once NSC-53306 has entered the cell.

The ability of a single dose of NSC-55156 i.p. or s.c. to block the antileukemic effects in vivo of a single massive dose of NSC-53306 has been reported previously (4). If the antagonist is given 30 min before, at the same time, or 30 min after the terephthalanilide, there is marked blocking of the antileukemic effect. Very little or no blocking is seen, however, when the antagonist is given 2, 4, 8, or 24 hr after the terephthalanilide.

The specificity of the blocking of certain terephthalanilides by certain antagonists both in vitro and in vivo suggests that further studies may lead to the discovery of an antagonistic pair in which the chronic toxicity will be blocked much more than the antileukemic effects.

REFERENCES
Prevention by Sulfonic and Phosphoric Analogs of the Terephthalanilide Inhibition of Leukemia P815Y \textit{in Vitro}


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