Determination of Mammalian Cell (Strain HeLa) Inhibition by an Agar Diffusion Technic

II. Paper Chromatographic Methods*

PAUL SIMINOFF AND VALENTINA S. HURSKY

(Bristol Laboratories Inc., Syracuse, New York)

SUMMARY

A procedure was described for paper chromatography of antitumor activities in fermentation materials employing HeLa cell bioautographs. The use of this tool to prevent duplication of effort on identical agents from different cultures and to correlate HeLa inhibition with other biological systems was illustrated.

Paper chromatography has proved to be a powerful tool in an antibiotic screening program (1). It serves the following purposes: (a) it provides an early characterization of the antibiotic activity as new or old, (b) it determines whether single or multiple activities are present, and (c) it predicts the solubility characteristics of the activity and probable extraction behavior. Thus, at a very early stage, important information is made available to both the microbiologist and biochemist which helps to determine interest in the antibiotic and to accelerate the studies leading to its isolation and purification.

The search for antitumor agents in such fermentation materials requires the same basic approach as does antibiotic screening. This report will describe one solution to such need: the use of mammalian cell bioautographs of antitumor agents isolated in crude or purified form from fermentation liquors of actinomycetes and chromatographed on paper strips.

* These studies were supported in part by funds supplied under contract SA-48-ph-1908 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

MATERIALS AND METHODS

Details of the preparation of seed cells from a substrain of the S-3 clonal strain of HeLa and a description of media were given in the previous paper (2).

Paper strips.—1/2-inch strips of No. 589 blue ribbon paper (Schleicher and Schuell) were used. Strips were marked at points 3, 4, 13, and 18 inches from the end. The test material was applied at the 4-inch point, and the chromatograms were developed by downward solvent movement in chromatography chambers. The developed strips were then dried and cut at the solvent front.

Cell bioautographs.—Ninety ml. of attachment medium containing 9 X 10⁴ cells were poured into sterile pyrex baking dishes. After a 3-hour attachment period, the supernatant fluid was removed and replaced by 60 ml. of first overlay agar which was allowed to set. Each developed paper strip was cut at the 3-inch mark and again at the 18-inch mark, and the two sections were laid on the agar as shown in Figure 1. Two sets of strips were routinely used per dish. The dishes were covered with sterile aluminum foil and incubated for 24 hours.

The strips were prepared by Mr. Harold Taylor, Department of Biochemistry, Bristol Laboratories Inc.

Fig. 1a.—Baking dish showing position of the strips. Cells have received the second overlay agar.

Fig. 1b.—Appearance of the S-3 HeLa cell monolayer after development of the bioautograph. A purified solid of culture NSC-A649 was chromatographed with two different solvent systems.
hours. With the strips in place, 40 ml. of second overlay agar was added, and the cells were incubated for 3 more days. The cells were fixed and stained with 24 ml. of Zenker's solution and 24 ml. of Giemsa's as previously described (2).

RESULTS AND DISCUSSION

Of twelve solvent systems studied, only two left toxic residues on the strip. One system contained 2 per cent lauric acid, and the other employed strips impregnated with 10 per cent sodium citrate. Systems with butanol, methanol, chloroform, isooamyl alcohol, methyl isobutyl ketone, ethyl acetate, piperidine, p-toluene-sulfonic acid, acetic acid, and ammonium hydroxide were quite innocuous.

A typical bioautograph is shown in Figure 16. These large, well defined zones were obtained with a dose of 0.3 ng. of A649 compound per strip. Since A649 is also active against bacteria, it was possible to make a direct comparison between the RF2 values for each solvent system as determined from bacterial and HeLa bioautographs. The two sets of values, as expected, agreed within experimental error (Table 1).

An important feature of the paper Chromatographic method is that it permits one to determine whether the bacterial and tissue cell culture tests are measuring the same activity in fermentation materials or concentrates. For example, culture NSC-A26125, active against Carcinoma 755 in mice, also inhibits Bacillus subtilis and HeLa. To determine whether the antibacterial and anti-HeLa cell activities are identical, strips were dosed with 5 µl. of a butanol extract of an active beer and duplicate chromatograms developed in parallel with each of six solvent systems, and HeLa and B. subtilis bioautographs were obtained. Results with the six systems are shown in Table 2. From a comparison of the RF values, it is possible to conclude that B. subtilis and HeLa were measuring the same activity. This conclusion was reinforced by the identical morphology of the inhibition spots for each solvent system in both cell cultures. Therefore, further work on culture A26125 is being carried on with B. subtilis as the assay organism.

### TABLE 1

<table>
<thead>
<tr>
<th>System no.*</th>
<th>HeLa (Rs)</th>
<th>B. subtilis (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.83</td>
<td>0.86</td>
</tr>
<tr>
<td>11</td>
<td>0.92</td>
<td>0.86</td>
</tr>
<tr>
<td>16</td>
<td>0.79</td>
<td>0.77</td>
</tr>
<tr>
<td>18</td>
<td>0.91</td>
<td>0.90</td>
</tr>
</tbody>
</table>

* System no. 1: Water.
  4: Butanol saturated with water.
  11: Butanol saturated with water + 5 ml. acetic acid/100 ml.
  16: 0.05 N ammonium hydroxide saturated with methyl isobutyl ketone.
  18: Ethyl acetate saturated with water.

### TABLE 2

<table>
<thead>
<tr>
<th>System no.*</th>
<th>HeLa (Rs)</th>
<th>B. subtilis (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.42</td>
<td>0.44</td>
</tr>
<tr>
<td>4</td>
<td>0.89</td>
<td>0.88</td>
</tr>
<tr>
<td>6</td>
<td>0.94</td>
<td>0.94</td>
</tr>
<tr>
<td>11</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>16</td>
<td>0.72</td>
<td>0.69</td>
</tr>
<tr>
<td>18</td>
<td>0.56</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* System no. 1: Water.
  4: Butanol saturated with water.
  6: 80% methanol-20% water + 10.5 ml piperidine/100 ml adjusted to pH 9.2.
  11: Butanol saturated with water + 5 ml acetic acid/100 ml.
  16: 0.05 N ammonium hydroxide saturated with methyl isobutyl ketone.
  18: Ethyl acetate saturated with water.

The use of paper chromatography to prevent duplication of effort has also shown tangible results. Culture NSC-A26126, which was active against Ca-755, B. subtilis, and HeLa, had received preliminary microbiological and biochemical study before paper chromatographic methods became available. Subsequent comparison of bacterial and HeLa cells papergrams showed the activity to be identical with that of A26125. Therefore, further work on this culture was suspended.

REFERENCES

Determination of Mammalian Cell (Strain HeLa) Inhibition by an Agar Diffusion Technic: II. Paper Chromatographic Methods

Paul Siminoff and Valentina S. Hursky


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/20/5_Part_1/618

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.