Experiences with the Tissue Culture System in Large-Scale Cancer Chemotherapy Screening*

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SUMMARY

Over 17,000 fermentation broth filtrates have been screened against three tumors in mice—Sarcoma 180, Carcinoma 755, and Leukemia L1210—and against human malignant cell cultures in vitro (HeLa or H.Ep. #2). Of the Ca-755 and L1210 active filtrates, 64 per cent and 68 per cent, respectively, were active against cell cultures at 1:32 dilution or greater. The proportion of S-180 active filtrates detected was lower; only 40 per cent of S-180 active filtrates were active in vitro at 1:32. The proportion of “false positives,” or percentage of tumor-negative filtrates active in vitro at 1:32, was 22 per cent. The results for 4300 filtrates screened against the human tumor H.S. #1 in the conditioned rat showed 65 per cent of the tumor-active filtrates to have tissue culture activity at 1:32 dilution or greater.

The significance of these results in relation to the potential of cell cultures as a primary screen for the detection of antitumor agents, as well as the usefulness of tissue culture as a bioassay method for the purification of antitumor compounds from cytotoxic filtrates, is discussed.

The high degree of association between tissue culture activity and experimental tumor activity found by Eagle and Foley (3-5) and other workers (7-9) has given strong impetus to the expanding use of tissue culture in cancer chemotherapy screening. Our laboratory is one of those under contract with the Cancer Chemotherapy National Service Center (CCNSC) engaged in a large-scale screening program as part of the search for new antitumor agents effective against human cancer. The greater part of our effort is the testing and purification of active materials from crude fermentation filtrates, with a secondary program of testing structurally defined compounds. All new substances are screened against three mouse tumors—Sarcoma 180, Carcinoma 755, and Leukemia L1210—and in the in vitro tissue culture system against cell cultures of human malignant origin (HeLa or H.Ep. #2). The human tumor H.S. #1 transplanted in the conditioned rat is also employed as a supplementary animal screen on a smaller number of samples.

This paper is a report of our experiences with the tissue culture system as part of this screening program. In particular, the discussion will center on the relation of tissue culture activity to activity against the four experimental tumors, the potential role of tissue culture as a prime screen for the detection of antitumor agents, and the usefulness of tissue culture as a bioassay method for the purification of antitumor compounds from crude fermentation filtrates.

MATERIALS AND METHODS

The tissue culture test procedure has been previously described in detail (12). Briefly, the method measures the cytotoxic activity of varying dilutions of broth filtrate against cell cultures of human malignant origin (HeLa or H.Ep. #2). Four dilutions of sample are routinely tested in each assay—1:10, 1:32, 1:100, and 1:320. The samples are diluted into a cell suspension of 15,000–20,000 cells/ml in Eagle’s medium (2) with 10 per cent human serum and plastic cup panels (11) used as the test containers. Each dilution is tested in duplicate, and a known positive control is run with each assay series. After 5 days’ incubation the cultures are examined microscopically for cytotoxicity,
with the use of an arbitrary 0 to 4+ scale of cell damage. Two end-points are determined: a cytotoxic end-point, or maximum dilution causing significant cytotoxicity, and a lethal end-point, or maximum dilution causing complete culture destruction.

A broth filtrate is adjudged as "tissue culture-active" and retested for activity if the cytotoxic endpoint extends to 1:32 dilution or greater. Similarly, the cut-off point for retesting synthetic compounds is a cytotoxic concentration of 100 μg/ml or less.

The experimental tumor assays for activity against S-180, Ca-755, and L1210 are done according to the protocols of the CCNSC (1). The results on mouse tumors reported in this paper were obtained either at Southern Research Institute, Hazleton Laboratories, or at our laboratories under the direction of Dr. J. J. Oleson. The H.S. #1 tests are done at our laboratories according to the procedure described by Marsh and Cullen (6).

RESULTS AND DISCUSSION

Relation of tissue culture activity to experimental tumor activity for fermentation filtrates.—As of November 1, 1960, 17,100 of the random broth filtrates fermented by our soil microbiology laboratory were tested for activity against the three mouse tumors—S-180, Ca-755 and L1210—and against human cell cultures (HeLa or H.Ep. #2). Of this total, 537 (3.1 per cent) were sequentially active in one or more of the mouse tumor screens according to the protocols of the CCNSC (stage 2 actives) (1). This activity was distributed among the three mouse tumors as shown in Table 1.

The tissue culture activity for the 17,100 filtrates was distributed as shown in Chart 1. According to the criteria of cytotoxicity at 1:32 dilution or greater, 3950 (23 per cent) of the filtrates were classified as "tissue culture-actives."

Chart 2 shows the relation between tissue culture activity and activity in the three mouse tumor screens. From these data the reliability (proportion of false-positive results) and sensitivity (proportion of tumor-actives detected) of the tissue culture screen can be evaluated for the 17,100 filtrates tested. Of the 16,563 filtrates which were inactive against the three experimental mouse tumors, 3841 (22 per cent of the tumor-negatives) were cytotoxic in tissue culture at 1:32 dilution or greater. Of course, as indicated in Chart 2, the proportion of "false positives" depends on the chosen cut-off point for tissue culture activity. At 1:100, 11 per cent of the tumor-negatives were active against cell cultures; at 1:320, only 3.5 per cent were tissue culture-active.

The proportion of tumor-active filtrates detect-
ed by the cell culture screen was also a function of the selected cut-off dilution. As shown in Chart 2, the percentages of cell culture-actives in the tumor-active group as a whole were 58 per cent at 1:32, 43 per cent at 1:100, and 26 per cent at 1:320. From the two considerations of reliability and sensitivity, it appears that the tissue culture system was moderately effective in detecting filtrates active in one or more of the mouse tumor screens. If tissue culture had been used as the primary screen during this testing period the proportion of tumor-active filtrates in the samples submitted for tumor assay would have been increased from the random 3.1 per cent to 7.8 per cent (309/3950) at 1:32 cut-off point for cell culture activity. At 1:100, 11 per cent (230/2020) of the tissue culture actives were tumor-active; at 1:320, 20 per cent (142/720).

When the composite tumor-active filtrates were analyzed for the individual experimental tumors, the selectivity of the tissue culture system was found to vary with the type of tumor. As indicated in Chart 3, tissue culture activity was a significantly more sensitive detector of Ca-755 and L1210 actives than it was of S-180 activity. Of the Ca-755 and L1210 actives, 64 per cent and 68 per cent, respectively, were active against cell cultures at 1:32, compared with only 49 per cent for the S-180 active filtrates. It was interesting to note that the tissue culture activity of the 70 filtrates active against more than one experimental tumor showed an even more impressive correlation. At 1:32 dilution 70 per cent of these highly active filtrates were active against cell cultures; at 1:100, 57 per cent; and at 1:320, 39 per cent.

The relationship between activity against the human tumor H.S. #1 carried in the conditioned rat and cytotoxicity in cell cultures is presented in Chart 4. Of the 123 H.S. #1 actives, 65 per cent were tissue culture-active at 1:32, 54 per cent at 1:100, and 31 per cent at 1:320. In the H.S. #1 negative group, the corresponding percentages were 22, 11, and 3.5, respectively.

Synthetic chemicals.—As part of our screening program we are testing a small number of structurally defined chemical compounds against the three mouse tumors and in tissue culture. As of November 1, 1960, 410 compounds have been tested, and only eight (2.0 per cent) have shown antitumor activity. The sensitive tumor for all eight compounds was Ca-753; there were no synthetics active in the S-180 or L1210 screens. The tissue culture activity for both the tumor-active and tumor-negative compounds is presented in Table 2. The small number of tumor-active synthetic compounds uncovered by the three mouse tumors does not permit a valid analysis of the relationship between cell culture activity and experimental tumor activity at this time.

Tissue culture as a primary screen.—It is apparent from the screening data presented in this report that approximately the same degree of correlation exists between tissue culture activity and experimental tumor activity for crude fermentation filtrates as has been reported for selected synthetic compounds (4). It is equally apparent that the correlation is not perfect—i.e., (a) there are substances highly active in tissue culture that are inactive against experimental animal tumors (at least those tested), and (b) there are substances highly active against one or more tumor systems.
that are inactive in cell cultures at related concentrations. It is this latter group of substances that appears to preclude the adoption of cell cultures as the only primary screen for the detection of antitumor agents—for even though the use of a tissue culture primary screen would greatly increase the number of available tumor-active materials at a substantial saving in time and expense, the risk would be taken that potentially useful materials could be missed (e.g., compounds transformed into active substances in vivo). Of the fermentation compounds isolated at our laboratories, duazomycin A (10) is an example of a highly tumor-active material without comparative cell culture activity. These conclusions relating to the role of tissue culture as a primary screen are subject to revision if it can be shown that tumor-active, cell culture-negative substances are restricted to particular chemical structures, or if future clinical trials strongly suggest that in vitro activity is associated with therapeutic effectiveness in man. It is proposed, however, that the high degree of association between cytotoxicity in cell culture and experimental tumor activity be taken advantage of by using tissue culture as a complete or partial primary screen for experimental tumor systems of limited capacity. These include the human tumor-heterologous host systems and spontaneous and induced animal tumor screens. The current program of the CCNSC with cell culture used as a prescreen for the standard mouse tumor systems for the large number of compounds available only in small quantities is also a promising application of tissue culture in cancer chemotherapy screening.

**Tissue culture as a bioassay.**—Whereas the role of cell cultures as a prime screen cannot be accurately defined at this time, there is little doubt about the usefulness of tissue culture as a bioassay in the purification of tumor-active substances from crude fermentation filtrates. It has been our experience that, with a few exceptions, the cell culture-active principle in a cytotoxic antitumor filtrate is responsible for the observed antitumor activity. The comparative speed of the tissue culture test (48–72 hours for tissue culture assay in purification studies versus 8–10 days for animal tumor assay) has permitted our organic chemists to proceed at a more rapid pace in the purification of antitumor agents from active crude filtrates. Table 3 illustrates the concentration of an active antitumor substance from broth A-32333 by intensive use of the tissue culture bioassay. For filtrates where tissue culture bioassay is applicable, the animal tumor tests are used to monitor and confirm the cell culture results.

**TABLE 3**

**PURIFICATION OF BROTH A-32333 BY TISSUE CULTURE BIOASSAY**

<table>
<thead>
<tr>
<th>Fraction</th>
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<tr>
<td></td>
<td>Cytotoxic end-point (µg/ml)</td>
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<tr>
<td>Broth freeze-dried solids</td>
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<tr>
<td>Purification Step 1</td>
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<td>Purification Step 2</td>
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<td>Purification Step 3</td>
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<td>Purified compound</td>
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</table>

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