Tissue Culture in Cancer Chemotherapy Screening*

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The sponsorship of this Symposium represents the first formal joint venture of the Tissue Culture Association and the American Association for Cancer Research. As these two groups meet together today to appraise each other's problems and accomplishments, it is appropriate to recall that tissue culture and experimental cancer research had their beginnings at about the same time and have developed over the years in close chronological parallel and with increasing mutual interaction.

The history of both of these fields dates back a little more than two generations, to the 1880’s. During that decade, many attempts were made to grow tissues or cells outside the body, as illustrated by the work of Wilhelm Roux, who in 1885 achieved the isolation and maintenance of the medullary plate of the chick embryo in warm saline solution (cf. 67). Four years later, Arthur Hanau (39) carried out the first successful homologous transplantation of an animal tumor, an achievement which signaled the start of modern experimental oncology. By 1903, Carl Jensen (49) was able to report that a mouse tumor could be transplanted for nineteen successive generations; this important laboratory technic was thus put on a secure basis and the availability of transplantable tumors facilitated the first attempts to develop systematic approaches to cancer chemotherapy.

The year 1907 was an important one in both fields of investigation. Paul Ehrlich (26) laid the foundations of modern chemotherapy in his Harper Lectures in London; Ross Harrison (40) made tissue culture a practical tool by explanting nervous tissues in a simple assembly and observing their development in isolation. By 1910 and 1911, Alexis Carrel and Montrose T. Burrows (16, 17) had started to maintain chick embryo fibroblasts continuously in tissue culture and were cultivating tumors in vitro; cancer chemotherapy also entered a period of intense activity following the reports of August von Wassermann and his associates (77, 78) on the inhibition of animal tumors by the intravenous or subcutaneous administration of selenium and eosin.

This is not the occasion for a detailed account of the growth and development of the two fields during the period between the two World Wars. However, it is of interest to recall a few of the events of the past dozen years. In 1946, a notable Conference on Tissue Culture was convened in Hershey, Pa., which brought together a group of active workers to evaluate the past, assess future needs and objectives, and establish the Tissue Culture Association. In 1953, Margaret R. Murray and Gertrude Kopech (58) completed the monumental task of bringing together the tissue culture literature up to 1950 in a classified bibliography of some 15,000 references; a first supplement covering the more recent literature is in preparation. In 1954, a conference on tissue culture technics in pharmacology at the New York Academy of Sciences (63) stimulated the
interest of experimental chemotherapists in this useful investigative tool. Two years ago, the Decennial Review Conference on Tissue Culture at Woodstock, Vt. (79), bore witness to the vastly increased scope and versatility of these technics in many diverse areas of investigation.

In experimental cancer chemotherapy, there has been a similar period of intense and ever-expanding research effort and periodic critical assessment. In the summers of 1945 and 1946, conferences dealing with the current status of the field were held at Gibson Island; much of this material was published in 1947 (56). Helen Dyer's very useful "Index of Tumor Chemotherapy" (20) appeared in 1949. A concerted national program in cancer chemotherapy was initiated by the U.S. Public Health Service in 1953, and the Cancer Chemotherapy National Service Center began operations 2 years later (28). A number of conferences at the New York Academy of Sciences and elsewhere afforded an evaluation of particular types of chemotherapeutic agents, e.g., folic acid antagonists and other antimetabolites (65, 81), 6-mercaptopurine (64), and alkylating agents (51), and of various tools and technics used for the selection of potential agents of chemotherapeutic interest (41, 73).

Of course, as both of these investigative areas, tissue culture and cancer chemotherapy, have expanded and flourished, more and more points of contact between them have become apparent. The availability of compounds with a documented inhibitory effect on tumor growth has been of great value in studies of the growth and behavior of cells in vitro; problems dealing with the chemistry of mitosis and the mechanism of action of mitotic poisons have concerned investigators in both fields; inter-comparisons of the direct in vitro effects and the often indirect in vivo action of chemical agents on cells have proved a fruitful area of research; the applicability of tissue culture technics to the search for carcinostatic and carcinolytic agents to the search for carcinostatic and carcinolytic agents has become an important question in cancer chemotherapy screening methodology.

Each of these topics merits review and thorough discussion, but only the last will be considered in detail in this survey. No systematic attempt has been made to deal with the multitudinous investigations in which nontumor cells or tissues (rat fibrocytes, chick and mouse embryonic tissues, organ cultures, normal cell lines, and so on) were exposed to various chemical agents. The pages to follow will deal primarily with those recent studies in which tissue cultures of human and animal tumors have been used; in considering the relevance of these in vitro results to in vivo tumor inhibition, the comments of several other reviewers (1, 15, 18, 50, 53, 57, 61, 71, 72) have been of great assistance.

**SYSTEMATIC APPLICATION OF TISSUE CULTURE TO SCREENING**

During the last decade, much information has been gathered on the effects of a variety of chemical compounds on tumor cells in tissue culture, with or without normal cell controls. Many of these experiments, however, have been concerned with one or two agents at a time; only a handful of studies have attempted a systematic exploration of larger series, thus permitting a more direct evaluation of in vitro technics as screening tools. A brief outline of the various systems which have been employed in this way will provide an indication of the diversity of approaches while furnishing some background information for the general comments which will be made later.

*Investigations of Biesele and associates.*—An impressive series of publications (2–14, 47, 48, 74) has provided data on the in vitro effects of about 300 compounds, particularly purines, purine nucleosides, pyrimidines, benzimidazoles, antifolics, and amino acid antagonists. In most of these studies, short-term primary explants of a transplantable mouse tumor, principally mouse Sarcoma 180, T241, or Ma387, were placed into roller tubes; in addition to several tumor fragments, each tube also contained explants of mouse embryonic skin as the normal control. The medium consisted of balanced salt solution, chick embryo extract, human placental serum, and horse serum. The agent to be tested was incorporated into the medium at several concentrations, ranging generally from 0.1 to 4.0 millimoles/ml, and allowed to act on the tumor and embryonic cells for 24 hours. Morphological evidence of damage was then sought, and counts of mitotic and pyknotic nuclei were often carried out to obtain a more quantitative result.

The main objective of these experiments has been to find compounds with selective or differential toxicity for tumor cells. Several agents, notably 2,6-diaminopurine, 6-mercaptopurine, and other 2- and 6- substituted purines, caused significant damage to tumor cells at concentrations which were entirely nontoxic to the embryonic controls, but most of them exhibited no such selectivity (cf. 3). A second objective has been to study the mechanism of action of these compounds by appropriate reversal experiments with presumed normal metabolites. Experiments with purine antagonists, in particular, have contributed to our understanding of nucleic acid synthesis and
its blockade by compounds like 6-mercaptopurine or 2,6-diaminopurine.

Some of the more recent studies (7–9) have been extended to human cell strains of cancer and normal tissue origin grown in the semisynthetic medium of Eagle (cf. below). In general, the results with these materials were similar to those reported earlier with mouse tumors on a nonsynthetic medium, but the human cell lines often appeared less sensitive.

As regards the comparison of these in vitro results with the anticancer effects of these compounds in vivo, it was pointed out repeatedly (e.g., 1, 4) that the correlation has not been particularly impressive. Selective toxicity to tumor cells in tissue culture, it appears, does not necessarily or predominantly go along with a satisfactory therapeutic index in the whole animal.

*Experiments of Eichorn and collaborators.*—This group of investigators (27) used short-term primary explants of mouse mammary carcinoma 72J and glioma 8110 in roller tubes, with mouse embryonic skin explants in separate control tubes. The cells were fed a medium consisting of horse serum and chick embryo extract for 24–48 hours; this was then replaced by a solution of 0.004 per cent serum albumin containing the agent to be tested at various concentrations. After 48 hours’ exposure, cytological evidence of damage was noted. The tissue culture response to eight compounds including triethylenethiophosphoramide and 6-mercaptopurine was compared with the effect of the same compounds on the same two transplantable tumors in the mouse.

In this short series of compounds, the correlation of in vitro and in vivo inhibition of the same tumor was very close. In tissue culture, the glioma responded to five of the eight agents, the mammary tumor only to three. There was no differential effect on tumors in comparison to the normal tissue control.

*Studies of Fjelde et al.*—Several recent reports (29–32, 70) have outlined experiments carried out with long established strains of human tumor cell lines H.S. #1, H.Ep.#1, or H.Ep. #2 in Porter flasks on a nonsynthetic medium consisting of balanced salt solution, chick embryo extract, human serum, human placental serum, and horse serum. No normal cell controls were employed. The effect of seven compounds on the growth of these cells was assessed by measuring the total tissue culture area at the beginning of the experiment and 7 days later by an areal growth projection technic. The minimum inhibitory concentrations ranged from $10^{-4}$ mg/ml for 2-azaadenine to $10^{-2}$ mg/ml for 6-mercaptopurine.

*Results obtained by Wright, Cobb, and associates.*—An attempt to employ tissue culture in a more direct approach to the problems of clinical chemotherapy, by-passing animal tumors entirely, has been made (19, 82, 88) by using a biopsy sample of a patient’s tumor for the in vitro tests and comparing the response of these primary explants with the clinical response of the same patient to the same drug. Patients with a wide variety of neoplastic diseases were used. Standard tissue culture technics were employed, the agent being added to the nonsynthetic medium at the smallest concentration producing inhibition of outgrowth in chick or mouse heart fibroblasts, which served as the normal controls. Morphological evidence of cytotoxicity was used as the criterion for in vitro effects. In more than half of the experiments, a good correlation of tissue culture results with clinical response was found; fifteen of nineteen instances of clinical improvement were accompanied by pronounced inhibition of the tumor in vitro, and, in nine of sixteen cases which failed to improve, the tissue culture results were also negative. Triethylene melamine and triethylene thiophosphoramide were used most frequently; preliminary results with several other agents of clinical interest were included.

*Investigations of Eagle and Foley.*—In the studies which have been outlined and in most other tissue culture experiments related to cancer chemotherapy, tissue explants were grown in a plasma clot in media composed of various natural materials, particularly plasma and tissue extracts. The recent work of Eagle and Foley (22–24) represents a radical departure from this procedure. On the basis of extensive investigations (cf. 21, 25, other references in 23), it was found that a variety of long established cell lines of normal or neoplastic origin could be grown as a monolayer directly on a glass surface in a relatively defined or semisynthetic medium. The latter contained thirteen essential amino acids, seven vitamins, salts, glucose, and 10 per cent human serum or 5–10 per cent horse serum for human and mouse cell lines, respectively. An inoculum of about 200,000 cells was used, and growth was allowed to proceed in culture flasks for 5–8 days in the presence and absence of various levels of the compound to be tested. Chemical determination of the protein content of flasks at the beginning and end of the experiment provided a quantitative measure of cell multiplication and, consequently, of the inhibitory effect of the agent. This was expressed in terms of an “ID₅₀,” the concentration at which 50 per cent inhibition of growth was achieved.
The response of this test system was assessed first (23) by exposing each of seven cell lines to fifteen compounds, including thirteen of known in vitro carcinostatic activity (four antifolics, two dihydrotiazines, azaserine, styloymycin, 6-mercaptopurine, actinomycin D, triethylene melamine, triethylenethiophosphoramide, and Daraprim), and two folic acid analogs of known in vivo ineffectiveness. Four of the cell lines were of neoplastic origin (human HeLa, KB, J, and mouse S-180), and three were derived originally from normal human tissues. Several interesting findings emerged from these comparisons. All seven cell lines exhibited essentially the same response to any one of the fifteen compounds. The thirteen carcinostatic compounds all were highly cytotoxic in vitro, i.e., their ID₅₀ was low; the two inactive compounds also were devoid of activity in tissue culture. The cytotoxicity of the active compounds varied over a 2000-fold range, with aminopterin, actinomycin D, and A-methopterin at the upper and triethylenethiophosphoramide and Daraprim at the lower end.

More recently, this comparison has been extended to 180 compounds of known activity or inactivity in vitro and of widely differing chemical constitution. One normal and one neoplastic cell line were employed; there was no significant difference in susceptibility to inhibition between them. In general, the active tumor inhibitors as a group were significantly more cytotoxic than the compounds without carcinostatic activity in animal tumor systems, but about one in five of the active agents failed to meet the criteria for a positive in vitro effect (inhibition at an ID₅₀ of 10⁻¹⁴ gm/ml or less), and a similar proportion of the inactive compounds exhibited activity in the tissue culture test.

The pronounced similarity of response of normal and neoplastic cell lines in this system has been one of the most intriguing features of these results, particularly if Bieseke’s success in demonstrating differential toxicity of a number of carcinostatic purines to short-term tumor explants in a different culture system is recalled and if it is kept in mind that positive carcinostatic activity in vitro presupposes a difference in response between the tumor and the normal host tissues. One of the possible explanations revolves around the idea that these long established lines of cells of liver or conjunctiva or intestinal epithelium can no longer behave as normal cells. Quite recently, however, Foley and Eagle (84) demonstrated that normal human and animal cells in first in vitro passage gave the same response to nineteen agents of varied effectiveness as did recently isolated or long established cell lines derived from them.

Studies with human and mouse brain tumors at Columbia University.—For the last half-dozen years, the author has participated in a collaborative program (86, 97, 42, 43, 45, 46, 59) of experimental cancer chemotherapy encompassing tissue culture studies, the assessment of carcinostatic activity of a variety of compounds in a spectrum of transplantable mouse tumors, attempts to determine tissue distribution and mechanism of action of some of these agents, and clinical evaluation where indicated. The animal tumor spectrum has included primarily the brain tumor Gliona 26, mammary adenocarcinomas 755 and E 0771, Sarcoma 180, the Ehrlich ascites carcinoma, and the ascitic form of leukemia L1210.

Compounds have been tested routinely at the maximum tolerated dose and evaluated statistically on the basis of the difference in tumor weights of treated and control groups of ten to twenty animals each. In general, it has been assumed, as a working hypothesis, that compounds with reproducible activity against a variety of transplantable tumors are of greater potential clinical interest than those which cause significant inhibition of only a single tumor in this or similar spectra of experimental neoplasms.

The in vitro experiments have utilized primary short-term explants of human glioblastoma multiforme and of the mouse brain tumor. Normal controls are generally omitted. The explants are carried in roller tubes for about 10 days until uniform growth is obtained, then transferred to Maximow slides, where they are maintained for 7–10 days in a plasma clot by the double coverslip lying-drop method. The feeding fluid consists of human placental serum, chick embryo extract, ox serum ultrafiltrate, and balanced salt solution. A solution or fine suspension of the compound to be tested is prepared to contain 10 times the desired concentration, and a tenfold dilution is then made with feeding fluid. The medium is renewed twice during the experiment. The cultures are examined daily for morphological evidence of damage and fixed and stained at the end of the test. The damage rating involves four...
main criteria— inhibition of migration, inhibition of mitosis, nuclear changes, and cytoplasmic changes (cf. 36, 59). The minimum inhibitory concentration is obtained by determining the effect of various levels; for routine comparisons, lack of cytotoxicity at $1 \times 10^{-6}$ M is considered a negative result, even though it is recognized that, with certain compounds, activity at higher concentrations may still be of physiological significance.

About 150 compounds have been examined in brain tumor tissue culture by this procedure. Table 1 summarizes the results obtained with 115 compounds which at this writing have also been studied in some detail in the mouse tumor spectrum in vitro. Since one of the features of this program has been a continuing interest in structure-activity relationships, most of these compounds fall into one of a few chemical classes, with nitrogen- and sulfur-containing heterocyclic structures predominating. The miscellaneous compounds include many which were studied intensively in a recent investigation of diverse systems for cancer chemotherapy screening (35).

Most of the quinolines, thiaxanthenones, and acridines exhibited pronounced in vitro cytotoxicity, but only one compound, the thiaxanthenone Miracil D, has shown significant carcinostatic activity against more than one of these experimental neoplasms in vivo (44). Conversely, the over-all tissue culture response to alkylating agents, benzimidazoles, purines, pyrimidines, and the group of miscellaneous agents was much less striking, although these groups included a considerable number of compounds with clear-cut activity in the mouse and some with clinical usefulness as well.

Table 2 details the in vitro activity of the eighteen compounds which affected more than one of the experimental tumors in vitro to a significant extent. Table 3 provides the complementary data on the in vivo results obtained with the sixteen compounds which had the highest activity in vitro. It is clear that under the particular conditions chosen for these comparative studies

### Table 1
**SUMMARY OF COMPARATIVE in Vitro AND in Vivo TESTS**

<table>
<thead>
<tr>
<th>Type of compound</th>
<th>Active in vitro at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^{-6}$ M</td>
</tr>
<tr>
<td><strong>Alkylating agents</strong></td>
<td>19</td>
</tr>
<tr>
<td><strong>Quinolines</strong></td>
<td>19</td>
</tr>
<tr>
<td><strong>Thiaxanthenones</strong></td>
<td>19</td>
</tr>
<tr>
<td><strong>Acridines</strong></td>
<td>12</td>
</tr>
<tr>
<td><strong>Benzimidazoles</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>Purines and pyrimidines</strong></td>
<td>9</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td>18</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>115</td>
</tr>
</tbody>
</table>

**Active in vivo**

- >1 Tu
- 1 Tu
- 0.1 Tu
- 0.01 Tu
- 0.001 Tu
- <0.001 Tu

**Tumor inhibited**

- 10
- 5
- 2
- 1
- 0

### Table 2
**In Vitro ACTIVITY OF COMPOUNDS WITH GREATEST in Vitro ACTIVITY**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tumors inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>HN2</td>
<td>++</td>
</tr>
<tr>
<td>Myleran</td>
<td>0*</td>
</tr>
<tr>
<td>Nonane</td>
<td>0</td>
</tr>
<tr>
<td>Benzimidazole mustard</td>
<td>0</td>
</tr>
<tr>
<td>Fluorobenzyl mustard</td>
<td>++</td>
</tr>
<tr>
<td>Purines and pyrimidines:</td>
<td></td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>0</td>
</tr>
<tr>
<td>8-Azaguanine</td>
<td>0</td>
</tr>
<tr>
<td>Thioguanine</td>
<td>0</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>0</td>
</tr>
<tr>
<td>Thiaxanthenone</td>
<td>+</td>
</tr>
<tr>
<td>Miracil D</td>
<td>+</td>
</tr>
<tr>
<td>Benzimidazole</td>
<td>5,6-Dichloro-</td>
</tr>
<tr>
<td>Miscellaneous:</td>
<td></td>
</tr>
<tr>
<td>N-Methylformamide</td>
<td>0</td>
</tr>
<tr>
<td>N-Methylacetamide</td>
<td>0</td>
</tr>
<tr>
<td>Urethan</td>
<td>++</td>
</tr>
<tr>
<td>Hydrocortisone</td>
<td>0</td>
</tr>
<tr>
<td>Ethionine</td>
<td>0</td>
</tr>
<tr>
<td>Colchicine</td>
<td>++++</td>
</tr>
<tr>
<td>1,5-Diaminobuuret</td>
<td>0</td>
</tr>
</tbody>
</table>

* 0 = inactive at $10^{-6}$ M.

### Table 3
**In Vivo ACTIVITY OF COMPOUNDS WITH GREATEST in Vitro ACTIVITY**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Tumors inhibited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acridine A</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>B</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>C</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>D</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>E</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>F</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>G*</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>Quinoline A</td>
<td>755</td>
</tr>
<tr>
<td>Quinoline B*</td>
<td>755</td>
</tr>
<tr>
<td>C</td>
<td>755</td>
</tr>
<tr>
<td>D</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>E</td>
<td>Ehrlich</td>
</tr>
<tr>
<td>N Mustard A</td>
<td>755</td>
</tr>
<tr>
<td>Thiaxanthenone A</td>
<td>L1210</td>
</tr>
<tr>
<td>B</td>
<td>L1210</td>
</tr>
<tr>
<td>C</td>
<td>L1210</td>
</tr>
</tbody>
</table>

* These two compounds, quinacrine and camoquin, have also been evaluated in a small series of patients with glioblastoma multiforme and found to be essentially inactive (to be published).
A detailed analysis of some of these findings has been or will be made elsewhere, but it is pertinent to mention here that, even within a particular chemical group, this correlation was often not impressive; Miracil D, for instance, the most active tumor-inhibitor among the thia-xanthenones studied to date, was only one tenth as active in tissue culture as at least twelve of its congeners. Another example is given in Table 4, which indicates that in a small series of closely related benzimidazole derivatives the brain tumor cytotoxicity in vitro correlates well with relative antiviral activity and to some extent with relative toxicity to mice, as determined by the maximum tolerated dose in a 5-day chronic toxicity test, but that none of these data bear any demonstrable relationship to the carcinostatic activity of these compounds in these particular transplantable tumors.

The inability of quinacrine to affect tumor growth significantly in vivo despite its pronounced cytotoxicity in vitro has been subjected to further analysis with the aid of a sensitive assay method for this representative of the acridine series. Comparative determinations in brain tumor cell explants after exposure to the minimum inhibitory concentration and in tumors collected from mice or patients after courses of quinacrine administration with various dosage regimens demonstrated that the lowest drug levels which are effective in tissue culture cannot be attained routinely in vivo without prohibitive toxicity to the host (45). Information gained by this type of experiment not only helps to explain discrepancies between the results of different screening technics but often aids also in the design or selection of chemotherapeutic agents which may have a more suitable distribution pattern and consequently a better therapeutic index in vivo.

### TABLE 4

<table>
<thead>
<tr>
<th>Benzimidazole Derivatives</th>
<th>Rel. antiviral activity*</th>
<th>Rel. tumor cytotoxicity†</th>
<th>Rel. mouse toxicity‡</th>
<th>Tumors inhibited in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Chlorobenzimidazole</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>E0771§</td>
</tr>
<tr>
<td>5,6-Dichlorobenzimidazole</td>
<td>14</td>
<td>5</td>
<td>2.5</td>
<td>755, Ehrlich</td>
</tr>
<tr>
<td>5,6-Dichlorobenzimidazole ribofuranoside</td>
<td></td>
<td>92</td>
<td>90</td>
<td>8§</td>
</tr>
<tr>
<td>4,5,6-Trichlorobenzimidazole ribofuranoside</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Data of Tamm (76).
† Data obtained with human and mouse brain tumors in vitro; for details, cf. text.
‡ Based on the reciprocal of the maximum tolerated dose in mice.
§ Data of Sugiura (75).
# Data reported by Kissman et al. (5@).
|| We are indebted to Dr. K. Folkers of Merck and Co. for these compounds.

### RELEVANCE OF TISSUE CULTURE RESULTS

A casual observer may ask in all fairness why comparative studies like the one just outlined have been continued for so protracted a period of time in the face of the patent failure of the tissue culture data to mirror in vivo activity. Another, less interested in assessing past performance than in charting future courses of action, properly may inquire whether any extensive further tissue culture observations should be made in the current quest for compounds of clinical usefulness and, if so, which particular in vitro technic should now be selected. The answer to these questions hinges on a consideration of some general aspects of cancer chemotherapy screening, which may be discussed briefly at this point.

**Objectives of screening.**—It is instructive as well as diverting to consult Webster's unabridged dictionary (60) for a serviceable definition of a "screen." This is a word of many meanings, with seventeen different entries relevant to subjects from archeology and cricket to motion pictures and physics. It goes without saying that the proper meaning for our uses is embodied best by the second definition ("a device used to separate coarser from finer parts") and that the first and principal meaning of the word ("that which shelters or conceals from view") is to be avoided with care.

A cancer chemotherapy screen, then, should permit the sifting of large numbers of compounds, with the retention of those which will exert a significant beneficial effect on cancer in man. It is axiomatic that in theory the cancer patient would provide the most suitable screen, but for many valid reasons this test system cannot be employed extensively in practice and a secondary
screen must be selected. In trying to evaluate these ancillary approaches, it cannot be emphasized too often that at present the correlation of the response of any laboratory screen with that of the disease in man is most tenuous. The overwhelming majority of the many thousands of compounds examined by one or more experimental screening technics have never been tested in the clinic; where results in human cancer are available, it is apparent that the response to treatment differs with the type of neoplasm, the state of the disease, the status of the patient, and numerous other factors. It is, of course, true that most of the chemotherapeutic agents now available for the clinical treatment of cancer were found first in one or another of the experimental screens, and this fact is a strong and telling argument for the continuation of screening in the laboratory. Not infrequently, however, one may be tempted to claim a special preference for a particular screening technic because it gives a positive response to many agents of clinical usefulness; this temptation must be resisted, since the few compounds which significantly affect neoplastic growth in man have so wide a spectrum of biological activity that almost every screening system can make this claim with equal justification.

**Intercomparisons of in vivo results.**—By far the most common screening systems are the transplantable rodent tumors whose successful establishment by Hanau and Jensen was mentioned at the beginning of this paper. A wide variety of experimental tumors have been employed over the years, and, while many compounds have been tested only against a single tumor, many intercomparisons among different tumors have also been carried out. These studies have shown that there are extensive differences in the response of different tumors to the same compound (cf. 35, 72, and many others). No single transplantable neoplasm has emerged which has an unusual capacity to predict the clinical usefulness of chemotherapeutic agents, and it is the current policy of major screening operations including those guided by the Cancer Chemotherapy National Service Center to test compounds against several transplantable tumors side by side, without letting the results in one tumor determine the extension of the tests to the others.

Numerous other in vitro systems have become available or are in the process of development (cf. 73). The examination of their response to many of the compounds which have already been tested in the more conventional systems cannot but add significantly to our understanding of cancer and the advancement of cancer chemotherapy.

**Intercomparisons of in vitro results.**—The survey of tissue culture screening studies which has been presented here has shown that, up to the present, only a few different systems have been exposed to more than a handful of chemicals. Some of the test systems have been designed to seek compounds with selective toxicity to tumor cells; others have been tailored to establish the minimum cytotoxic concentration of various agents under specified culture conditions. Even with the sparse comparative information now available, discrepancies among various in vitro results are beginning to appear. These differences may be a function of the nature of the culture medium; for example, compounds such as 6-mercaptopurine, 8-aza- guanine, thioguanine, and 1,9-dimethanesulfonfolyxynonane were quite active inhibitors of long established cell lines on a semisynthetic medium (22) but failed to affect primary explants of brain tumor cells on a nonsynthetic medium at concentrations of $10^{-4}M$ or lower (Table 2); 5-fluorouracil, another agent without effect on the brain tumor cultures, was recently shown to be a powerful inhibitor of H.Ep. #1 cells in Eagle’s medium (66); A-methopterin inhibited the growth of Sarcoma 180 in Eagle’s basal medium, but this inhibition could be prevented by the addition of hypoxanthine + thymidine + glycine even if the folic acid antagonist was added at concentrations 10,000 times that ordinarily required for complete inhibition (38). The nature of the tumor may also play a role, as shown by the significantly greater effectiveness of 8-aza- guanine against Brown-Pearce tumor cells than against glioblastoma cells in the same tissue culture medium (33). As more tests are done and other tissue culture systems are added (e.g., 54, 68), additional discrepancies will undoubtedly emerge.

**Comparison of in vitro and in vivo results.**—Since well under 1000 of the more than 25,000 compounds tested in vitro have been examined in vivo at this writing, there is as yet no real basis for comparison. However, if a tentative conclusion is to be drawn, the correlation of the response of tumors in tissue culture and in the animal has, in general, not been impressive. This has been illustrated adequately in the survey of the brain tumor studies; but other instances may also be cited, e.g., the failure of the test system of Eagle and Foley (22) to respond to hydrocortisone, Myleran, or N-methylformamide; the early data of McConnell et al. (55) demonstrating that...
emetine and *S. marcescens* polysaccharide both damaged Sarcoma 37 in vivo but only the former affected the same tumor in vitro; or the recent report of Schindler and Welch (69) of the failure of Sarcoma 180 cells in tissue culture to respond to 6-azauracil, a potent inhibitor of this tumor in vivo, because of the inability of the cultured cells to carry out the required conversion of 6-azauracil to 6-azauridine.

There are, of course, many reasons for discrepancies between in vitro and in vivo results. For one thing, the toxicity of a compound to tumor cells, the primary criterion of activity in tissue culture, is only one of the several component parts of the therapeutic index which is the basis of the demonstration of carcinostatic activity in the animal. Moreover, an agent after administration to the tumor-bearing host may be transformed rapidly into a derivative with greater or lesser effectiveness against tumor cells or with higher or lower toxicity to normal cells; its distribution and localization in tissues and body fluids and its rate of excretion are crucial factors in an in vivo test but are of little significance in tissue culture; problems of cumulative toxicity and the development of resistance plague the chemotherapist working with animal tumors or cancer patients but are of small import in the in vitro studies; tumor-host interactions and indirect, endocrine-mediated effects may play a vital role in the response of the neoplasm to chemotherapy but cannot, of course, be reproduced in tissue culture; cells may be endowed with quite different permeability to drugs when bathed by body fluids or by tissue culture feeding fluids; the chemical compound may be more stable in one of these suspending media than in the other; an antagonist to the antitumor action of the chemotherapeutic agent present in vivo may be lacking in vitro, or the converse may be true. Some of these factors which favor discrepancies between tissue culture and the animal have been documented or at least suggested by experimental evidence, others are still entirely speculative, but when they are taken together they make general agreement between these two types of biological test systems less rather than more likely.

**Conclusions**

With this brief discussion, it is now pertinent to return to the questions raised at the beginning of this section and to attempt an answer. It has been seen that there are discrepancies in the response of different neoplasms in man to a chemotherapeutic agent, in the effectiveness of a compound against various animal tumors, in the damage caused by a chemical to tumor cells in different types of tissue culture. Differences abound in the intercomparisons of experimental with human neoplasms and of in vitro data with both. The rule of this microcosm, as of any other, seems to be diversity and variety.

It may be submitted that too much effort has gone into looking for similarities of response of different ancillary screening methods, in the virtual absence of a primary yardstick by which they might be judged. What is needed is the simultaneous examination of large series of compounds of widely varying chemical structure and biological activity in different in vitro systems, in ascites cells, in solid transplantable neoplasms, in spontaneous tumors, in heterologous human transplants, and in every other system which can contribute meaningful information on their carcinostatic or carcinolytic activity under every reasonable set of conditions. At the present imperfect state of our knowledge, the most fruitful approach to the complexities of cancer chemotherapy may well be the imaginative exploitation of the discrepancies which are bound to emerge from these comparisons. If cancer chemotherapy screening is designed with these objectives in mind, it is clear that tissue culture will continue to be an integral part of the investigative effort in this challenging field.

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