INTRODUCTION

When Alexis Carrel performed his famous experiments [1] which demonstrated the feasibility of growing and maintaining cells from the mammalian organism in artificial systems, one of the obvious applications of cell and tissue culture was the study of the neoplastic cell and its genesis under relatively well-controlled conditions outside of the living organism. After Carrel's initial experiments, a number of investigators demonstrated that neoplastic tissues isolated from an organism could be maintained in cell culture.[2,3] It was not until the 1930s, however, that Earle and his associates first attempted in a meaningful manner the conversion of normal to neoplastic cells entirely *in vitro*.[4] Part of this delay resulted from the need for purified chemicals capable of initiating and promoting the neoplastic transformation. When the polycyclic hydrocarbons were isolated and their structure determined in the early 1930s,[5] the basis for Earle's experiments was complete. Unfortunately, the studies by Earle and his associates were inconclusive, since the neoplastic transformation occurred both in control cells not treated with the carcinogenic hydrocarbon and in cells treated with this chemical.[6]

Earle and his associates worked for many years believing that this result was possibly due to minute contamination of control cultures by the carcinogens added to the treated cultures. However, after numerous experiments he and his associates came to the conclusion that cells would "spontaneously" transform to neoplastic tissue in cell culture. Because of this complication, other investigators did not pursue this subject strenuously until about 15 years ago. At that time, Berwald and Sachs[7] described the transformation of hamster embryo cells with polycyclic hydrocarbons in cell culture. In those experiments, control cells showed little or no spontaneous transformation.

Since the initial work of Berwald and Sachs, Heidelberger [8] as well as DiPaolo [9] and several others [10,11] have extended these studies in this country. One of the principal difficulties that has arisen during studies of the *in vitro* neoplastic transformation is the establishment of criteria, both morphologic and biologic, that characterize cell transformation in culture. Two years ago, as part of a symposium on the testing of chemicals for carcinogenesis, a group of scientists published "accepted" characteristics of cells transformed in culture.[12] These characteristics are reproduced in Table 1. The essential conclusions were: a) the ultimate standard of the malignant transformation in any event is the growth of cells *in vitro* as a biologic neoplasm with lethal potential for the host and b) no single criterion of those listed in Table 1 may be utilized to ensure absolutely that cells transformed *in vitro* are biologically neoplastic. The committee also made some arbitrary suggestions concerning the number of the characteristics listed in Table 1 that need to be satisfied before definitive conclusions concerning the neoplastic characteristics of the cell can be made.

Although demonstration of the transformation of cells *in vitro* to the neoplastic state of itself is extremely interesting and important for studying the mechanisms of carcinogenesis, such systems may potentially offer excellent rapid test assay systems for environmentally and experimentally produced chemical carcinogens. The systems described in this symposium indicate that not only mesenchymal tissues (such as those utilized by Sachs, Heidelberger, and others) may be transformed by chemicals in cell culture, but also that more highly differentiated epithelial tissues such as liver also undergo the same neoplastic change *in vitro*. At the present time the popular rapid assay system for mutagenesis is that described by Ames and his associates.[13] Cells grown in culture, especially those derived from liver, which possess most of the metabolic activating systems for precarcinogens,[14] may in the final analysis offer the best systems for the assay of carcinogenic substances either by measuring the acute effect of the carcinogen on the metabolism of DNA or by effecting the transformation of liver cells

Table 1—Criteria of Neoplastic Transformation *In Vitro**

1. Production of biologically malignant neoplasms *in vitro* by inoculation of 10^6 or less cells into syngeneic hosts, in the absence of neoplasms produced by inoculation of comparable numbers of cells not treated with the *transforming* agent. Some *transformed* cell lines do not conform to this criterion, and some embryonic cells injected into immunosuppressed or syngeneic hosts will grow to the size of a gross tumor. The time of growth in the syngeneic host of transformed cells to detectable size may vary tremendously.
2. *Immortality* of transformed cells in culture. This is characteristic of almost all biologically neoplastic cells although in some instances those having immortality *in vitro* do not give rise to tumors *in vivo*.
3. Growth of transformed cells in soft agar. With the exception of some mouse cell strains, e.g., Heidelberger's strain C3H/10T1/2 and transformed mouse prostate cells, transformed cells exhibiting this characteristic also produce neoplasms on inoculation into a suitable host. On the other hand, a number of biologically neoplastic tissues grown *in vivo* will not grow in soft agar in culture.
4. Colonies of transformed cells exhibit different morphologic and growth characteristics in culture compared with normal cells grown in culture. *Nontransformed* cells grow in an "ordered" way, whereas transformed cells tend to "pile up" with crisscross patterns and a higher degree of pleomorphism. However, this criterion applies only to fibroblastic cells grown in culture. So few epithelial cells have been transformed in culture that morphologic criteria of transformation have not been determined accurately.
5. Loss of *contact inhibition* of cell replication and increase in saturation density by transformed cells. Again, this characteristic appears not to hold for epithelial cell cultures, and a significant number of nontransformed cells in culture demonstrate no contact inhibition.
6. Transformed cells in many, but not all, instances may be agglutinated by plant lectins. Not all cells agglutinated, however, demonstrate biologic neoplasia *in vivo*.
7. Cells transformed by chemicals or viruses in culture exhibit antigenic alterations. *Spontaneous transformants* show no antigenic alterations.
8. Transformed cells may show karyotypic changes. However, cell lines that produce no tumors *in vivo* may be quite aneuploid, as are many *revertants* in culture.
9. Transformed cells usually have a greater efficiency of cloning than nontransformed cells.

* Taken from the Report of Discussion Group 14 in Pitot.[12]

in vitro. In any event, the transformation of cells in culture from the normal to the neoplastic state by the direct addition of chemical agents to the medium offers an exciting tool for the experimental oncologist and potentially a method for the rapid assay of carcinogens in our environment.

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References

1. Carrel A, Burrows MT: Cultivation of adult tissues and organs outside of the body. JAMA 55:1379–1381, 1910
2. Fischer A: Charaktereigenschaften von Krebszellen *in vitro*. Klin Wochenschr *in vitro*. Br J Exp Pathol 10:312–319, 1929
3. Fischer A: Charaktereigenschaften von Krebszellen *in vitro*. Klin Wochenschr 7:6–20, 1928
4. Earle WR, Nettleship, A: Production of malignancy *in vitro*. V. Results of injection of cultures into mice. J Natl Cancer Inst 4:213–227, 1943

5. Cook, WJ, Hewett CL, Hieger I: The isolation of a cancer-producing hydrocarbon from coal tar. J Chem Soc 395–405, 1933
6. Sanford KK, Earle WR, Shelton E, Schilling EL, Duchesne EM, Likely GD, Becker MM: Production of malignancy *in vitro*. XII. Further transformations of mouse fibroblasts to sarcomatous cells. J Natl Cancer Inst 11:351–373, 1950
7. Berwald Y, Sachs L: *In vitro* cell transformation with chemical carcinogens. Nature 200:1182–1184, 1963
8. Heidelberger C: Chemical oncogenesis in culture. Adv Cancer Res 18:317–366, 1973
9. DiPaolo JA, Donovan P, Nelson R: Quantitative studies of *in vitro* transformation by chemical carcinogens. J Natl Cancer Inst 42:867–874, 1969
10. Benedict WF, Gielen JE, Nebert DW: Polycyclic hydrocarbon-produced toxicity, transformation and chromosomal aberrations as a function of aryl hydrocarbon hydroxylase activity in cell cultures. Int J Cancer 9:435–451, 1972
11. Igel HJ, Freeman AE, Spiewak JE, Kleinfeld KL: Carcinogenesis *in vitro*. II. Chemical transformation of diploid human cell cultures: A rare event. In Vitro 11:117–129, 1975
12. Pitot HC: Criteria of neoplastic transformation: Report of discussion Group No. 14. Carcinogenesis Testing of Chemicals. Edited by L Golberg. Cleveland, CRC Press, Inc., 1974, pp 113–114
13. McCann J, Ames BN: Detection of carcinogens as mutagens in the *Salmonella*/microsome test: Assay of 300 chemicals: Discussion. Proc Natl Acad Sci USA 73:950–954, 1976
14. Miller JA: Carcinogenesis by chemicals: An overview—G. H. A. Clowes Memorial Lecture. Cancer Res 30:559–576, 1970