Title
Quantitation of the number of cells within tumor colonies in semisolid medium and their growth as oblate spheroids.

Journal
Cancer research, 44(1)

ISSN
0008-5472

Authors
Meyskens, F L, Jr
Thomson, S P
Moon, T E

Publication Date
1984

Peer reviewed

[CANCER RESEARCH 44, 271–277, January 1984]

Quantitation of the Number of Cells within Tumor Colonies in Semisolid Medium and Their Growth as Oblate Spheroids[1]

Frank L. Meyskens, Jr.,[2] Stephen P. Thomson, and Thomas E. Moon

Department of Internal Medicine and Cancer Center, University of Arizona, Tucson, Arizona 85724

ABSTRACT

The number of cells within tumor colonies has not been determined accurately in prior reports because, in all but small clusters, cells grow too closely and stacked to allow direct counting of cells by inverted microscopy. Therefore, we stained colonies formed in agar from 38 tumor cell samples of diverse histological origin, removed them with a micropipet, and directly counted the number of cells. The number of cells within colonies increased geometrically with colony diameter and inversely with the size of the cells within the colonies. The relationship can be described using linear regression:

$$[\ln(\text{no. of cells/colony}) = 0.87 - 2.80 \ln(\text{colony cell diameter}) + 2.38 \ln(\text{colony diameter})]$$

which gave an R^2 of 0.92. Measurements of colonies in agar showed that they grew as oblate spheroids rather than spheroids. In an average sample, 60-μm-diameter colonies contained eight to 10 cells; the range for the 38 samples was 1.2 to 48.5. These results precisely define colonies in terms of cell number. They allow calculation of the total number of cells formed from clonogenic cells, a more complete estimate of proliferation than conventional cloning efficiencies which only measure initial proliferation. Furthermore, because of the dependence on the size of the colony cells, if colonies are defined only by a specific diameter then they do not contain similar numbers of cells. Calculations which assume spherical colonies, rather than the oblate spheroid shape we found, greatly overestimate the number of cells within colonies. Our data can increase the accuracy of quantitation in the colonogenic system and thus improve the interpretation of the proliferation of clonogenic tumor cells.

INTRODUCTION

Clonogenic assays have been widely used as an *in vitro* marker for transformation (9,10) and as a method to identify normal and abnormal hematopoietic progenitor and animal tumor stem cells (11, 17). More recently, this technique has been applied to selectively grow clonogenic human tumor cells (6). We and others have provided evidence that the colonies are composed of tumor cells (13, 14), and that a subset of the clonogenic cells are stem cells as demonstrated by the growth of colonies injected into nude mice into tumors or by replating of primary colonies *in vitro* (2–4, 19). A number of investigators have also offered evidence that the fractional survival of clonogenic tumor cells after a chemotherapeutic treatment *in vitro* can be related to clinical effect (1, 12, 15, 20). However, a number of methodological problems with the assay have been identified, and its routine use to identify chemosensitivity for individual patients requires further refinement of the assay; particularly, improvements in preparation of single cell suspensions and growth conditions are needed (16).

Quantitative application of clonogenic assays require, as a minimum, accurate enumeration of the growth of clonogenic tumor cells. The numbers of clonogenic cells are commonly quantitated by cloning efficiencies. They are calculated by dividing the number of colonies by the number of cells plated, with colonies defined as multicellular growth units above a designated number of cells or diameter. Therefore, cloning efficiencies measure only the number of cells which undergo an initial proliferation. They do not measure the extent of proliferation of clonogenic cells past the minimum size set by the definition of a colony. Cloning efficiencies may be adequate to quantitate the number of clonogenic cells, but they are incomplete measures of clonogenic cell growth. Thus, it is not known what "size" of multicellular growth unit should be measured in clonogenic assays.

A more complete estimate of the proliferation of clonogenic cells within the assay system would be obtained if the total number of cells which formed from the clonogenic cells was determined. This cell number could be calculated by multiplying the frequency of each size colony by the number of cells within colonies of each size, if they were known, and summing. However, the number of cells within colonies of each size commonly is not known in detail, thus preventing calculations of the more complete estimate of proliferation. Thus, a major quantitative problem for clonogenic systems is not knowing the number of cells within clusters or colonies of different diameters. The geometrical arrangement of cells in colonies with greater than 20 cells is too close and stacked up on each other to allow discrimination of single cells. Thus, the number of cells within colonies can not be accurately enumerated by conventional viewing of the colonies in agar with inverted microscopy. This places potential limitations on the quantitative aspects of clonogenic systems. It prevents the determination of the total number of cells formed. It also complicates the enumeration of the frequency of each size of colony defined as more than 20 cells. For example, the 40 or 50 cells commonly used as the minimum number to define a colony is difficult to use if there are many growth units with 30 to 60 cells each, because one cannot readily distinguish between "colonies" of different sizes. Furthermore, defining colonies by a constant diameter is difficult because it is not known which, or if, a constant diameter can define colonies with a constant number of cells. Several laboratories have used a diameter of 60 μm to define colonies without knowing exactly how many cells they contained.

In this study, we have examined and defined the relationship between the diameter and number of cells within clusters and colonies using 38 samples of clonogenic tumor cells of diverse histological origin from fresh biopsies and cell lines. The number of cells within colonies was closely correlated with colony diam-

[1] This work was supported in part by grants from the American Cancer Society (PDT184) and from the NIH (CA27502, CA21839, CA17094, and CA23074).

[2] To whom requests for reprints should be addressed.

Received May 16, 1983; accepted September 28, 1983.

eter and inversely with the size of the cells within the cluster or colony. We also found that colonies grew as oblate spheroids. These results allow calculation of the total number of cells formed and can be used to accurately define colonies in terms of cell number.

MATERIALS AND METHODS

Preparation and Culture of Cells from Biopsies. Tumor tissue was obtained under aseptic conditions from patients with different types of human tumors (protocol approved by the University of Arizona Committee on Human Subjects). Tumor tissue was cut free of necrotic and normal tissue and minced into 1-sq-mm pieces or less by extensive slicing with scissors. The tissue was placed into a 50-ml conical tube containing Ham's F-10 medium (Grand Island Biological Co., Grand Island, N. Y.) with 10% heat-inactivated fetal calf serum (KC Biologicals, Inc., Lenexa, Kans.), penicillin (100 μg/ml), and streptomycin (100 units/ml; Eli Lilly, Indianapolis, Ind.) and was inverted several times. Tumor pieces and macroscopic clumps were allowed to settle to the bottom of the tube for 5 to 10 min at unit gravity, and the supernatant containing the single cells was aspirated. Tumor pieces were resuspended in media, and the process was repeated several times until the supernatant was clear. Cells were pooled, counted, tested for viability by exclusion of 0.4% trypan blue (Grand Island Biological Co.) and plated in the upper layer of an agar bilayer (35-mm-diameter Petri dishes) supplemented with various nutrients (6, 13). The single-cell nature of the plated cells was assured by checking for cellular aggregates 1 hr after plating. Cells from established lines routinely had less than one aggregate/plate, and no plate containing more than 5 aggregates was used. Cells were cultured for 10 to 14 days in a well-humidified, 5% CO_2 and 95% air atmosphere at 37°.

Enumeration of Number of Cells per Cluster or Colony. Clusters or colonies were stained with toluidine blue then directly plucked intact, placed on a slide with 4 μl of media, and overlaid with a coverslip; a procedure which has been described in detail (19). The number of cells was directly enumerated using conventional × 40 to × 100 light microscopy (Fig. 1). Clusters and colonies with mean diameters of 60, 100, and 150 [± 5 μm (S.E.)] were identified, and the diameters of the cells within colonies were measured. The mean number of cells within 5 to 10 colonies of each size for each tumor sample was determined. The average S.E. was 9.18%. The mean cell diameter was determined by measuring 20 cells from each of 5 to 10 colonies. The average coefficient of variation for the cell diameters was 16.3%. By counting 20 colony cells/sample, the S.E. averaged only 0.78 μm.

Shape of Colonies. Theoretical number of cells per colony of different shapes was calculated by dividing the volume of the shape by the volume of different sizes of single cells using:

$$\text{Sphere} = \text{Volume of } 4/3\ \pi\ r_1^3$$

$$\text{Oblate sphere} = \text{Volume of } 4/3\ \pi\ (r_1)^2\ r_2$$

$$\text{Oblate sphere} = \text{Volume of } 4/3\ \pi\ (r_1)^2\ r_2 C$$

where C is space correction factor, the length of r_1 is the maximum radius of the colony in the horizontal dimension (major semiaxis) and was measured directly in the agar, and r_2 is the radius of the colony in the vertical dimension (minor semiaxis) and was empirically determined by cutting out small rectangular pieces of agar and tilting them 90° to measure the vertical radius of colonies intact in agar. The space correction factor C represents the proportion of the colony volume that is actually occupied by cells and is discussed below and derived in "Appendix." The orientation of r_1 and r_2 is shown in Fig. 2.

RESULTS AND DISCUSSION

We have measured the diameter of cells within colonies, the diameter of clusters or colonies, and the number of cells per cluster or colony grown from clonogenic cells from 38 different sources. Data from 14 samples in which colonies as large as 150 μm in diameter were grown are summarized in Chart 1*A* and are graphed so that the diameter of the cluster or colony (*abscissa*) related directly to the number of cells per cluster or colony (*ordinate*). As one would expect, the number of cells was related to colony diameter: colonies 150 μm in diameter have more cells than 100-μm-diameter colonies, which have more than 60-μm-diameter colonies. However, the increase in cell number with colony diameter was exponential and not linear.

We have found that cell diameter was closely related to the number of cells in colonies of the same size but from different samples. For example (Chart 1*A*), colonies 150 μm in diameter contained 6 to 552 cells which entirely reflected the size of the cells within the colony. A pictorial presentation of this point for two 100-μm-diameter colonies from different samples and containing cells of different diameters is provided in Fig. 3. Our data suggest that these phenomena are dependent only on the size of the cell and not on the tumor cell type, although a much larger number of different tumors will need to be measured before a definite statement can be made. The S.D. for cells from each colony was not large, relative to the range of cell diameters studied, and reflects in part the size of the cell in different phases of the cell cycle.

To explicitly relate the number of cells in a colony with the diameter of the cells and the diameter of the colony, the following regression equation was derived. It gives a very good fit (R^2 = 0.92; Chart 2, *solid line*) to the data for all sizes of colonies:

$$\ln(\text{no. of cells/colony}) = A + B \ln(\text{mean cell diameter}) + C \ln(\text{mean colony diameter}) \quad \text{(A)}$$

Coefficient	S.E.	(95% confidence interval)
A = 0.874	0.245	(0.394, 1.354
B = −2.804	0.496	−3.776, −1.832)
C = 2.378	0.446	(1.504, 3.252)

This equation can be reexpressed as:

$$\text{No. of cells/colony} = 2.40\ \frac{(\text{colony diameter})^{2.378}}{(\text{cell diameter})^{2.804}} \quad \text{(B)}$$

This mathematical presentation demonstrates several important features of colonies: (*a*) The number of cells per cluster or colony was directly related to an exponential function of cluster or colony diameter and was not linearly related. (*b*) The number of cells per cluster or colony was inversely related to an exponential function of the cell diameter. (*c*) Colonies of the same diameter can contain severalfold different amounts of cells depending on the diameter of the cells.

Using this information from the equations, we have developed a nomogram to relate cluster and colony diameter and the diameter of the cells to the number of cells within the clusters and colonies (Chart 1, *B* and *C*). Thus, colony diameter, which is often used to define the "size" of a colony, does not predict the number of cells within a colony without consideration of the mean size of the cells within the colony. This means that, if one defines a clonogenic cell as a cell that can divide a minimum number of times, the colony "size" must be defined in terms of cell numbers which cannot be predicted with measurements of

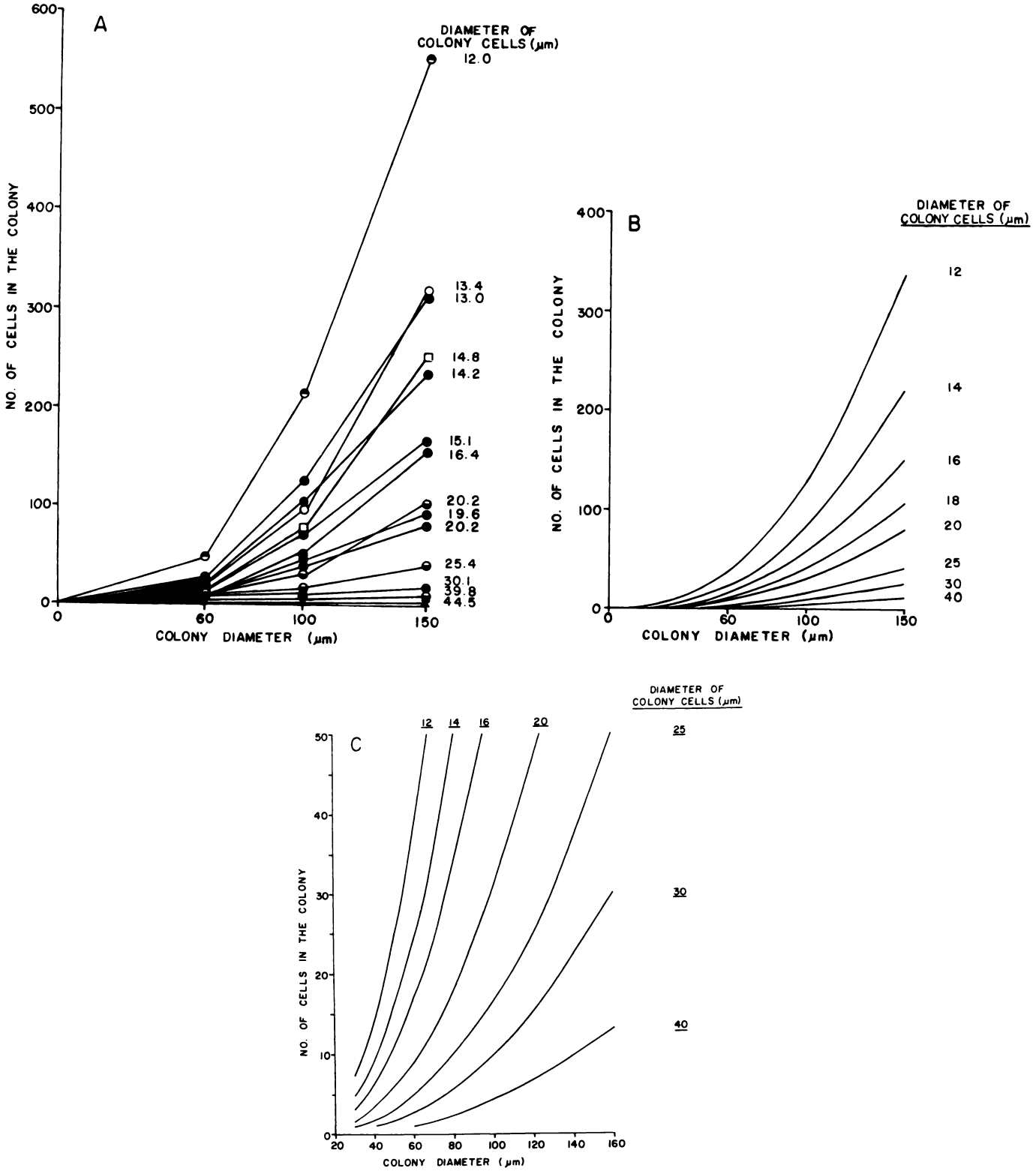

Chart 1. Relationship between cluster and colony diameter, cell diameter, and number of cells per cluster or colony. *A*, data from 14 sources of tumor cells in which colonies of diameters of 60, 100, and 150 μm grew. Mean diameter is of cells within clusters and colonies. Cells from biopsies of melanoma (●), ovarian (◒), lung (×), and sarcoma (◓) tumors; cells from cell lines of murine melanomas CCL 53.1 (○) and T470 breast (□). *B*, theoretical nomogram relating cluster and colony diameter, cell diameter, and the number of cells within the cluster or colony. The nomogram was constructed using the equations derived from the data shown in Charts 1*A* and 2 and discussed in the text. *C*, theoretical nomogram relating cluster and colony diameter, cell diameter, and the number of cells within the cluster or colony. This represents expansion of the lower part of the curves in Chart 1*B*.

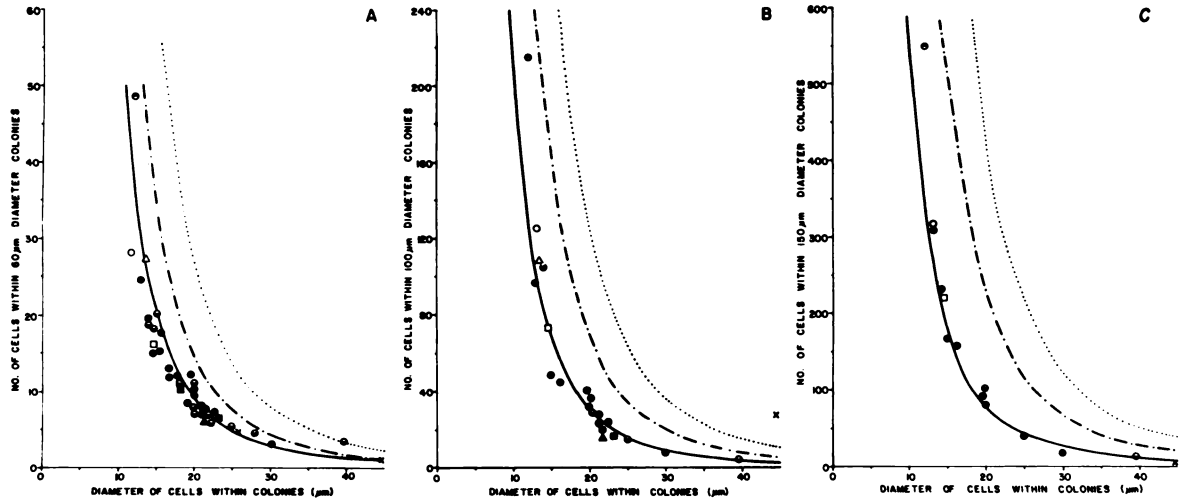

Chart 2. Shapes of colonies resembles an oblate spheroid. The data from Chart 1 and from 24 other sources of tumor cells demonstrate the relationship between the mean diameter of cells in colonies and the number of cells in colonies with defined diameters (±5 μm). Shown are colonies with different major axis diameters: *A*, 60 μm; *B*, 100 μm; *C*, 150 μm. The number of cells is the mean of 5 to 10 clusters or colonies, and the cell diameters are the mean of 20 cells from different colonies of the sample. Average mean, ±9.18%. Colony shapes (as described in "Materials and Methods"): ····, sphere; –·–, oblate spheroid; ——, Equation B in text. Cells from biopsies of melanoma (●), ovarian (⊖), lung (×), and sarcoma (◓) tumors; cells from biopsies of breast (■), colon (▲), and renal (△) tumors; and cells from a colon (WIDR) cell line (□).

diameter alone. Thus, a single diameter cannot be used to define colonies for different samples.

We have also made morphological observations of colonies in agar which have shown that nontumor cells were not present and that the shape of the growth units was not a spheroid as usually occurs in liquid medium (5), but more closely resembled an oblate spheroid. This can readily be appreciated in Fig. 2. The relationship of r_2 to r_1 was determined by directly measuring r_2 and r_1 in 99 colonies from 2 samples. For Sample 1:

$$r_2 = r_1/(1.82 \pm 0.09)$$

and, for Sample 2:

$$r_2 = r_1/(1.84 \pm 0.09)$$

The data in Chart 2 express the relationship between the mean cell diameter and number of cells in colonies as well as permits a graphical comparison of the actual data to the possible theoretical shape of the colonies. The mean diameter of cells in colonies is presented on the abscissa and related to the number of cells in colonies with diameters of 60 μm (Chart 2*A*). The data were clearly not represented by the shape of a sphere (Chart 2*A*, *dotted line*). If one assumes that colonies grow as spheroids, then estimates of the number of cells they contain (calculated by dividing their volume by the volume of single cells) would be 3- to 4-fold too large. This difference was greatest for colonies containing smaller diameter cells and for the larger diameter colonies (Chart 2, *B* and *C*).

Similarly, the assumption that colonies grow as oblate spheroids that are totally occupied by tumor cells yields an estimate of total number of cells that is also too large when compared to the actual total cell count within colonies (Chart 2). However, the assumption that colonies grow as oblate spheroids with space between tumor cells is consistent with the data shown in Chart 2. The proportion of the colony volume that is actually occupied by cells is derived in "Appendix." The proportion is called the space correction factor *C* and is related to the cell radius *p* and the colony radius (major semiaxis) r_1 by:

$$C = 3.27 \frac{p^{0.196}}{r_1^{0.622}}$$

Insights into the spacing of cells in a colony are suggested from the above equation. (*a*) As the cell radius increases, the space correction factor also increases. (*b*) The space correction factor *C* decreases when colony radius increases. This suggests that the proportion of colony volume occupied by cells will decrease (thus increasing the space between cells) as colony diameter increases. (*c*) The quantitative change in *C* is more greatly influenced by changes in the colony size and relatively less by changes in cell size.

The implication of these relationships are considerable for quantitation of *in vitro* tumor cell clonogenic growth. Tumor colonies have been routinely enumerated either by visual observation (6, 12, 15) or with an optical scanner (7, 20) with the tacit assumption that colonies in agar grow as spheroids (5). Accurate direct counting of the number of cells in tumor colonies by inverted microscopy is not possible because the cells are stacked in all but small clusters with few cells. Therefore, the designation of a colony with moderate or many cells by determination of the diameter of the colony alone is restricted in its meaning, because the number of cells within the colonies is unknown. Thus, if the "size" which defines a cluster or colony was the diameter of the growth unit instead of the number of cells within it, then the definition does not define colonies in terms of similar cell numbers, which reflect the minimum number of cell divisions.

Tumor colonies with a minimum specified diameter (40 to 60 μm) have been widely used to measure changes in colony survival. Our results indicate that the median number of cells in these colonies was 10, and ranged from one to 48, depending on the diameter of the cells within the colony. This means that previous measurements of colony survival using a 60-μm-diameter criteria have enumerated clonogenic cells which have divided an average of 3 times and a range of 1 to 6 times. The meaning of this for the interpretation of clonogenic cell survival remains to be defined, particularly since the relationship between the extent of clonogenic cell division within clonogenic assays and

true proliferative capacity and self-renewal is largely unknown.

A stem cell model of human tumor growth has been proposed recently (8). It suggests that analysis of the "size" of colonies in terms of cell number may distinguish between clonogenic cells that represent transitional *versus* stem cell populations. Our data can precisely determine the "size" of colonies, the proliferative units in terms of cell number, and thus facilitate the determination of the "cut off" point, in colony sizes, between stem and transitional cell populations. This approach relates measurements of cellular proliferation to self-renewal and, therefore, should be a more accurate estimate of stem cells than either measurements of tritiated thymidine incorporation into colonies (18) or dye exclusion (21). However, these latter 2 tests are considerably simpler, and only *in vitro-in vivo* correlative studies will establish the relative merit of these assays in the clinic.

Our results suggest that comparisons of increases or decreases in colony survival secondary to inhibitors or stimulators should accurately account for cell and colony diameter. This would be particularly important in the assessment of "biological modifiers" which may not alter the initial rate of growth (*i.e.*, the first 3 or 4 divisions) but may markedly alter the ability to proliferate past 5 divisions. If simple cloning efficiency was used alone, then this decrease in proliferative capacity would not be detected. Thus, agents that slow or terminate growth after a few divisions would be detected using measurements of total proliferative capacity, while they would be undetectable using cloning efficiency alone.

In conclusion, use of the empirically derived formula described in this report will allow the precise quantitation of the number of cells within colonies. The total number of cells in colonies can now be calculated by obtaining the frequency of clusters and colonies of different sizes and multiplying by the number of cells in each cluster or colony size and summing. Thus, one can obtain an estimate of how many cells were formed, a more complete estimate of proliferative capacity of clonogenic tumor cells than cloning efficiencies that only measure initial proliferative capacity. Because clonogenic assays are being widely used to describe the biology and chemosensitivity of human tumors, these observations are particularly important.

ACKNOWLEDGMENTS

We thank R. Markmann for secretarial assistance, J. Buckmeier for graphic preparation, and S. Salmon, J. Trent, and particularly B. Durie for helpful comments.

APPENDIX

Derivation of Colony Space Correction Factor. The colony space correction factor C is defined as the proportion of colony volume that is actually occupied by cells. Thus, C can be expressed as:

$$C = \frac{\text{Volume of actual number of cells in colony}}{\text{Volume of oblate spheroid}} = \frac{(\text{No. of cells in colony}) \times (\text{volume of a cell})}{\text{Volume of oblate spheroid}}$$

Using the results presented in this paper, each of the quantities necessary to calculate C can be explicitly expressed as:

$$\text{No. of cells in colony} = 2.40 \frac{(\text{Colony diameter})^{2.378}}{(\text{Cell diameter})^{2.804}}$$

for:

$$\text{Volume of a cell with a radius } p = 4/3\pi\, p^3$$

Table 1

	Space correction factor[a]			
		Cell radius		
	μm	5 μm	10 μm	15 μm
Colony Radius	30	0.54[a]	0.62	0.67
	50	0.39	0.45	0.49
	75	0.31	0.35	0.38

[a] Fifty-four % of a colony (radius, 30 μm) is occupied by cells (radius, 5 μm).

then:

$$\text{Volume of oblate spheroid} = 4/3\pi\, r_1^2\, (r_1/1.83)$$

where r_1 is the major semiaxis, and $r_1/1.83$ is the minor semiaxis. Substitution of these quantities in the expression for C yields:

$$C = \frac{\left(2.40 \frac{(2r_1)^{2.378}}{(2p)^{2.804}}\right) \times \left(\frac{4}{3}\pi p^3\right)}{\frac{4}{3}\pi r_1^3 \left(\frac{1}{1.83}\right)} = \frac{(2.4)\,(2^{2.378})\,(1.83)\, p^{0.196}}{2^{2.804}\, r_1^{0.622}} = 3.27 \frac{p^{0.196}}{r_1^{0.622}}$$

Table 1 illustrates the quantitative relationship between the space correction factor C, colony radius r_1, and cell radius p.

REFERENCES

1. Alberts, D. S., Chen, H. S. G., Soehnlen, B., Salmon, S. E., Surwit, E. A., Young, L., and Moon, T. E. *In vitro* clonogenic assay for predicting response of ovarian cancer to chemotherapy. Lancet, *2:* 340–342, 1980.
2. Buick, R. N., and Mackillop, W. J. Measurement of self-renewal in culture of clonogenic cells from human ovarian carcinoma. Br. J. Cancer, *44:* 349–355, 1981.
3. Buick, R. N., Minden, M. D., and McCulloch, E. A. Self-renewal in culture of proliferative blast progenitor cells in acute myeloblastic leukemia. Blood, *54:* 95–104, 1979.
4. Carney, A. F., Gazdar, A. F., Bunn, P. A., and Guccion, J. C. Demonstration of the stem cell nature of clonogenic tumor cells from lung cancer patients. Stem Cells, *1:* 149–164, 1981.
5. Conger, A. D., and Ziskin, M. C. Growth of mammalian multicellular tumor spheroids. Cancer Res., *43:* 556–560, 1983.
6. Hamburger, A. W., and Salmon, S. E. Primary bioassay of human tumor stem cells. Science (Wash. D. C.), *197:* 461–463, 1977.
7. Kressner, B. E., Morton, R. A., Martens, A., Salmon, S., Von Hoff, D., and Soehnlen, B. Use of an image analysis system to count colonies in stem cell assays of human tumors. *In:* Cloning of Human Tumor Stem Cells. S. E. Salmon (ed.), pp. 179–193. New York: Alan R. Liss, Inc., 1980.
8. MacKillop, W. J., Ciampi, A., Till, J. E., and Buick, R. N., A stem cell model of human tumor growth: implications for tumor cell clonogenic assays. J. Natl. Cancer Inst., *70:* 9–16, 1983.
9. Macpherson, I., and Montagnier, L. Agar suspension culture for the selective assay of cells transformed by polyoma virus. Virology, *23:* 291–295, 1964.
10. McAllister, R. M., and Reed, G. Colony growth in agar of cells derived from neoplastic and nonneoplastic tissues of children. Pediatr. Res., *2:* 356–362, 1968.
11. Metcalf, D. Recent Results in Cancer Research, Vol. 61. Berlin: Springer-Verlag Publishing Co., 1977.
12. Meyskens, F. L., Jr., Moon, T. E., Dana, B., Gilmartin, E., Casey, W. J., Chen, H. S. G., Franks, D. H., Young, L., and Salmon, S. E. Quantitation of drug sensitivity by human metastatic melanoma colony-forming units. Br. J. Cancer, *44:* 787–794, 1981.
13. Meyskens, F. L., Jr., Soehnlen, B. J., Saxe, D. F., Casey, W. J., and Salmon, S. E. *In vitro* clonal assay for human metastatic malignant melanoma. Stem Cells, *1:* 61–72, 1981.
14. Persky, B., Thomson, S., Meyskens, F. L., Jr., and Hendrix, M. J. C. Methods for evaluating the morphological and immunohistochemical properties of human tumor colonies grown in soft agar. In Vitro (Rockville), *18:* 929–936, 1982.
15. Salmon, S. E., Hamburger, A. W., Soehnlen, B., Durie, B. G., Alberts, D. S., and Moon, T. E. Quantitation of differential sensitivity of human tumor stem cells to anticancer agents. N. Engl. J. Med., *298:* 1321–1326, 1978.
16. Selby, P., Buick, R. N., and Tannock, I. A critical appraisal of the "human tumor stem-cell assay." New Engl. J. Med., *308:* 129–133, 1983.
17. Steel, G. G. Growth and survival of tumor stem cells. *In:* G. Steel (ed.), Growth Kinetics of Tumors, pp. 217–267. Oxford, England: Clarendon Press, 1977.
18. Tanigawa, N., Kern, D. H., Hikasa, Y., and Morton, D. L. Rapid assay for evaluating the chemosensitivity of human tumors in soft agar culture. Cancer Res., *42:* 2159–2164, 1982.

19. Thomson, S. P., and Meyskens, F. L., Jr., Method for measurement of self-renewal capacity of clonogenic cells from biopsies of metastatic human malignant melanoma. Cancer Res., *42:* 4606–4613, 1982.
20. Von Hoff, D. D., Casper, J., Bradley, E., Jones, D., and Makuch, R. Association between human tumor colony forming assay results and response of an individual patient's tumor to chemotherapy. Am. J. Med., *70:* 1027–1032, 1981.
21. Weisenthal, L. M., Marsden, J. A., Dill, P. L., and Macaluso, C. K. A novel dye exclusion method for testing *in vitro* chemosensitivity of human tumors. Cancer Res., *43:* 749–757, 1983.

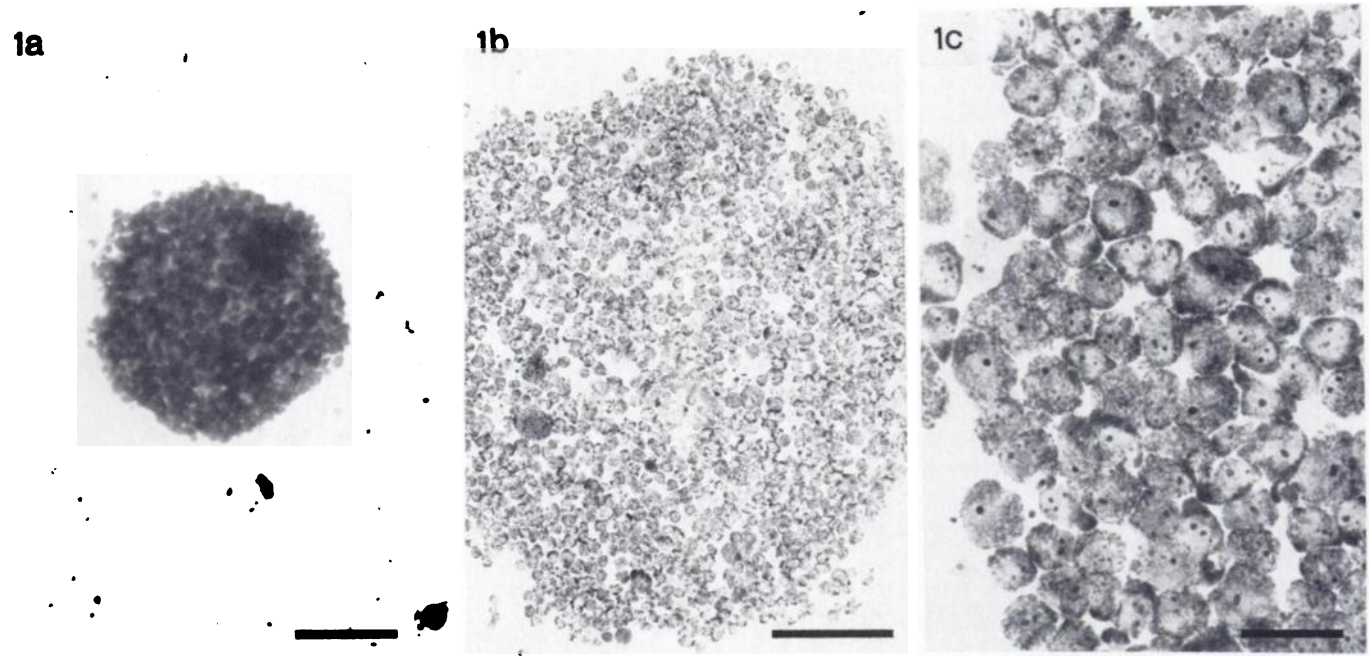

Fig. 1. Determination of the number of cells within clusters and colonies. Colonies were identified, and the diameter of the horizontal axis was measured. *A*, toluidine blue (0.4%) stain was added, and the colony was plucked with a micromanipulator and expelled intact in 4 μl of medium onto a microscope slide. A coverslip was added, which flattened the colony into a layer of single cells (*B*), and the number of cells (*B* and *C*) was counted at × 400. ——, 200 m (*A* and *B*); 25 μm (*C*).

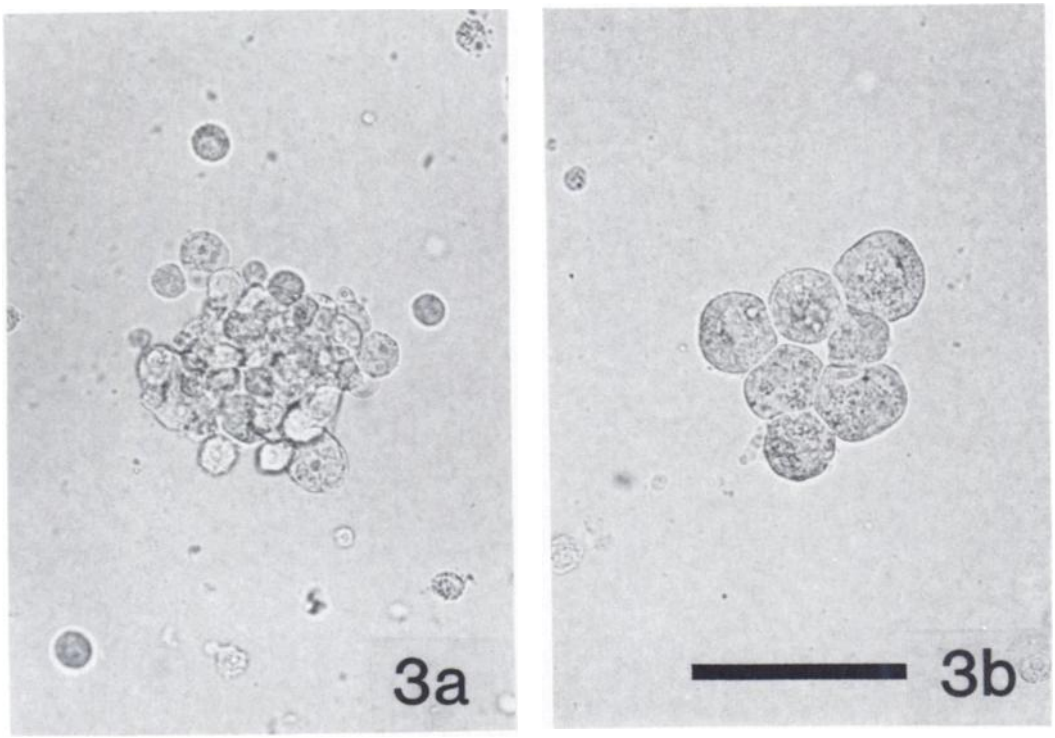

Fig. 3. Photomicrographs of 2 colonies with the same diameter containing cells of different diameters. Colonies of the same diameter with cells of different diameters contained 38 cells (*A*) or 7 cells (*B*). ——, 100 μm.

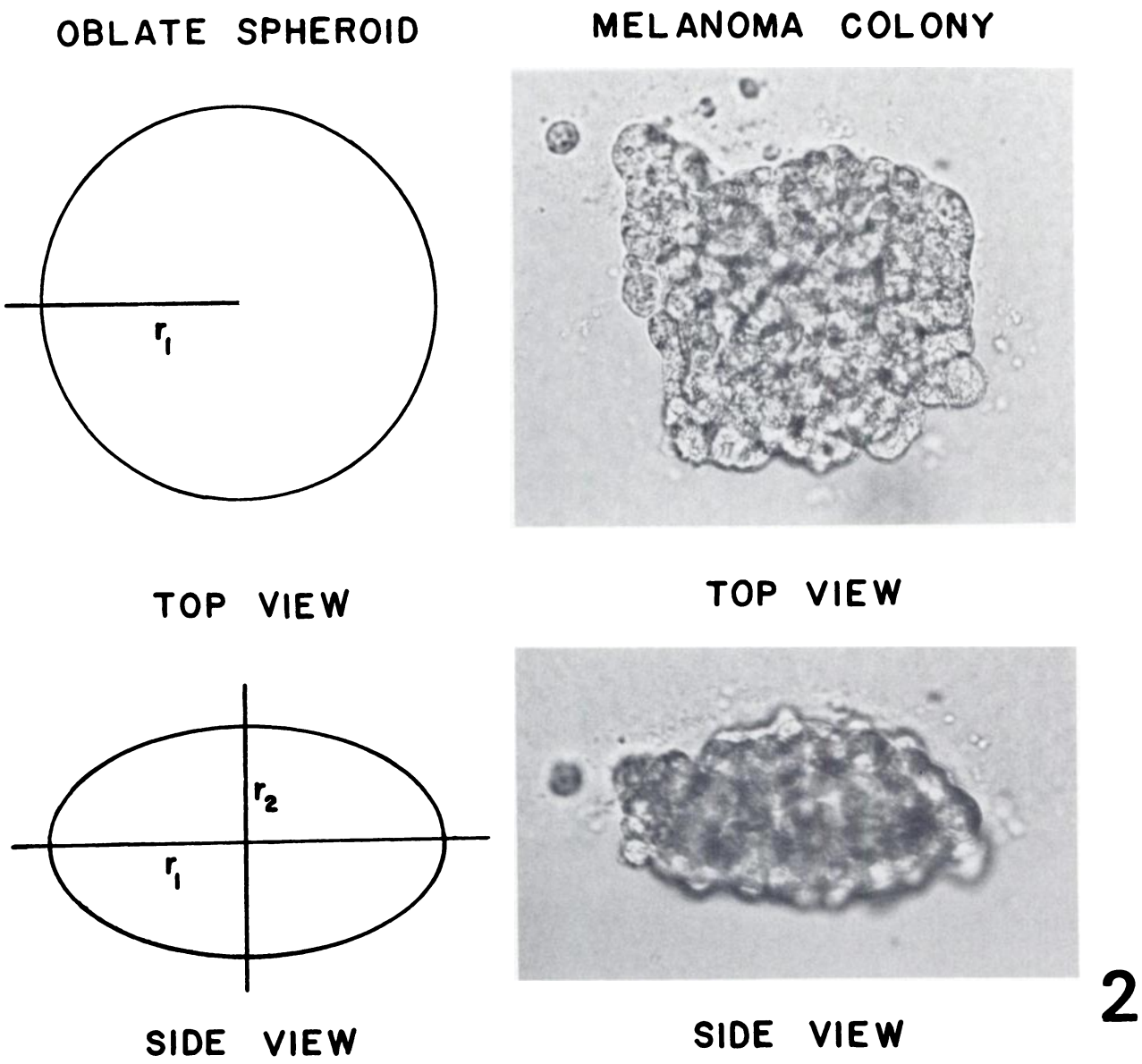

Fig. 2. Horizontal and vertical view of colony as an oblate spheroid. The horizontal (*TOP*) view of the 150-μm-diameter colony represents the conventional inverted microscopic view. The vertical (*SIDE*) view was obtained by cutting out small rectangular pieces of agar and tilting them 90° to measure the vertical diameter of colonies intact in agar; r_1, major axis; r_2, minor axis.

Quantitation of the Number of Cells within Tumor Colonies in Semisolid Medium and Their Growth as Oblate Spheroids

Frank L. Meyskens, Jr., Stephen P. Thomson and Thomas E. Moon

Cancer Res 1984;44:271-277.