On Carcinoma Growth and Vascular Supply: A Study of Mouse Mammary Tumor Strain MTG-B[1] (35875)

RANDY JIRTLE[2] AND KELLY H. CLIFTON[3]

Radiobiology Laboratories, Departments of Radiology and Pathology, University of Wisconsin Medical School, Madison, Wisconsin 53706

The relationship of the average tumor cell to its vascular supply may play a critical role both in the rate of tumor growth and its response to therapy. We have reported on the rate of decrease in the specific activity of the deoxyribonucleic acid of transplanted MTG-B mouse mammary carcinomas prelabeled with ^{125}I-5-iodo-2′-deoxyuridine as an index of the *potential* tumor cell population growth rate throughout the tumor growth period (1). These studies were limited to the grossly viable tissue of the tumors on the assumption that most, if not all, new cells arise in such regions. The potential growth rate was found to be constant in tumors containing 22 to 1200 mg or more of such "cleaned" grossly viable tissue, despite the fact that large areas of necrotic, hemorrhagic and cystic material were dissected from the cleaned samples of the larger tumors before analysis. It was postulated that the difference between the potential growth rate so estimated and the observed growth rate was the result of an accelerating rate of cell loss which reached more than 50% of the potential new cells born/day in large tumors.

The current data deal with a study of the vascular supply during growth of the same mouse tumor strain. They illustrate a marked constancy between functional vascular space and cleaned tissue weight over a wide range of viable tumor cell masses. These two groups of data support the postulate that an equilibrium between the viable proliferative areas and their vascular supply is established early in tumor growth and persists until near death of the host animal. Within those areas which remain grossly viable in large tumors, the functional vascular space appears adequate to support the same metabolic activity and proliferative rate as in smaller tumors.

Materials and Methods. Red blood cells labeled with ^{59}Fe were obtained from young adult C3BF1/Wr and BC3F1/Wr mice injected with 0.04–0.05 μCi of radioferrous citrate/g of body weight four times at 3-day intervals. Blood was collected from the axillary vessels. The cells were washed three times with isotonic saline and 0.1 ml of a 45% suspension of labeled cells was injected into the lateral tail veins of similar mice which bore MTG-B tumors grafted in their thighs (1). One hour later (2), reference blood samples were drawn from the tumor-bearing mice; tumors were removed, dissected free of grossly necrotic, cystic, and hemorrhagic tissues; and the viable "cleaned" portion was weighed. The levels of radioactivity in the blood and cleaned tumor tissue samples were determined with a sodium iodide crystal scintillation counter equipped with a pulse height analyzer. This method was chosen in preference to those involving dyes, macromolecules, or surface-labeled blood cells, in that the incorporated label is thought to be released only on destruction of the red cells (2), and in that the volume measured is restricted to that into which the labeled red cells enter during the 1-hr equilibration period (2, 3).

Results. The results of measurements of 190 grafted tumors show the functional vascular space per unit viable mass to be remarkably constant in MTG-B tumors containing 50 to 1900 mg of cleaned, presumably viable tissue (Fig. 1). Although the absolute value

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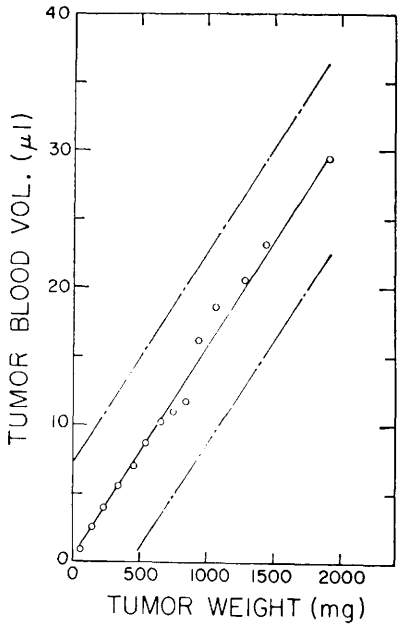

FIG. 1. Regression (—) of 190 measurements of vascular space on the weight of cleaned, presumably viable, MTG-B carcinoma tissue: (O) the means of 6–28 observations pooled for presentation; (— · —) 95% confidence limits for *individual* measurements. Slope of the regression is 1.53×10^{-2} μl blood volume/mg of tissue (95% confidence limits: 1.40 to 1.65×10^{-2}). Regression significant at $p<.01$; deviations from regression are insignificant, $p>.05$.

of 1.5×10^{-2} μl blood volume/mg of viable tissue is subject to error from any differences in hematocrit between tumor vessels and those from which the reference samples were drawn, it seems reasonable to expect this error to be quite constant. In experiments in progress, the blood volume per milligram of tumor has also been found to be constant in C57BL mouse gastric adenocarcinoma 328 over the weight range of 50 to 3500 mg of cleaned tissue.

Discussion. Insofar as the viable proliferative centers of the MTG-B carcinoma is concerned, these and our previously reported data (1) indicate that the relative functional vascular supply and the potential cell population growth rates are both quite constant from the time tumors are small until near death of the host mice when the tumors contain large areas of necrosis, cysts, and hemorrhage. In contrast, when measurements have been made on whole tumor masses without regard to their content of nonviable tissue a decrease in relative vascular space has been observed with increasing tumor size (4–6). These latter results are consistent with the findings of Tannock and Steel (3) that capillaries which appeared structurally intact within necrotic regions of tumors were, however, largely nonfunctional.

The current data bear on the mechanism of deceleration in tumor growth rate commonly observed with increasing tumor size (7). Laird (8) has suggested that a process akin to senility develops in large tumor cell populations; whereas Summers (5) has related the declining growth rate to a decreasing nutritional and/or oxygen supply to the average tumor cell. Frindel *et al.* (9) and Frindel and Tubiana (10) found that the mean cell cycle time of the NCTC tumor in the solid form did not change with tumor growth. They attributed deceleration of solid tumor growth both to a decrease in growth fraction and to cell loss. Tannock (11) in contrast, has described an experimental tumor in which the viable cells occurred in a cylindrical arrangement around capillaries. These cords of viable tissue were separated by areas of necrosis. Although the growth fraction decreased from the region close to the capillary within a cord to the region adjacent to the necrosis, the cell cycle times of the dividing cells were not significantly different in these two areas. The mean growth fractions and cell cycle times varied little from cord to cord. These findings extended the earlier report of Thomlinson and Gray (12) who described a similar morphologic relationship between capillaries and viable tumor tissue in human pulmonary tumors.

Growth of the viable cell population in tumors of the structural type described by Tannock (11) would appear to depend on linear growth of the tumor cell cords in equilibrium with concurrent growth of the capillaries. Our data suggests that similar equilibria may be more widespread, obtaining in tumors such as the MTG-B strain in which the morphological relationship of viable cells and capillaries is not as obvious. Given a constant rate of new cell formation,

widespread necrosis, degeneration, and hemorrhage would be expected to occur when the absolute number of new tumor cells formed per unit time exceeded the capacity for formation of new capillaries.

If these data and considerations are reflective of similar equilibria in some carcinomas in man, marked changes in the percentage of viable cells which are severely hypoxic would not be expected to develop with growth in such tumors, despite the accumulation of nonviable masses when they are large. Accordingly, insofar as the sensitivity of the viable tissue centers in which most, if not all, of the new tumor cells arise, is influenced by their relative vascular space, one would expect their response to radiation or other therapy to be similar per unit mass over a wide range of tumor sizes.

Summary. The vascular supply of the grossly viable portions of grafts of the MTG-B mouse mammary carcinoma was measured with ^{59}Fe-labeled red cells. The relative vascular space was constant, estimated as 1.5×10^{-2} μl/mg in tumors yielding 50 to 1900 mg of "cleaned" tissue from which necrotic, cystic, or hemorrhagic areas had been removed. We have previously reported that the potential growth rate in the grossly viable portions of MTG-B tumors is also constant from the time tumors are small until near death of the host animals. It is postulated that an equilibrium between the viable portions of such tumors and their vascular supply is established when tumors are small. This equilibrium persists well into the period when tumors are large; and necrosis, hemorrhage, and cysts are common. It is thus further suggested that such degenerative changes become marked when the absolute number of new tumor cells born exceeds the capacity for new vascular tissue formation. However, the functional vascular supply of the regions from which most, if not all, new cells arise in large tumors of this type is apparently adequate to support the same metabolic and proliferative rates as in small tumors, despite the occurrence of widespread degeneration in adjacent areas. Insofar as the response to therapy depends on the relative vascular supply and proliferative rate, the sensitivity per unit viable tissue would be expected to be similar in large and small tumors of this type.

1. Clifton, K. H., and Yatvin, M. B., Cancer Res. **30**, 658 (1970).

2. Friedman, J. J., Proc. Soc. Exp. Biol. Med. **103**, 80 (1960).

3. Tannock, I. F., and Steel, G. G., J. Nat. Cancer Inst. **42**, 772 (1969).

4. Gullino, P. M., and Grantham, F. H., Cancer Res. **24**, 1727 (1964).

5. Summers, W. C., Growth **31**, 333 (1966).

6. Rogers, W., Edlich, R. F., and Aust, J. B., Angiology **20**, 374 (1969).

7. Mendelsohn, M. L., Cell Tissue Kinet. **3**, 405 (1970).

8. Laird, A. K., Brit. J. Cancer **18**, 841 (1964).

9. Frindel, E., Malaise, E. P., Alpen, E., and Tubiana, M., Cancer Res. **27**, 1122 (1967).

10. Frindel, E., and Tubiana, M., C. R. Acad. Sci. Paris Ser.D **265**, 829 (1967).

11. Tannock, I. F., Brit. J. Cancer **22**, 258 (1968).

12. Thomlinson, R. H., and Gray, L. H., Brit. J. Cancer **14**, 39 (1955).

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