Quantitative Evaluation of Growth Rates in Tumors before and after Radiation*

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The author is engaged in developing a classification of tumors according to their rates of growth, metabolic activities, and radiosensitivities. The need and feasibility of such a classification arose from the observations made in previous studies (9, 10, 12), in which it was demonstrated that the above characteristics may differ significantly even among tumors of almost identical morphological structure.

The purpose of the research reported herein is a quantitative evaluation of growth activity in tumors on a biological basis. As will be shown, the usual external physical measurements, made with a caliper, are not accurate criteria for judging the growth rates of tumors, particularly in relation to mitotic activity. For this investigation two tumors which had been previously studied extensively by the author were used. These were the mammary tumors of the Bar Harbor strains of mice, dba and CSH, both diagnosed as adenocarcinomas. For the sake of brevity, these tumors will be referred to as the dbB and CSH tumors, respectively. A brief summary of the pertinent characteristics of each will follow.

The dbB mammary tumor has a latent period of about 5—6 days, i.e., it reaches a size of about 6 mm. in diameter 5—6 days after implantation of a tumor graft. Within the following 8 days, the tumor increases rapidly in size, reaching a diameter of about 20—80 mm., and kills the animal within 5 weeks following implantation. The CSH mammary adenocarcinoma has a latent period of 14—18 days upon implantation of a tumor graft. The tumor increases in size slowly and kills the animal within about 3 months.

The rates of growth of these tumors were originally determined in the usual manner, i.e., by external measurement of two or three dimensions with a caliper. The question arose whether the difference in the increase in size of the above-mentioned tumors can be accounted for by a quantitative difference in mitotic activity or by other factors.

To throw light on this problem, a procedure which evaluates the growth activity of tumors on a more quantitative biological basis than external measurements alone is required. Such a procedure is particularly important for the evaluation of tumor therapy. It was decided to test the applicability of the method devised by Chalkley to the quantitative study of mitotic activity in tumors (4). This method permits a quantitative evaluation of spatial distribution of morphologic tissue components in an extended volume of tissue. It has been applied in studying the quantitative relationships of various tissue components by a number of investigators (2, 3, 5, 7, 14). However, to the knowledge of the author, it has not been employed for studies of the nature herein reported.

PART I

THE DETERMINATION OF VOLUME RATIOS OF RESTING TO MITOTIC CELLS IN THE CSH AND dbB TUMORS

EXPERIMENTAL

Small fragments of young, actively growing dbB and CSH mammary tumors, fixed in Zenker's solution and prepared in the usual manner, were used. Sections of about 4 μ in thickness were cut and stained with hematoxylin and eosin, and Feulgen. The latter has been helpful in identifying cells in active division.

Originally, due to the complexity of tumor tissue, several factors were chosen as criteria, such as a number of "hits" on resting cells, on cells in active division, on disintegrated cells, on hemorrhagic areas, and on empty spaces; but it was soon realized that such a variety of factors made the analysis too complex. The most reproducible ratios obtained were those of mitotic to intact
resting tumor cells. It was decided to use these ratios as the criterion in the present study. The term "mitotic cell," as used in this paper, refers to those cells in division, from the earliest recognizable prophase to the separation of daughter cells. The term "resting cell" includes those cells which are intact and not in active division.

### TABLE 1

**Volume Ratios of Mitotic to Resting Cells of dbbTumors**

<table>
<thead>
<tr>
<th>Number of mouse</th>
<th>Age of tumor (days)</th>
<th>Mitotic index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5</td>
<td>1:48.8</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>1:58.8</td>
</tr>
<tr>
<td>III</td>
<td>10</td>
<td>1:50.0</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>1:58.8</td>
</tr>
<tr>
<td>V</td>
<td>15</td>
<td>1:46.7</td>
</tr>
</tbody>
</table>

Average of 5 tumors: 1:49.6

*Ratio of dividing to resting cells in an extended volume of tissue.

Microscopic fields, showing intact portions of the tumors, were chosen at random for analysis. The number of mitotic cells hit during the counting of 700 hits on resting tumor cells within these intact portions formed the basis for the ratios. The choice of 700 hits for individual analyses of tumors is based on Chalkley's original investigations, in which he demonstrated that stabilizing ratios could be obtained from 500 hits, taking nuclei and cytoplasm as objects (4). To be even more precise, counts based on 700 hits were taken as a criterion for this investigation. The ratio for each tumor section so analyzed was obtained by dividing the 700 hits on resting cells by the number of hits on mitotic figures. The data obtained from five actively growing dbb tumors are recorded in Table 1.

The reproducibility of the counts on one slide studied by several individuals and the narrow range of the results from five tumors of different ages justify the reporting of these observations.

### RESULTS

Analysis of the data in Table 1 reveals that the volume ratios of mitotic to resting tumor cells range from 1:43.8 to 1:53.8, the average being 1:49.6. The age of the tumor, i.e., the time elapsing from implantation of the tumor fragments to the removal of the tumor for cytological analysis, varied from 5 to 15 days. This period is the time during which the tumor increases in size most rapidly. The average increase in tumor size in five mice within this period was 25 x 15 x 10 mm. The relatively small variation of the volume ratios of mitotic figures to resting cells of these five tumors during their most active period of proliferation seems to indicate that mitotic activity alone does not entirely account for the rapid increase in dbb tumor size. The presence of a considerable number of cystic empty spaces in the tumor sections (Fig. 1) offers a possible explanation for this sudden increase in tumor size, which is not due to mitotic activity. This point will be considered further in the discussion.

Table 2 records the relative volume ratios of mitotic to resting cells of five transplanted mammary adenocarcinomas of the CSH strain of mice. These ratios average 1 mitotic cell to 72.5 resting cells, ranging from 1:60.6 to 1:87.5.

Since the CSH tumor is the more slowly growing one, these tumors are considerably older than the dbb tumors. The greatest increase in size occurs within 3 or 4 weeks following implantation, averaging about 9 x 7 x 6 mm. From the data noted in Table 2, it appears that this tumor (CSH) reaches a rather even growth activity within 3 weeks following the latent period, as seen from the ratios of mitotic to resting cells.

### TABLE 2

**Volume Ratios of Mitotic to Resting Cells of CSH Tumors**

<table>
<thead>
<tr>
<th>Number of mouse</th>
<th>Age of tumor (days)</th>
<th>Mitotic index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>1:87.5</td>
</tr>
<tr>
<td>II</td>
<td>14</td>
<td>1:60.6</td>
</tr>
<tr>
<td>III</td>
<td>41</td>
<td>1:77.7</td>
</tr>
<tr>
<td>IV</td>
<td>41</td>
<td>1:68.6</td>
</tr>
<tr>
<td>V</td>
<td>57</td>
<td>1:70.0</td>
</tr>
</tbody>
</table>

Average of 5 tumors: 1:72.5

*Ratio of dividing to resting cells in an extended volume of tissue.

### PART II

**The Determination of Volume Ratios of Resting to Mitotic Cells in dbbTumors Following Irradiation**

In studying the effects of irradiation, there is a great need for an index evaluating the efficacy of radiation on living tissues, particularly where therapy of malignant tumors is concerned. It has been shown that not all cells can be destroyed outright, unless tremendous radiation doses are applied. This information was obtained from experiments carried out in tissue culture in vitro (6, 8, 12, 13, 15) and in vivo (10). However, large doses cannot be employed in vivo in one exposure due to the damaging effect on normal tissues, primarily on the skin. Consequently, in radiation therapy the total dose required for tumor destruction has to be divided, i.e., fractionated into
smaller ones and applied within certain intervals of time.

In applying fractionated treatments, one faces the problem of permitting the uninjured tumor cells to continue to grow and the partially injured tumor cells to recover before a subsequent radiation dose is given. It is of importance, therefore, to ascertain the extent of damage done to the tissue and the extent of its recovery following each radiation dose, in order to adjust the over-all time within which the course of treatment should be carried out and thus achieve the desired results. A quantitative procedure for evaluating such a situation would be of great value. The satisfactory results obtained from the evaluation of growth activity of nonirradiated tumors by the use of Chalkley's method prompted the application of this method to the study of irradiated tumors. The dbrB tumor was selected for this experiment because of its more uniform rate of growth; for example, its latent period is usually about 5–6 days.

Actively growing dbrB tumors, grown in dba mice, were exposed, in situ, to various doses of x-radiation (the physical factors: 200 kv; 20 ma; 0.5 mm. Cu + 1.0 mm. Al filtration and HVL = 1.1 mm. Cu. The tumors were irradiated at a distance of 18.5 cm. from the x-ray source, and the average intensity was 602 roentgens/min). The irradiated tumors were excised within various intervals of time following irradiation, and portions from several areas were immediately fixed in Zenker's solution. With the routine procedure, paraffin sections 4 μ in thickness were stained with hematoxylin and eosin, and Feulgen. Only intact areas of the tumor section in the microscopic field were chosen for analysis. This was done for two reasons: First, it was deemed of greater significance to know the activity of the intact portions of tumor tissue remaining after irradiation than to know how much had been destroyed; such a criterion may serve as a guide in planning further treatments with additional doses of irradiation. Second, this was necessary in order to be able to compare the results with those obtained from the nonirradiated, control tumors for which the same criterion was used.

In the irradiated tumors, as in the controls, the number of mitotic cells hit during the counting of 700 hits on intact resting cells within the same microscopic fields determined the ratios. No attempt is made here to describe in detail the histologic changes occurring in the tumors following irradiation, because such changes have been described elsewhere (10).

In Table 3 are recorded the results obtained from five dbrB tumors irradiated with different doses of x-rays and removed for analysis following various intervals of time. Analysis of the data revealed the following: A ratio of 1 mitotic cell to 41.0 resting tumor cells was found in intact portions of an 11-day-old dbrB tumor 24 hours following exposure to 5,000 r. This ratio lies close to the range noted in the control nonirradiated dbrB tumors. Whether these cells escaped being hit by x-rays or recovered following slight injury, or whether they might have shown some effect later on, known as "delayed effect" produced by radiation, cannot be determined at the present time.

A ratio of 1 mitotic figure to 87.5 resting cells was found in a dbrB tumor 18 days following irradiation with 5,000 r. This ratio indicates a decrease in mitotic activity, as compared to the average ratio of normal control tumors, which was 1:49.6. A delayed effect of irradiation may be indicated in this case.

As expected, with the increase of the x-ray dose a decrease in mitotic activity occurred. A ratio of 1:100 was calculated from a tumor 26 days following exposure to 10,000 r, indicating a decrease in active proliferation of the intact portions of the tumor as compared to the average mitotic activity of control, nonirradiated tumors. A ratio of 1 mitotic to 87.5 resting intact tumor cells was found in a dbrB tumor 50 hours following exposure to 12,000 r, and a ratio of 1 mitotic to 350 resting tumor cells was calculated from a tumor 22 hours following exposure to 16,000 r.

As anticipated, there were variable amounts of disintegrated portions in the irradiated tumors, depending upon the dosage applied and the length of time following irradiation. However, this was not taken into consideration, because the main interest lies, not in the amount of tumor tissue destroyed, but in the proliferative potentialities of the tumor after irradiation.

The method was further applied to throw light on the problem of "indirect effects" of radiation.

<table>
<thead>
<tr>
<th>Number of Mouse</th>
<th>Dose (roentgens)</th>
<th>Time Index</th>
<th>Mitotic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5,000</td>
<td>24 hours</td>
<td>1:41.0</td>
</tr>
<tr>
<td>II</td>
<td>5,000</td>
<td>13 days</td>
<td>1:87.5</td>
</tr>
<tr>
<td>III</td>
<td>10,000</td>
<td>20 days</td>
<td>1:100</td>
</tr>
<tr>
<td>IV</td>
<td>15,000</td>
<td>50 hours</td>
<td>1:87.5</td>
</tr>
<tr>
<td>V</td>
<td>16,000</td>
<td>22 hours</td>
<td>1:350</td>
</tr>
</tbody>
</table>

*Ratio of dividing to resting cells in an extended volume of tissue.

1 "Indirect effect" as used here refers to the effect resulting from toxic substances produced by irradiation and does not
using mitotic activity as a criterion. The theory advanced by some investigators concerning the effects of radiation on living matter is that a tumor situated in a region remote from the one which is being irradiated might be affected indirectly. For example, Ahlstrom et al., working with phosphorus 32, showed changes in the nuclear DNA in a tumor opposite the one which was exposed to radiation (1). Such an "indirect effect" of radiation is explained by the presence of toxic substances circulating in the animal organism which were produced by irradiation.

The problem of "indirect effects" of radiation will not be discussed here. However, if such effects actually influence tumor growth, they should be reflected in the mitotic activity. Since detailed data bearing on these "indirect effects" will be presented elsewhere, only one example typical of these findings will be mentioned here.

A number of dba mice received grafts on both sides, between the groin and subaxillary regions, of dbB tumor particles of similar size. The tumors on both sides developed equally well in all mice bearing implants, and the tumors situated on the right sides of the mice were exposed to lethal doses of x-radiation, while the rest of the animal organism was protected by the device described in a previous report (10). The example referred to above is as follows: A tumor on the right side received a total dose of 30,000 r, applied in four exposures of 5,000 r each, with a 24-hour interval between each exposure. Six days following the last treatment the tumor had regressed, and only some scar tissue remained, while the untreated tumor on the left side continued to increase in size (Fig. 2). The latter was excised and fixed; sections 4 µ thick were stained for cytological study. A ratio of 1 mitotic figure to 41 resting tumor cells was found in these sections. This volume ratio of mitotic to resting cells falls close to the range of those ratios found in normal, untreated dbB tumors.

Numerous observations have been made in this laboratory of continued growth of untreated tumors, both adjacent to and remote from irradiated tumors which regressed following radiation. Examples of such observations were previously reported by the author (10, 11). The present finding, based on mitotic activity, is in accord with these observations. This suggests that the supposedly "indirect effect" of irradiation, presumably produced by toxic substances circulating in the organism was not, in this case, sufficient to destroy tumor cells or even to affect the rate of growth of a tumor autogenous to the strain of the mouse. The discrepancy between these observations and those reported by Ahlstrom et al. may be due to a different experimental procedure in irradiating the animals.

**SUMMARY AND CONCLUSIONS**

Attempts were made to evaluate the rates of growth of tumors before and after radiation on a quantitative biological basis. Chalkley's method, which permits a quantitative evaluation of spatial distribution of morphologic tissue components in an extended volume of tissue, was used for this study.

Two mammary tumors of the Bar Harbor strains of mice, dba and C3H, both diagnosed as adenocarcinomas and referred to in the text as the dbB and C3H tumors, respectively, were employed as test objects. Analysis of five dbB tumors yielded an average ratio of mitotic to resting cells of 1:49.6 (range: 1:45.8–1:53.8), while analysis of five mammary tumors of the C3H strain yielded an average ratio of mitotic to resting cells of 1:72.5 (range: 1:63.6–1:87.5). It is indicated that the relatively greater increase in size of the dbB mammary tumor, as compared to the C3H tumor, is due not only to the greater mitotic activity of this tumor but also to its inherent secretory tendency, which produces engorgement of the glandular lumina. Consequently, it is thought advisable to take such a biological characteristic into consideration in evaluating the increase in tumor size as measured externally.

Volume ratios of mitotic to intact resting cells of five dbB tumors, exposed to various doses of x-radiation and removed following various periods of time, are presented. Microscopic fields of stained sections of intact portions of the tumor, chosen at random, were used for analysis. A decrease in mitotic activity was noted which depended upon the dose of irradiation applied and the lapse of time between exposure and removal of the tumors. For example, 1 mitotic figure to 41.0 resting cells was found in the intact portions of a dbB tumor 24 hours following a dose of 5,000 r; a ratio of 1:87.5 was found in another dbB tumor which received the same dose of radiation but which was removed 13 days following exposure. The ratio of 1:41.0 is very close to the range of mitotic indices found in untreated dbB tumors, while the ratio of 1:87.5 indicates a decrease in mitotic activity as compared to the normal. A delayed effect is indicated.

The effectiveness of a large dose of radiation is illustrated by the ratio of 1 mitotic figure to 350...
resting cells found in a dbBR tumor which was removed and fixed 22 hours following irradiation with 16,000 r.

The results obtained indicate the possibility of applying Chalkley's method to the quantitative evaluation of growth rates of tumors, taking the mitotic index as a criterion. This method also proved helpful in evaluating the effectiveness of a given dose of radiation on a quantitative basis.

ACKNOWLEDGMENT

Sincere appreciation is herewith expressed to Dr. Harold W. Chalkley for his valuable advice and criticism of this paper.

REFERENCES

FIG. 1.—A section of dbrB tumor. Note the dilatation of the lumina of the acinic structures, some of which still contain mucoid material, and the empty spaces. X80.

FIG. 2.—A mouse which received grafts in both subaxillary regions of tumor particles. The tumor on the right side regressed following x-radiation. Note epilation, regrowth of gray hair and some scarring on this site. Note the continuous growth of the untreated tumor seated in the left subaxillary region.
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