Associations between Growth Rate, Mitotic Frequency, and Chromosome Number in a Plant Tissue Culture*

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SUMMARY

Twelve subcultures derived from one explant of normal, white spruce tissue, and 40 subcultures derived from one explant of tumorous, white spruce tissue, were studied over seven successive transfers. The tumor subcultures showed greater variation than the normal subcultures with respect to chromosome number, rate of cell division, and rate of growth.

In both tissues, by far the greater variance in growth rate was found between subcultures rather than within subcultures over successive transfers. The intra-class correlation was +.98 for the tumor subcultures and +.67 for the normal subcultures, both significant at the .001 level. These correlations indicate that the subculture variation in growth rate is mostly of a genetic nature.

Significant correlations were found among growth rate, rate of cell division, and chromosome number in both the tumor and normal subcultures. The most important finding is the association between chromosome number and growth rate over the entire aneuploid series. As a plausible hypothesis to explain these associations the authors suggest that there is a cause-and-effect relationship from chromosome number through rate of cell division to growth rate.

The physiological, nutritional, and metabolic aspects of a massive type of tumorous growth on *Picea glauca* have been studied for a number of years (3, 13, 15, 16). It has been shown, *inter alia*, that the tumorous tissues in culture are cytologically unstable (4), and because of the extremely wide variation found in the preliminary studies, further investigation of this phenomenon has been undertaken. Data presented in this paper give chromosome numbers of a series of subcultures of tumorous and normal tissues in relation to the rates of growth that they have exhibited.

*Picea glauca* is a particularly satisfactory material to work with because it is possible to isolate cultures from closely adjacent normal and tumorous tissues with almost no danger that either one will contaminate the other.

Studies of different types of tumors on various plant species have been extensively reviewed (1, 2, 5, 11). In some studies the chromosome complement of the tumor tissues has been noted (7, 8, 10), but systematic studies, especially with relationship to growth in culture, have been very few.

MATERIALS AND METHODS

Small blocks of tumorous and normal parts of *Picea glauca* (white spruce) were brought into the laboratory. The bark was cleaned with a sharp wire brush, and the block was washed with 95 per cent alcohol, which was then burned off. The washing and burning were repeated once. Then with a very sharp knife the corky bark was peeled off and discarded; the surface was washed again with 95 per cent alcohol, and flamed off. The stem was then laid on a sterile block, and the remaining bark carefully dissected away to within about 1 mm. of the cambium. From this sterile surface a series of strips were separated by deep longitudinal cuts 1.5 cm. apart. These strips (1.5 × 1.5 cm. by about 3 mm. in thickness) were used as explants. All the cultures used in the experiment...
to be described were derived from two such explants—one tumorous, the other normal. The first explants were placed on a nutrient substratum on June 3d, 1959. They were subcultured on the same medium, with extensive subdivision through four successive transfers (Charts 1 and 2), and the growth of the resulting cultures (40 from tumorous tissue and twelve from normal tissue) was estimated by weighing. The data presented here were obtained on a medium designated “F” (3) which was found to give the best growth with tumor tissue. Details of the nutrient experiments will be presented elsewhere.

Pyrex lipless test tubes, 25 X 150 mm., were prepared for the cultures in the following way: narrow strips of Whatman No. 1 filter paper were folded repeatedly to form strips about 5 cm. long and just a little wider than the diameter of the culture tube. When the filter paper had been placed in all the tubes, 15 cc. of the medium was pipetted into each tube. The tubes were autoclaved under 15 pounds’ pressure for 30 minutes. After being cooled the tubes were held obliquely, and one culture was placed on the filter paper in each tube about 1.5 cm. from the top. The tube was tightly covered by a piece of aluminum foil and flamed. This method of closing the tubes provided adequate sterility. The tubes were kept in an oblique position, so that the culture rested on moist filter paper but was not immersed in the fluid. The cultures were maintained at room temperature during the entire course of the experiment.

Measurements were started at the fourth successive transplant in October. Tissue masses weighing 250 mg. were used in inoculating all of the 40 tumor subcultures to be described, as well as the twelve normal subcultures. Each month thereafter each subculture was weighed, and, after being weighed, 250 mg. of tissue was cut off and transferred into fresh medium. The 250-mg. piece was wedge-shaped, cut like a piece of pie from the larger tissue mass. When it was necessary to correct to 250 ± 25 mg., additions or subtractions were made with smaller wedge-shaped cuts. The values given in Table 1 represent the monthly increases in weight over the initial 250 mg. in each of the seven successive transfers for each subculture. Since these cultures were continued for further studies, all the given data are wet weights. Because the growth increments were measured at monthly intervals, growth increment is here considered synonymous with growth rate.
Chromosomes were counted by the method previously reported (4). The finding of 22 chromosomes per cell was characteristic of 51 per cent of cells examined in vitro. In tumor tissues, chromosome numbers were often observed to exceed 70; above this number chromosome counts cannot be made with much accuracy. Counts exceeding 70 were therefore recorded as 70. Probably a larger number of those counts were 4n = 88. Thus, for larger mean chromosome numbers there was a bias in the low direction.

RESULTS

Table 1 shows the mean growth increments of the seven successive transfers for each of the 40 subcultures of tumor tissue and the twelve normal subcultures. They have been arranged in the table according to rank of mean growth. Considering that all the tumor material was derived from a single explant, the variation in growth rate between subcultures was remarkably large, but it was relatively low within subcultures. The growth ranks shown in Charts 1 and 2 indicate the extent of the variation of growth potential within the original explants. The subcultures in the right quadrant of Chart 1 tended to fall in the upper ranges of growth rank, whereas the other quadrants showed considerable variation within themselves. However, there tended to be striking simi-

TABLE 1

THE MEAN INCREMENT OF GROWTH (MG.) OVER SEVEN TRANSFERS FOR THE 40 TUMOR TISSUE SUBCULTURES AND TWELVE NORMAL TISSUE SUBCULTURES

<table>
<thead>
<tr>
<th></th>
<th>Tumor tissue</th>
<th>Normal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subculture</td>
<td>X  S_2</td>
<td>X  S_2</td>
</tr>
<tr>
<td>1</td>
<td>28.8±4.8</td>
<td>154.7±36.8</td>
</tr>
<tr>
<td>2</td>
<td>48.9±18.4</td>
<td>1447.6±26.0</td>
</tr>
<tr>
<td>3</td>
<td>419.3±35.3</td>
<td>1571.9±27.2</td>
</tr>
<tr>
<td>4</td>
<td>496.7±58.9</td>
<td>1584.0±17.6</td>
</tr>
<tr>
<td>5</td>
<td>574.9±50.9</td>
<td>1796.7±50.6</td>
</tr>
<tr>
<td>6</td>
<td>637.3±58.3</td>
<td>2008.1±10.2</td>
</tr>
<tr>
<td>7</td>
<td>834.7±41.6</td>
<td>2022.0±26.5</td>
</tr>
<tr>
<td>8</td>
<td>945.9±50.4</td>
<td>2035.1±47.5</td>
</tr>
<tr>
<td>9</td>
<td>1049.1±47.4</td>
<td>2069.9±50.8</td>
</tr>
<tr>
<td>10</td>
<td>1065.4±59.5</td>
<td>2458.0±26.5</td>
</tr>
<tr>
<td>11</td>
<td>1157.9±28.4</td>
<td>2521.7±55.8</td>
</tr>
<tr>
<td>12</td>
<td>1225.4±40.1</td>
<td>2604.9±37.7</td>
</tr>
<tr>
<td>13</td>
<td>1308.9±61.3</td>
<td>2783.6±54.2</td>
</tr>
<tr>
<td>14</td>
<td>1388.1±67.7</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 2

VARIANCE OF GROWTH INCREMENTS (MG.) OF THE SUBCULTURES

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Tumorous tissue</th>
<th>Normal tissue</th>
<th>Variance ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within subculture variance S_1^2</td>
<td>188,388</td>
<td>1,601</td>
<td>F_1^2 = 86.4 (P &lt; .001)</td>
</tr>
<tr>
<td>Between subculture variance S_2^2</td>
<td>13,219,069</td>
<td>24,650</td>
<td></td>
</tr>
<tr>
<td>Between subculture component of variance S_3^2 = S_2^2 - S_1^2</td>
<td>1,867,699</td>
<td>3,392</td>
<td>F_3^2 = 567.3 (P &lt; .001)</td>
</tr>
</tbody>
</table>

N = No. of subcultures = 7.
larities of rank within the small families at the periphery of the chart. In the normal subcultures, the growth rank relationships depend entirely on the distance of relationship—the closer the growth similarity, the closer the genealogical relationship. Charts 3a and 4a show the mean increments of the seven transfers for each subculture. The values for the tumor series did not appear to cluster about any single segment of the total range; there was thus no apparent mode. The values did appear to fall into groups with more or less similar growth rate (lettered A, B, C, D, E, and F in Chart 3a), but, in the absence of informa-

d through the points which represent the total average increments for the 40 subcultures over the seven successive transfers. The curvilinearity of this regression is significant at the .01 level, indicating that, with continued transfers, the increments would reach a plateau. After the 7th transfer in May, 1960, the subcultures were transferred monthly without cutting them back and without measurement until October, 1960, when they were again cut back to 250 mg. and transferred. Transfer numbers 14 and 15 constitute the two successive monthly transfers at that later date. At that time, the subcultures seem to have reached

![Chart 3](image)

**Chart 3.**—Characteristics of the tumor subcultures. Mean growth increment (growth rate) over seven transfers ($Z_T$), mean number of dividing cells per slide ($X_T$), and mean number of chromosomes per counted cell ($Y_T$).

In Chart 5 are also drawn the average increments for each of the six arbitrary groupings described above. With the exception of Group A, all groups seemed to show a similar regression of growth on transfer; it did not seem to matter how high the growth increment was in the first transfer—the subcultures followed a similar course of growth increase and tended toward a plateau. This is surprising, for it would seem more likely _a priori_ that the subcultures which started off with a large increment in the first transfer would not show proportionately as much increase in the growth rate as would those which
started with a very low initial value. The two most slowly growing subcultures (Group A) did not seem to follow the same pattern as the other groups.

Chart 6 shows the corresponding values for the normal subcultures during successive transfers. The normal subcultures did not behave during transfers as uniformly as did the tumor cultures (Table 2), although there was a general tendency to increase to the third or fourth transfer and then to decline. The growth increments for transfers means that by far the greater part of the total variance was attributable to differences between the subcultures—i.e., the variation of growth within subcultures during successive transfers was very small compared with the variance between subcultures. The variation within subcultures was due largely to the increase in increment value in successive transfers. If this increase were taken out by fitting each value to the regression equation computed for the entire series of 40, the correlation would be even higher.

![Graph](chart.png)

Chart 4.—Characteristics of normal subcultures. Mean growth increment (growth rate) over seven transfers (Zₙ), mean number of dividing cells per slide (Xₙ), and mean number of chromosomes per counted cell (Yₙ).

14 and 15 attained a value in the middle of the range, with relatively low standard errors.

The subcultures showed a marked individualism with respect to their range of growth values. Each subculture grew during successive transfers at a rate limited within rather strict confines. Table 2 gives the variances observed both between and within the subcultures for the seven transfers. The intraclass correlation computed by the formula:

\[ \frac{S^2_G}{S^2_P + S^2_B} \]

(see Table 2) was +.93 for the tumor series, significant at the .001 level. This high correlation means that by far the greater part of the total variance was attributable to differences between the subcultures—i.e., the variation of growth within subcultures during successive transfers was very small compared with the variance between subcultures. The variation within subcultures was due largely to the increase in increment value in successive transfers. If this increase were taken out by fitting each value to the regression equation computed for the entire series of 40, the correlation would be even higher.

Normal tissue also grew on continued transfer at rates limited within rather strict confines. The intraclass correlation for the normals was +.67, significant at the .001 level. The variance, both within and between subcultures, was much lower in the normals than in the tumorous tissues. The variation within subcultures in the normal was lowered mainly because the subcultures did not show the continued rise in increments during the seven transfers. By comparing the ratio of the variances for the tumorous and normal series, it can be seen that the reduction in the component of variance between series was also lower in the normals. The ratio of these variances of the tumorous and normal tissues indicates that the
greater reduction was in the component of variance between series. The great reduction in this component was also reflected in the lowered intraclass correlation for the normals.

Charts 3b and 4b show the association between the increment in weight and number of dividing cells per slide. Charts 3c and 4c show the association between these same values and the average numbers of chromosomes per dividing cell.

In determining the number of dividing cells and the chromosome counts, the 40 subcultures were first coded. From the coded material, similar masses of tissue were excised to make slide preparations by the method of de Torok and White (4). In each case the entire slide was searched for dividing cells. The correlations between the three variables for the tumor series are given in Table 3.

A significant association was found between mean growth and the mean number of chromosomes counted in the dividing cells. The same associations held for the normal tissues (Table 3).

Here the association between mean growth and chromosome number was larger. The lower correlation in the tumorous tissue may have been partially a result of the ceiling on chromosome number imposed by the inability to determine the exact number over 70. This ceiling was never approached in the normal tissues, and thus no bias existed in those chromosome counts.

Linear regression lines, as shown on Charts 3b, 3c, 4b, and 4c, were computed so that mean growth increment could be estimated by mean

### Table 3

<table>
<thead>
<tr>
<th>Correlations Between the Variables:</th>
<th>Tumor tissue</th>
<th>Normal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of dividing cells per slide (X)</td>
<td>r_{X,Y} = +.814*</td>
<td>r_{X,Y} = +.784*</td>
</tr>
<tr>
<td>Mean chromosome number per dividing cell (Y)</td>
<td>r_{X,Y} = +.411*</td>
<td>r_{X,Y} = +.584†</td>
</tr>
<tr>
<td>Mean growth during seven transfers (Z)</td>
<td>r_{X,Z} = +.420*</td>
<td>r_{X,Z} = +.856*</td>
</tr>
</tbody>
</table>

*P < .01.
†.05 < P < .10.
number of dividing cells per slide or mean number of chromosomes per counted cell. Only in Chart 5e might a curvilinear regression line better fit the data. However, because of the downward bias in the higher chromosome number counts, the points may in reality describe a linear relationship. Accurate chromosome counts above 70 would be necessary to determine whether a curvilinear or linear regression would better apply.

**DISCUSSION**

In studies on animal tissues Parker (9) found that cultures of fibroblasts derived from different organs differed in functional properties, such as growth rate, but that each such culture behaved consistently through repeated transfers. Puck and Fisher (12) found consistent differences in the nutritional requirements of cells in the original uncloned HeLa tissue culture stock. Strong (14) found variation in growth capacity between individual isolates from the same tumours which could not be explained alone by genetic differences of the host animals. In the present study the normal and tumorous subcultures were found to differ between themselves in growth rate, but each subculture so established was relatively consistent through repeated passages. In both normal and tumour tissues there is a wide range of growth potentials in different subcultures from a single original explant. These individual differences were present at the time of the first transfer and were maintained essentially unchanged through subsequent passages; that is, subcultures which grew slowly at the initial transfer grew slowly in the subsequent transfers, whereas those which grew more rapidly at the outset continued to grow rapidly in subsequent transfers. The large differences between subcultures and the low variability within subcultures, upon successive transfers, indicate that the subcultures differ in their genetic control of the characteristics studied.

A significant fact emerges here: when the mean growth rates for the subcultures over the seven transfers were ranked, it was noted that families of similar growth ranks existed (Charts 1 and 2); the closer the relationship between the subcultures, the greater was their similarity of rank of growth rate. In the tumour tissues this association is pronounced but not perfect. This would indicate that the original explants from which the entire series was derived—namely, one block of normal tissue and one block of tumour tissue—were heterogeneous from the outset, being made up of cells of widely varying growth potential. The fact that the genealogical relationship of subcultures in the tumor series does not always result in similarities of growth rate rank indicates that growth potential continues to be heterogeneous through subsequent transfers. The normal tissues seem to have been able to evolve differences in growth rate in vitro, but the extent of the variation was greatly restricted compared to the wide differences between subcultures of the tumorous tissue. Another explanation might be that the original tumor tissue explant contained cells of a far greater heterogeneity of growth potential than did the original normal tissue explant.

The growth rate of the tumorous tissues steadily increased with successive transfers; a plateau was approached but was not reached in the first seven transfers. One would expect the faster growing subcultures to reach a maximum of growth rate more quickly than the more slowly growing ones. On the contrary, with the exception of the two most slowly growing subcultures, all seemed to increase in growth rate at the same rate during the successive transfers. The authors have no explanation for this phenomenon. An increase and plateauing of growth rate during the first successive transfers of a tumor was also found by Strong (14) in his studies on the transplantable anaplastic carcinoma of the mouse. In the present study, whatever the cause of the increase in the tumor tissues, a different situation existed in the normal subcultures, where growth rate reached a maximum at the fourth transfer and then declined to the seventh transfer.

The question arises, are there characteristics correlated with growth rate which might shed light on the factor or factors through which a genetic variation in growth rate is mediated? The second significant observation in this study is the high positive correlation between growth (rate of weight increase and rate of cell division) and chromosome number. This association appears in both normal and tumor tissues. The tumor tissues are not consistently polyploid, but show a continuous aneuploid series (4). The observed subculture growth potentials are correlated with the chromosome numbers over the entire range of the aneuploid series. The high correlation between growth rate and rate of cell division would indicate that growth rate here is largely a function of the rate of division of cells and not of cell size, although it is possible that cell size plays a minor role. It is difficult to obtain a reliable measurement of the amount of protoplasm in plant cells in which the vacuole sizes vary so much. Although it would seem plausible from these results that increased growth rate is a result of increased chromosome number, it is also possible that the variability of both characteristics is largely the result of
qualitative differences in the genetic material influencing both traits.

In studies in vivo of the hyperdiploid Ehrlich ascites tumor ELD and its polyploid derivative ELT, Hauschka et al. (6) found that both tissues multiplied at the same rate but that the ELT showed a greater accumulation of mass initially, because of its greater cell size. Their study was performed in vivo under conditions which caused progressive nutritional limitation and therefore does not conflict with the findings of the present study.

Earlier workers have for the most part compared chromosome numbers and growth rate between various types of tumors and not between subcultures of the same type. The existence of a high correlation between chromosome number and growth rate could be established here, because the entire material was derived from a single explant and subsequently divided into several subcultures. This material has an additional advantage for such studies in that it forms aneuploids over the entire range up to tetraploid levels and possibly beyond (4).

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