The Determination of Maximum Tumor Growth Rates in the Rat*

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The aspects of the tumor-host relationship concerned with the determination of the maximum rate of tumor growth supportable by the host animal (6, 9) and elucidation of the factors regulating this maximum growth rate have received little attention.

Barrett and Hansen (4) have recently confirmed the early observations of Bittner (5) and later others (9), who reported that, in mice bearing multiple, simultaneously transplanted tumors, each tumor grew independently of the others in the same host. It seemed conceivable to us that such an independent relationship might not be maintained if the animal were bearing multiple rapidly growing tumors, in view of the excessive metabolic demands that would be placed on the host. Thus, it was postulated that a maximum supportable rate of aggregate tumor growth would be attained in a single host; this rate might be independent of the number of tumors simultaneously growing on a single host animal.

The tumor-host system comprised by the rapidly growing Walker 256 carcinoma (8) in the Sprague-Dawley rat is well suited for such studies. For comparison, duplicate studies were made with the relatively slow-growing Rutgers I sarcoma (2, 3, 11). The term system, as used in this paper, refers to the integrated biological entity composed of both the host and the tumor tissue.

These studies were initiated to demonstrate, measure, and compare the maximum aggregate rates of tumor growth supportable by the rat which is host to either type of tumor in the form of single or multiple transplants. It was also desirable to ascertain whether the individual transplants of one given tumor in an animal with multiple transplants grow independently of one another, or whether several similar tumors in one host mutually stimulate or inhibit one another. Lastly, it was of interest to determine, in an animal containing both rapidly growing and slowly growing dissimilar tumors, whether each individual tumor species maintains its growth characteristics, or whether there is a measurable influence of one upon the other.

MATERIALS AND METHODS

To avoid the difficulty of evaluating the effects of a growing tumor transplant upon the normal growth of the immature host, 100 adult female rats (300–380 gm.) of the Sprague-Dawley strain were used throughout this study. Tumors were transplanted into 90 rats as outlined below, and ten rats served as nontumor controls. In such a stable population the average weight change of the ten control rats was insignificant over a 3-week period. All animals were fed Purina Laboratory Chow ad libitum.

Transplantation technic.—Firm, white, non-necrotic tumor tissue was dissected from the cortex of the donor tumor immediately after the host had been exsanguinated under light ether anesthesia. A suspension was then prepared by straining this cortical tumor tissue through sterile 30 mesh wire screening and mixing with equal parts (by weight) of saline containing 2,000 units of penicillin/ml. A single donor tumor suspension was used for all transplants of that particular type of tumor. All tumors were transplanted on the same day. Aliquots (0.2 ml.) of the tumor suspension were injected subcutaneously according to the following scheme: for single Walker transplants, the right flank of the animals was used; for the double Walker transplants, both right and left flanks were
used; for quadruple Walker transplants, both right and left lateral thoracic regions and right and left flanks were used. The same positional relationships were maintained for the single, double, and quadruple transplants of the Rutgers tumor. Previous studies in this laboratory had indicated that these tumors grew equally well in any of the four positions mentioned above.

In the experiments with the Walker tumor, ten recipient rats were used for each of the three methods of transplantation. This same design was repeated with the Rutgers tumor, with the exception that the number of rats given the single transplant of Rutgers tumor was increased to twenty to allow for the occasional regressions that occur with this tumor. In addition, twenty rats were given transplants of both of the dissimilar tumors, i.e., the Walker given at the right flank and the Rutgers at the left flank of the same animal (Table 1).

Tumor measurements were made by calipers beginning on the 7th day after transplantation and continued until the 17th day, at which time the experiment was terminated. The tumor weight in grams was calculated by multiplying the product of the three diameters of the tumor by the factor 0.54, a relationship of volume and tumor specific gravity established by Schrek (13) and confirmed in this laboratory, where estimation techniques have been repeatedly calibrated against actual tumor weights.

The maximum tumor growth rates were obtained by determining the greatest slope of the tumor growth curve covering a minimum of three successive measurement periods. The rate of growth during this period was then considered to be the maximum rate which the host could support for that particular tumor.

RESULTS

The 90 rats received transplants in a total of 210 sites with 100 percent successful takes. Of these, all grew well except one, a single transplant of Rutgers tumor which regressed after about 11 days and was omitted from the group.

Although the Walker tumor will readily metastasize in its later stages of growth, no evidence of gross metastases was noted at the time of termination of the experiment; neither were there any signs of metastases in the animals bearing the Rutgers tumors. No effort was made to pair-feed the animals or to limit the availability of food, inasmuch as it was desired to permit unrestricted feeding in this initial study.

The growth of the Walker and Rutgers tumors, expressed in terms of aggregate tumor mass/host in single and multiple simultaneous transplant systems is indicated in Chart 1. The Walker tumor grew more rapidly than the Rutgers tumor in both the single or multiple tumor transplant experiments. Analysis of the growth curves revealed that the average "maximum tumor growth rate" for the single Walker tumor, as measured by the greatest slope of this growth curve, was about 10.0 gm of tumor/day. Doubling the number of Walker tumors growing on one host resulted in an over-all increase in the amount of total tumor present at any given time, but the maximum aggregate rate of growth of this larger tumor mass still remained at 9.9 gm/day. Likewise, rats bearing four Walker tumors also supported a larger total mass, but here again the maximum rate of growth did not exceed 10.0 gm/day. Thus, it appears that the maximum Walker tumor growth rate which the host could support was about 10 gm/day. In contrast, the more slowly growing Rutgers tumor showed a lower maximum aggregate growth rate which was not attained unless two or more tumors were growing simultaneously in the same rat. These maximum tumor growth rates, and the statistical evidence for probability (P values) of significant differences between them, are summarized in Table 1.

It should be pointed out that, because of the central necrosis seen in either of these two types of tumors, not all the total tumor mass present in an animal at a given time is viable, proliferating tumor tissue. If the individual tumors of a rat bearing double or quadruple transplants were to grow independently, the total tumor mass of the animal would be theoretically 2-4 times that of the singly transplanted animal. However, this was not the case.

TABLE 1

<table>
<thead>
<tr>
<th>Maximum total tumor growth rate* (gm/day)</th>
<th>Tumor Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Walker W</td>
<td>10.0</td>
</tr>
<tr>
<td>Double Walker WW</td>
<td>9.9</td>
</tr>
<tr>
<td>Quadruple Walker WWWW</td>
<td>10.3</td>
</tr>
<tr>
<td>Single Rutgers R</td>
<td>4.0</td>
</tr>
<tr>
<td>Double Rutgers RR</td>
<td>8.2</td>
</tr>
<tr>
<td>Quadruple Rutgers RRRR</td>
<td>8.6</td>
</tr>
<tr>
<td>Single Walker and single Rutgers WR/W</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>W 6.6</td>
</tr>
<tr>
<td></td>
<td>R 2.4</td>
</tr>
</tbody>
</table>

According to probability (P) values based on "t" test:

No difference between groups: W-WW (P > .90); W-WWWW (P > .90); WW-WWWW (P > .90); RR-RRRR (P > .90). Highly significant difference between groups: RR-RR (P < .005); RRBBRR (P < .001).

* Determined from the region of maximum slope of the tumor growth curves (see Charts 1 and 4).
with either the Walker or the Rutgers tumors. The degree of departure from this theoretical behavior is depicted in Chart 1, in which the theoretical growth curves for multiple tumor systems were constructed by multiplying the estimated weights of the single tumors by factors of two and four. It was interesting to note that only the double transplant of Rutgers tumor approached this theoretical behavior closely. This is in accord with the observation above that the maximum growth rate for the Rutgers tumor had not been attained by the single tumor.

These effects of multiple simultaneous transplants upon the growth rates of the individual tumors in the same host are depicted in Chart 2. It is apparent that the presence of more than one Walker tumor transplant growing on a host affects the individual tumor sizes. Up until about 11 days there might be an enhancement of growth of the individual tumors of the multiple transplant system, compared with the growth of the single Walker tumor. However, after 11 days, this relationship reversed itself, and the growth rates of the individual tumors of both multiple systems gradually diminished, thus accounting for the failure to attain the theoretical aggregate growth for the multiple transplant systems. With the Rutgers tumor, no repression of individual tumor growth was noted unless more than two tumors were simultaneously growing on the host.

Investigation of the general condition of the host animals revealed significant findings. By subtracting the calculated tumor weight from the known combined weight of the tumor and host, one can approximate rather closely the true or net carcass weight of the host alone. The degree of weight depletion of the host tissue in the later stages was found to be proportional roughly to the total aggregate mass of tumor tissue present (Chart 3). During this same 17-day period, ten nontumor-bearing control rats showed no significant body weight changes. The depletion of the

**Chart 1.**—Comparative aggregate growth curves of single and multiple transplanted tumors and the relationships between the actual and theoretical aggregate tumor growth of multiple transplants based on the growth of the single tumor transplant. \(W\) = single Walker transplant; \(WWW\) = total tumor of double Walker transplant; \(WWWW\) = total tumor of quadruple Walker transplant; \(R, RR, RRRR\) designate similar curves for the Rutgers tumor. The theoretical \(WW\) curve was constructed by multiplying the actual curve for the single Walker tumor \((W)\) by 2. The theoretical \(WWWW\) curve was constructed by multiplying the actual curve of the single \(W\) by 4. The same method was used to obtain the theoretical double and quadruple Rutgers \((R)\) tumor growth curves.
Chart 2.—Effects of multiple transplants upon the average growth of the individual component tumors of multiple transplant systems. $W =$ single Walker transplant; $WW =$ average growth of the individual tumors of a double Walker; transplant $WWWW =$ average response of the individual tumors of a quadruple Walker transplant. $R$, $RR$, $RRRR$ represent similar curves for the respective individual component Rutgers tumors.

Chart 3.—The relative effects of single and multiple transplants of Walker tumor, and of single and multiple transplants of Rutgers tumor upon the rate of carcass weight depletion. The initial weights of all animals were between 300–380 gm. $W$, $WW$, $WWWW$ represent curves of progressive weight changes of animals bearing the single, double, and quadruple Walker tumors. The corresponding designations are used for the animals bearing the Rutgers tumors. The carcass weights were obtained by subtracting the calculated weight of the tumor from the total tumor-host weight.
host occurs earlier and to a more severe degree with the Walker tumors than with the Rutgers tumors. The two most severely depleted rats bearing quadruple Walker tumor transplants died between the 14th and 17th days.

In view of the report of Graff et al. (9), it became of interest to determine whether the presence of dissimilar tumors, when growing simultaneously in the same host, produced any changes in the growth characteristics of each specific type of tumor. The results of such a study are shown in Chart 4. The average total tumor weights of animals bearing a Rutgers tumor in one flank and a Walker tumor in the contralateral flank may be compared with tumor weights of animals bearing only bilateral Rutgers tumors or bilateral Walker tumors. It was not surprising to note that the growth of aggregate Walker-Rutgers transplant system was intermediate between that for the bilateral Walker tumors and bilateral Rutgers tumors. The individual tumors of this Walker-Rutgers system maintained their own characteristic growth rates until about 11 and 14 days for the Rutgers and Walker tumor, respectively (Chart 5). Thereafter, compared with the typical growth of the individual tumors of the two bilateral systems, the Rutgers component grew more slowly than if the contralateral tumor had been another Rutgers tumor, and the Walker tumor grew more rapidly than if the contralateral tumor had been another Walker tumor. This may be a competitive dissimilar tumor system wherein the Walker tumor dominates the tumor-host metabolic relationship to the extent that it is more successful than the Rutgers tumor in trapping the growth factors derived from the host tissues. The Rutgers tumor apparently did compete to an appreciable extent, since the single Walker tumor in this mixed Walker-Rutgers system grew more slowly than if no Rutgers tumor had been present on the same host (Table 1).

**DISCUSSION**

By using the technic of simultaneously transplanting multiple tumors in the same host animal, it has been possible to demonstrate and measure the maximum aggregate tumor growth rate which the host can support. Knowledge of the maximum tumor growth rate for a particular tumor in a defined tumor-host system has aided in the characterization of these tumors and should further the understanding of the usual tumor-host relationship.

Although some of the determinants of growth rate are inherent within the genetic architecture of the cells comprising the tumor, the ability of the host to supply the demands of the total tumor mass may be distinctly limited. Consequently, the
factor which limits the maximum growth rate of
the tumor then may be the availability of host-
derived growth essentials to each tumor cell.

The fact that the maximum aggregate tumor
growth rates were different for the two tumors
studied may indicate either that different de-
mands are made upon the host by different tu-
mors (10) or that one tumor is more efficient than
the other in obtaining and utilizing growth factors
or metabolic substrate from the host. Further
work to elucidate the factors regulating the maxi-
mum supportable growth rate is indicated.

With respect to the growth curves of the single,
double, and quadruple transplants of Rutgers tu-
mor, it is apparent that, under these experimental
conditions, the full capacity for growth of the
single transplant of tumor in that host is attained.
Likewise, when two Rutgers tumors are growing in
the same host, each tumor grows independently
and at the same rate as a single transplant of tu-
mor. This would be expected, since, under the con-
ditions of the experiment as described, the maxi-
mum capacity of the host to support growth is
completely challenged by two Rutgers tumors. It
is only when more than two Rutgers tumors are
growing on the same host that one observes that
the limited potential of the host to support tumor
growth has been reached. A maximum rate of
growth is thus imposed upon the total tumor mass
by limiting host factors.

The growth characteristics of the Walker tu-
mor demonstrate this phenomenon even more
dramatically. With this tumor the maximum rate of
growth which the host can support is soon at-
tained by the single tumor, and this rate remains
constant despite doubling or quadrupling the
number of tumors.

Remaining unexplained for the present is the
suggestion (Chart 2) that, in the early stages of
growth of the multiple transplants of Walker tu-
mors, the presence of more than one tumor per
host may stimulate the early growth of each tu-
mor beyond what one would expect for a single
Walker tumor. This may be related to a shortening
of the induction period. Such factors as, for ex-
ample, a more rapid institution of a highly vascu-
lar bed in the subcutaneous tissues (1) or other sys-
temic responses to the presence of the foreign tissue
(7, 12) may account for a shortened induction
period.

Evidence to support the view that different transplanta-
tion animal tumors vary in their abili-
ties to parasitize their host is obtained from the
data on animals simultaneously bearing the two
dissimilar rat tumors. During the early stages of
tumor growth, each tumor was growing at its own
characteristic rate, and making only minimal de-
mands upon the host (as manifested by host
weight changes). However, the Walker tumor rap-
idly grew to a large size and soon dominated the
entire tumor-host system. The net effect was an
inhibition of the Rutgers tumor, which was unable
to realize its characteristic potential for growth.
The fact that the early growth of each tumor was
not influenced by the presence of the other favors
the view that the domination of the system by the
Walker tumor noted in the later stages was due to
dissimilar tumor-host relationships for each type
of tumor, rather than just to relative differences in
the masses of the two tumors.

SUMMARY

1. The maximum aggregate tumor growth rates
for both the Walker 256 carcinoma and the Rut-
gers I sarcoma have been demonstrated and meas-
ured in the Sprague-Dawley rat.

2. Individual tumors of a multiple transplant
tumor-host system do not grow in an independent
manner after the maximum supportable growth
rate of the total tumor mass has been attained.

3. An apparent early stimulation of the growth of
individual tumors of the multi-transplanted
Walker system has been observed.

4. In rats simultaneously bearing these two dis-
similar rat tumors, the individual tumor species
maintained their growth characteristics during the
early stages. In the later stages the Rutgers tumor
was apparently unable to compete equally in terms
of growth rates with the contralateral Walker
tumor.

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