The Therapeutic Response of Three Human Tumor Lines Maintained in Immune-suppressed Mice

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SUMMARY

Studies were made of the growth and therapeutic response of three lines of human tumor serially transplanted in immune-suppressed mice. They included a well-differentiated colonic carcinoma (HX 13), a poorly differentiated colonic carcinoma (HX 18), and undifferentiated small-cell carcinoma of the bronchus (HX 29). Their histological appearance and growth rates were stable, with volume-doubling times ranging from 6 to 12 days. Studies by the technique of labeled mitoses showed that the growth kinetics of the three tumor lines were very similar, with median intermitotic times in the range of 26 to 35 hr. An analysis of the incidence of single and double takes revealed evidence for variation in susceptibility among the recipient mice. One tumor (HX 18) was transplantable with single-cell suspensions but $10^6$ cells were required for 50% takes. The response of the tumors to a range of chemotherapeutic agents was studied. There was evidence that drugs that are known to be effective in the treatment of patients did well, in particular 5-fluorouracil against the colonic tumors and cyclophosphamide against the bronchial carcinoma. Long-term regressions induced by cyclophosphamide in the bronchial carcinoma may reflect assistance from host defense mechanisms.

INTRODUCTION

At present there is considerable interest in the properties of human tumors transplanted into immune-deficient mice. The achievement of growth in such xenografts has now been reported from a number of laboratories (2, 4, 6-8, 11, 16), and information on their response to treatment has also appeared (1, 3, 9, 20). At this stage little is known about the biological characteristics of the xenografts, and the extent to which their therapeutic response reflects that of the tumor within the original patients or indeed of any human tumors is not known.

The work reported here derived from an earlier study of the growth kinetics of xenografted human tumors (17). This study demonstrated that the growth rate and cell population kinetics of xenografts of 8 colorectal tumors probably differed in some respects from the growth kinetics of the tumors in the original patients. Nevertheless, in view of the greater disparity in kinetic state between human tumors and most experimental murine tumors, it was felt that the xenografts deserved further investigation. We have therefore examined the response of 3 xenografted lines of human tumors to a range of chemotherapeutic agents, with the initial objective of comparing their response with the spectrum of responses that might be expected in the treatment of the corresponding disease categories in man.

MATERIALS AND METHODS

Immune-deprived Mice

The technique of immune suppression was identical with that used in our earlier study (17). Male and female CBA/Lac mice of the Institute of Cancer Research colony were thymectomized at 3 to 4 weeks of age, and 2 to 4 weeks later they were given 920 rads whole-body irradiation at a dose rate of 65 rads/min from a $^{60}$Co source. Within 6 hr they were given an i.v. injection of $5 \times 10^6$ bone marrow cells taken from thymectomized donors. They were maintained on Spratt’s No. 1 diet and tap water ad libitum and were housed in rooms that although separate from the rest of the animal laboratories, were not sterile.

Tumor Transplantation and Measurement

Between 2 and 4 weeks after the irradiation and bone marrow replacement, bilateral implants of tumor fragments were made into the s.c. tissue of the flanks of the recipient mice. The size of the implanted fragments was about 8 cu mm. Sterile precautions were used and the handling of fresh tumor tissue was done in a room reserved for this purpose. When palpable tumor nodules appeared, the flanks of the mice were shaved with an electric clipper, and the size of the tumors was measured 2 or 3 times a week thereafter using vernier calipers. The tumor volume was estimated as $(a/6)(a \times (mean\ diameter))$. For the end-point dilution experiments on the HX 18 tumor, cell suspensions were made using a method developed by V. D. Courtenay (manuscript in preparation). This involved trypsinization of finely chopped tumor tissue, followed by brief, hard shaking in medium without trypsin. The cell yield was approximately $2 \times 3 \times 10^7$ cells/g.
Source of the Tumors

The tumors used in this study were selected from the group of human tumor xenografts that were established in this laboratory by Pickard et al. (17). Three well-growing xenografts were selected to include widely different histopathological characteristics.

HX 13. This was a well-differentiated columnar cell carcinoma of the colon (Fig. 1a). The tumors took longer to reach treatment size than the other 2 xenograft lines, but this was largely due to a longer latent period before detectable growth. They also had the lowest take probability, averaging 29% between the 4th and 6th passage.

HX 18. This was a poorly differentiated colonic carcinoma (Fig. 1b) which was obtained from recurrent tumor at the anastomosis site of a hemicolectomy. The tumors were very cellular with a delicate stroma and showed an early tendency towards central necrosis. Growth was rapid, and the take probability was high (80% within the 9th to 14th passage). HX 18 was readily made into a single-cell suspension which could be transplanted i.m. using inocula of 10⁶ cells or more.

HX 29. This was an undifferentiated small-cell carcinoma of the bronchus, originating from a liver metastasis (Fig. 1c). It maintained the distinctive characteristics of a small-cell anaplastic carcinoma through all the passages thus far studied. There was little evidence of stroma. The experiments recorded here were on the 6th to 8th passages, during which the tumor had a take probability of 55%.

Karyotype Analysis

Tissue from HX 18 and HX 29 was grown in monolayer culture for 1 week and chromosome preparations were examined. The modal chromosome number was 46 in both tumors. In HX 18 there were 2 of 47 cells with a chromosome number less than 44; in HX 29 the proportion was 8 of 66. In all cases the chromosomes had the morphological characteristics of human chromosomes.

Chemotherapy

Groups of 6 to 10 tumors were selected for chemotherapeutic treatment at a mean tumor volume of 0.1 to 0.3 ml. Single-dose i.p. treatments were mainly used. Seven chemotherapeutic agents were used: VLB² (Velbe: Eli Lilly and Co., Ltd., Basingstoke, England), CY (Endoxana: WB Pharmaceuticals Ltd., London, England), DTIC (NSC 45388: Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.); 5-FU (Roche Products, Ltd., Welwyn Garden City, England); CCNU (NSC 79037: Division of Cancer Treatment, National Cancer Institute); MeCCNU (NSC 95441; Division of Cancer Treatment, National Cancer Institute). All except CCNU and MeCCNU were administered as a solution in 0.9% NaCl solution. The nitrosoureas, because of their aqueous insolubility, were dissolved in dimethyl sulfoxide in 5% Tween 80 (1:10, v/v). The therapeutic tests against xenografts were preceded by toxicity studies on the basis of which the approximate dose lethal to 10% of animals was used.

RESULTS

Drug Toxicity. Although we anticipated that mice that had been subjected to thymectomy plus lethal whole-body irradiation followed by bone marrow grafting would not be able to tolerate doses of cytotoxic agents that could be withstood by normal mice, in fact it was found that the doses lethal to 10% of animals were not much lower than normal.

The treatment of animals bearing HX 18 tumors was complicated by an effect of the tumor on the level of drug toxicity. With both 5-FU and DTIC it was found that HX 18 animals succumbed to doses that were tolerated by non-tumor-bearing mice or mice bearing either of the other 2 xenografts.

Growth of the Xenografts. The growth of untreated tumors is illustrated in Chart 1. Within the range of passage numbers that were used, the growth characteristics were reasonably stable as was also the histological appearance of the tumors. Only 2 spontaneous regressions were observed, 1 in HX 18 and 1 in HX 29, of a total of over 500 xenografts that were followed until tumor growth necessitated euthanasia.

Each of the 3 xenograft lines was studied over a period of 12 months, during which 7 to 10 experiments were begun on each. The proportion of positive takes observed in these experiments was almost always below 100%. There was no significant trend in the success rates and the average take probabilities were HX 13, 0.30; HX 18, 0.82; HX 29, 0.61. The low proportion of takes is a disturbing feature of the results. It may imply that the donor tumors were heterogeneous in their growth capacity, that the mice were not uniformly immune-suppressed, or that there were unidentified critical factors in the establishment of positive growth in s.c. implants of human tissue in the mouse. Some evidence for incomplete immune suppression comes from the data shown in Table 1. This is an analysis of the proportion of single and double takes throughout the series of experiments. The results imply that in each of the 3 tumors the proportion of single takes was less than the proportion that would be expected on binomial theory, and in each case this difference was statistically significant at the \( p = 0.05 \) level. These results indicate that there was a tendency for the 2 implants in each mouse either to take or not to take. At the time of tumor transplantation the procedure was first to prepare a large batch of small tumor fragments which were then selected at random for implantation into each mouse. It is therefore possible to rule out the explanation that the pairs of tumor fragments came from the same part of the donor tumor. We conclude that the recipient mice must have varied in their receptiveness, probably due to differences in the level of residual immunity.
Chart 1. Growth curves of untreated tumors from the 3 lines. Symbols denote successive transplants in the order ●, ○, □, △ (6 to 8 tumors/group).

Table 1

| No. of takes | No. of mice | Observed take probability | Expected take probability
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>HX 13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>0.654</td>
<td>0.490</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>0.092</td>
<td>0.420</td>
</tr>
<tr>
<td>2</td>
<td>39</td>
<td>0.255</td>
<td>0.090</td>
</tr>
</tbody>
</table>

92 takes out of 306 implants: overall take probability = 0.30

HX 18
0        21          0.100                      0.033
1        35          0.166                      0.298
2        155         0.735                      0.669

345 takes out of 422 implants: overall take probability = 0.82

HX 29
0        56          0.303                      0.151
1        32          0.173                      0.475
2        97          0.524                      0.373

226 takes out of 370 implants: overall take probability = 0.61

*a* On the basis of a binomial distribution, if *p* is the take probability per single implant, the expected proportions of 0, 1, or 2 takes out of 2 trials per mouse are \((1 - p)^2\), \(2p(1 - p)\), and \(p^2\), respectively. In each case the observed and expected distributions of take probability are significantly different at the 5% level.

**Cell Kinetic Studies.** Cell proliferation in the 3 xenograft lines was studied using the technique of labeled mitoses. The scoring of mitotic figures was restricted to cells that were presumed to comprise the parenchyma; mitoses in the stroma were rarely seen. The experimental details of these experiments were as reported by Pickard et al. (17), and the results on HX 18 were given in that publication. The results are shown in Chart 2. In each case the data have been analyzed by the method of Steel and Hanes (23) in which the best-fitting theoretical curve is sought, based on a simple conservative 3-compartment model of the cell cycle. The parameters of the best-fitting curves and deductions from them are given in Table 2. It can be seen that these data are remarkably similar among the 3 xenograft lines. The labeling indices were in the range 19.0 to 26.1%; the median intermitotic time was shortest in the most rapidly growing line (HX 18) and longest in the well-differentiated adenocarcinoma (HX 13).

**End-Point Dilution Studies.** As part of an attempt to develop stem-cell assays for the xenografted tumors, serial dilutions of single-cell suspensions of HX 18 were prepared and implanted i.m. into immune-suppressed mice. In 2 experiments 10⁶ irradiation-killed cells were added to each inoculum in the hope that the considerable growth enhancement seen in experimental mouse tumors (22) might also be obtained. In 1 experiment the killed cells were omitted. Animals were observed for a period of at least 2 months after implantation and the proportion of positive takes was recorded. The results are shown in Chart 3. The full line is a cumulative Poisson distribution, fitted to the data by eye. The line does not fit the data well. Too few takes were observed at high-cell inocula and too many at low-cell
**Table 2**

Summary of the cell kinetic results on the 3 xenograft lines

<table>
<thead>
<tr>
<th>Passage</th>
<th>Volume-doubling time(a)</th>
<th>Labeling index (%)</th>
<th>Mitotic index (%)</th>
<th>Cycle phase duration(b)</th>
<th>Median inter-mitotic time (hr)</th>
<th>Growth fraction (%)</th>
<th>Cell loss fraction (%)</th>
</tr>
</thead>
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<tr>
<td>HX 13</td>
<td>6th</td>
<td>11.8</td>
<td>22.2</td>
<td>1.07</td>
<td>2.5 (3.4; 3.2)</td>
<td>17.0 (17.4; 4.2)</td>
<td>35.0</td>
</tr>
<tr>
<td>HX 18</td>
<td>2nd</td>
<td>6.0</td>
<td>19.0</td>
<td>1.0</td>
<td>4.6 (5.2; 2.6)</td>
<td>11.9 (12.6; 4.4)</td>
<td>26.1</td>
</tr>
<tr>
<td>HX 29</td>
<td>7th</td>
<td>8.2</td>
<td>26.1</td>
<td>2.22</td>
<td>5.2 (5.7; 2.5)</td>
<td>12.4 (13.6; 5.9)</td>
<td>30.3</td>
</tr>
</tbody>
</table>

\(a\) Volume-doubling time in days determined from the slope of a Gompertz equation fitted to the growth data at a volume of 0.3 ml.

\(b\) Values given are the median phase duration in hr as well as the (mean : S.D.)

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**Chart 3.** Relation between observed proportion of takes and the size of the inoculum for i.m.-implanted cells isolated from HX 18. ○, results with viable cells alone; ●, results with the addition of \(10^7\) lethally irradiated tumor cells per implant. Error bars, binomial standard errors of the mean. Curve, cumulative Poisson distribution fitted by eye to data for the upper 3 inoculum levels.

inocula. Discrepancies of this type have been observed before (18), and the simplest explanation is that the implant sites were non-uniform with regard to the number of cells required for a positive take. If this variation is ignored, it may be seen that the number of cells required for 50% takes was about \(8 \times 10^4\), with very little reduction in this value by the addition of killed cells.

**Response to Chemotherapy.** The response of the well-differentiated colonic carcinoma HX 13 to single doses of 6 cytotoxic agents is shown in Chart 4. In most cases there was an initial growth delay, without significant regression, after which growth resumed at a rate that was similar to that of the controls. In the case of CY the data show a permanent retardation in growth. The most effective agent was 5-FU.

The results for the anaplastic colonic carcinoma HX 18 are shown in Chart 5. The effects of individual drugs were no greater than against the well-differentiated tumor. CY and 5-FU were the most effective agents, and again CY appears to have had a more permanent effect on growth rate than is seen with the other drugs. Two drug combination experiments were performed: CY + 5-FU and CY + adriamycin. The effects of these combinations were very similar and were greater than for the single agents. Estimates of growth delay are given in Table 3. In the case of CY + 5-FU the growth delay of the combination was roughly the additive growth delay of the separate drugs.

With CY + adriamycin the growth delay was slightly more than additive.

The bronchial carcinoma, HX 29, gave results that are summarized in Charts 6 and 7. The effect of adriamycin was initially greater than against the other 2 tumors, but subsequently the treated tumors appeared to accelerate. Two experiments with repeated adriamycin doses were performed; the results are given in Table 3. 5-FU was not very effective but CCNU showed a relatively good response. The effects of CY against this tumor were dramatic. All tumors treated with doses of 180 mg/kg or greater showed clear volume regression. Subsequently, the tumors either regrew or regressed to below 2 mm diameter, which was the limit of detection. There was only 1 case in which a tumor regressed completely and then regrew. It therefore seemed that there was a sharp distinction between one group of tumors, which soon regrew, and the other group, which achieved long-term control (i.e., no regrowth within 60 to 90 days of treatment). The data presented in Chart 7 show that there was a positive dose-response relationship in the extent of regression of the tumors that subsequently regrew and in the proportion of tumors that was cured.

Table 3 summarizes the estimates of growth delay in the 3 tumor lines. The time at which each tumor reached twice its treatment size was recorded, and the median times to double in volume \((TD)\) are given in the table. As an estimate of growth delay we have calculated

\[
\text{Growth delay} = \frac{TD_{\text{treated}} - TD_{\text{control}}}{TD_{\text{control}}}
\]

which may be regarded as the number of tumor-doubling times that were saved by the treatment. This ratio may be used as an index of effectiveness, although of course it does not indicate whether the effect was predominantly an arrest of growth or a change in tumor growth rate.

**DISCUSSION**

The testing of the action of chemotherapeutic agents against grafts of human tumors in immune-deprived mice involves the assumption that such xenografts resemble the corresponding human tumor in those respects that determine responsiveness. The objective of the present project...
was to investigate the response to chemotherapy of 3 different lines of xenografted tumor and, where possible, to draw conclusions about the relationship of the results to the response of tumors in man.

We have chosen, as a 1st step in this investigation, to examine the chemotherapeutic response of 3 well-established xenografts and to relate this to their growth kinetics. To perform such a study on early xenograft passages of tumors from patients who then go on to receive chemotherapy is a 2nd and larger stage in the investigation, which we are now beginning.

The main positive indication that the present xenografts resemble human cancer is their histological appearance. The tumor designated HX 13 retained through 6 passages the typical appearance of a well-differentiated adenocarcinoma of the colon (Fig. 1). HX 29 after 8 passages was almost
### Table 3

**Growth delay induced by chemotherapeutic treatment**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Passage</th>
<th>Proportion of deaths</th>
<th>$TD^c$</th>
<th>Growth delay $^c$</th>
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</thead>
<tbody>
<tr>
<td>HX 13</td>
<td>Control</td>
<td>4</td>
<td>7.5</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>5</td>
<td>10.3</td>
<td></td>
<td></td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6</td>
<td>11.8</td>
<td></td>
<td></td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>5-FU</td>
<td>200</td>
<td>4</td>
<td>0/3</td>
<td>28.8</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>CY</td>
<td>240</td>
<td>4</td>
<td>0/4</td>
<td>21.3</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>Adriamycin</td>
<td>12</td>
<td>5</td>
<td>1/5</td>
<td>18.5</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>CCNU</td>
<td>40</td>
<td>5</td>
<td>0/4</td>
<td>17.0</td>
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<tr>
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<td>DTIC $^d$</td>
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<td>6</td>
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<td>18.0</td>
<td>0.5</td>
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<td>6</td>
<td>1/4</td>
<td>28.0</td>
<td>1.4</td>
</tr>
<tr>
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<td>9</td>
<td>5.0</td>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>4.0</td>
<td></td>
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<td>b</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13</td>
<td>6.0</td>
<td></td>
<td></td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>6.3</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td>CCNU</td>
<td>40</td>
<td>9</td>
<td>1/4</td>
<td>7.3</td>
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</tr>
<tr>
<td></td>
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<td>240; 100$^e$</td>
<td>13</td>
<td>0/5</td>
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<tr>
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<td>240; 12$^e$</td>
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<td>11.9</td>
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<td>6 &amp; 7</td>
<td>1/9</td>
<td>7/13</td>
<td>b</td>
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</tbody>
</table>

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*Within 30 days of treatment; all deaths attributed to drug toxicity.

*The median time from treatment volume to twice the treatment volume (days).

*Estimate of the number of tumor-doubling times ($TD$) gained by treatment: ($TD_{treated} - TD_{control}$) / $TD_{control}$.

*Daily doses (for 2 or 3 days).

*Seven day interval.

indistinguishable from a human small-cell carcinoma of the bronchus as seen at autopsy. The anaplastic appearance of HX 18, both in the patient and as a xenograft, makes judgment on its resemblance more difficult. The studies that we have made of the chromosome constitution of dividing cells in the xenografts have shown the cells to be unquestionably human and this, taken with the evidence of histological similarity, leaves us in no doubt that the parenchymal cells in the grafts are of human neoplastic origin.

The biological behavior of the xenografts was less characteristic of human neoplasia. The tumors showed little evidence of invasiveness, except occasionally into the muscle layers overlying the peritoneum. Often they were seen to be surrounded by a thin fibrous capsule. Round-cell infiltration was seldom observed and metastatic spread was not detected.

The most obvious indication of the artificiality of the xenografts was their growth rate. Their volume-doubling times at a size of 0.3 ml varied from 6 days in the case of HX 18 to 12 days in the well-differentiated HX 13, with the oat cell tumor intermediate. In a recent survey that we have made of the available data on the growth rate of tumors in man, we have found 56 measurements on pulmonary metastases of colorectal tumors which had a mean volume-doubling time of 95 days. Fifty-five studies on primary undifferentiated bronchial carcinoma yielded a mean dou-
The other disturbing feature of the xenografts is their low take probability. It is well known that primary xenografts show a low and variable proportion of takes (3, 4, 16). We would have expected that, after 4 to 14 passages in mice, tumors would have been selected that were capable of 100% take probability, but the maximum achieved was 80% with HX 18. An obvious explanation of this may lie in the possibility that the recipient mice varied in their degree of immune suppression. The results presented in Table 1 confirm this possibility and suggest that tumors may differ in the extent to which their take probability is influenced by variation in residual immunity. HX 13 had a low take probability and showed the greatest dependence on mouse variation; HX 18 had a high take probability and showed the least dependence on mouse variation. In the end-point dilution experiments with HX 18, the deviation from a cumulative Poisson distribution was not large but it was significant. It may be that many primary xenografts show an even stronger dependence on residual mouse immunity and that their take probability would be improved by efforts to reduce this. Despite the evidence for residual immunity, the proportion of spontaneous regressions observed in the present work was very low. The present experiments have included over 500 xenografts that were followed until tumor growth necessitated euthanasia, and among these spontaneous regression was observed in only 2 cases.

Our opinion at this time is that the low take probability of primary and subsequent xenograft passages may only in part reflect inadequate immune suppression. In order to grow, a xenograft must resist the local macrophage response at the implantation site; it must also stimulate connective tissue cells within the host to proliferate and form a stroma. It may well be that different tumors, or cells from different parts of a tumor, vary in their ability to promote angiogenesis and the formation of a connective tissue framework (10).

The labeled mitoses data are useful in the negative sense that they rule out the possibility that differences in the response of the 3 xenograft lines to particular cycle phase-dependent drugs might be due to differences in the
The timing of the mitotic cycle. If the data reported here are taken in conjunction with those reported in our earlier paper (17), it is remarkable that among xenografts from widely different source tumors there should be so little difference in the timing of the mitotic cycle. The 3 labeled mitoses curves shown in Chart 2 are probably indistinguishable within the scatter in the data, as also are the estimates of growth fraction and cell loss factor. As discussed in our previous publication, the comparison between the intermitotic times of cells in the xenografts and in tumors in man is hampered by the lack of good data on human tumors in situ. The recent work of Muggia et al. (15) has provided useful data on metastatic undifferentiated small-cell bronchial carcinoma. In 12 patients the median labeling index was 16.7% (compared with 26.1% for HX 29 in the present study) and the average S-phase duration was 18.8 hr in 1 patient (compared with 12.4 hr in HX 29). These data therefore support our earlier conclusion that the cells in xenografts probably proliferate more rapidly than the corresponding cells in situ.

The chemotherapeutic studies were largely restricted to single-dose treatments, using a maximum tolerated dose to the mouse. The colorectal tumors (HX 13 and 18) in all cases continued to grow, after an arrest that varied with the type of drug and its dose (Charts 4 and 5). The 2 most effective single agents were CY and 5-FU. The combination of CY and 5-FU was studied in 1 experiment against HX 18. The order of the drugs (CY first) and the 7-day gap between them was chosen on the basis of concurrent toxicity studies which showed that this was the best tolerated sequence and the shortest possible gap within which a full 2nd treatment could be given. The results showed that the growth delay achieved with the 2 agents was approximately the sum of the growth delays achieved with the single agents. Similar results were obtained when adriamycin was substituted for the 5-FU, and in view of the fact that adriamycin seemed less effective than 5-FU when used alone, there may be slight evidence for CY-adriamycin synergism.

The most interesting results were obtained with xenografts of the bronchial carcinoma (HX 29). CY was identified as a very effective agent against this tumor, and yet the response curves for the individual tumors (Chart 7) are puzzling in 1 respect. In each of 3 replicate experiments the tumor responses could be separated into 2 discrete categories: tumors that temporarily regressed and then regrew; and tumors that showed permanent regression. The puzzling feature is that the temporary regressions were relatively slight, and backward extrapolation of the regrowth curves implies a surviving fraction of no less than perhaps $10^{-2}$ to $10^{-3}$. At this level of cell kill one would not have expected long-term tumor control if small numbers of surviving tumor cells could initiate regrowth. We are thus led to conclude that these results imply that a depression of viable cell numbers by only a few decades yielded apparent control of some tumors. The most likely conclusion is that these xenografts were growing in the face of a host defense mechanism that could eradicate up to perhaps $10^4$ or $10^6$ cells if left intact at the end of treatment. An alternative explanation, that only 1 cell in $10^4$ could act as a stem cell for regrowth in situ seems unlikely in view of the anaplastic nature of this tumor and the fact that it has recently been possible to clone the tumor cells in soft agar with a plating efficiency of about 1% (V. D. Courtenay, private communication).

Although in the course of this project we have observed a number of properties of the xenografts that cast doubts on their resemblance to human cancer, probably the final test is whether their response to treatment reflects the experience of the cancer chemotherapist. In this respect the results are promising. The response of the colorectal xenografts to the 7 drugs that we have used is probably not very different from the responses that are often achieved in the clinic, bearing in mind the difference in tumor growth rate. The number of tumor-doubling times gained by the single-dose treatments (Table 2) varied from virtually zero to about 4, and in man this would indicate an increase of up to perhaps 1 year in survival time. This level of response might be large compared with what would be expected from single-dose treatment in man, but it should be borne in mind that in our choice of dose level we have been able to risk greater toxicity than would be possible in the clinic. 5-FU is also indicated as probably the most effective single agent against xenografts of colorectal tumors, as is often the case in clinical experience (12, 14). With the bronchial carcinoma (HX 29) the good response to CY also corresponds with the effectiveness of this treatment in the clinic (19). A similar conclusion has been reached by Mitchley et al. (13) in their studies of the response of another xenografted human bronchial carcinoma to hexamethylmelamine. These studies lead us to conclude that further studies of the predictiveness of the response of xenografts to chemotherapy and radiotherapy are justified. The results have also helped to concentrate attention on the biological factors that limit the success of xenograft transplantation, in particular the level of residual host immunity and the ability of human tissues to stimulate the development of a satisfactory stroma from mouse tissues.

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Fig. 1. Photomicrographs of sections of the 3 tumors. a. HX 13; b. HX 18; c. HX 29. H & E. × 100.
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