Hormonal Control of Growth and Progression in Tumors of Nb Rats and a Theory of Action

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SUMMARY

A continuation of previous studies of hormone-dependent tumors in various organs in Nb rats concerns the effects of removal of the hormone stimulus from animals with growing tumors. Tumor regression usually followed this procedure, and in various models it was associated with an increased survival of the animal. A regressed tumor could be caused to grow at any time by estrogen treatment, and the resulting tumor remained hormone dependent, although some progression might occur. Continuous breeding rarely affected the growth or progression of transplanted adrenal or breast carcinomas. When spontaneous regression of tumors took place following removal of the estrogen stimulus, all types of tumors (except leiomyomas of the uterus) showed progression usually to autonomy, and in the case of male rats bearing breast carcinomas it was inevitable. The substitution of pellets containing a reduced level of estrone, to determine which prevented regression and allowed uninterrupted growth, offered an assessment of the type or amount of hormone required for the growth of different tumors. By means of such a model of breast cancer in male rats, it was possible to demonstrate that a reduction in hormone levels sufficient to prevent advancing tumor growth, but adequate to reduce the extent of regression, also reduced the frequency or prevented the development of autonomous change. Although regression per se was not a prerequisite for autonomous change, the paradox was evident that progression towards autonomous growth was accelerated with procedures expected to check tumor growth and was minimal with procedures that accelerated it. Liver metastases of hormone-dependent adrenal carcinomas continued growth and could not be influenced by removal of estrogen, although the primary transplant regressed. When such metastases were transplanted, they were not found to have progressed to autonomy but retained a hormone-dependent status. Some tumors, when maintained in estrogen-conditioned hosts, apparently showed a reversion to a more hormone-dependent cell type rather than the expected progression towards autonomy. A theory is suggested to explain the experimental findings on the development and control of estrogen-responsive tumors.

INTRODUCTION

Preceding papers have described a series of estrogen-dependent transplantable tumors which arose spontaneously or were obtained from primary tumors arising after prolonged estrogenization in 14 different organs of Nb rats (13). HD³ transplants with a growth rate related to the level of estrogen treatment were maintained with little change for many years, and transplantation to normal and conditioned rats allowed a classification to be suggested of 5 types of response including tumors inhibited by estrogen. Although the growth rate increased in most tumors with successive transplants from estrogenized hosts, progression towards more autonomous growth (requiring less estrogen) occurred very slowly over many years (except for pituitary and some mammary carcinomas). An analysis of the progression occurring in all the HD tumors suggested that tumors maintained in estrogenized hosts, when transplanted, either did not show spontaneous growth, remaining dormant in normal animals, or that they progressed extremely slowly towards autonomy (hormonal progression). HS tumors that showed a slower growth rate in normal animals than in estrogenized hosts also progressed over a number of generations, so that growth rates were increased when compared with those in the case of transplant made from normal animals. Such spontaneous progression was more rapid and predictable than was progression during hormone treatment (14).

This study has been directed to changes that may occur in growing transplants after the estrogen stimulus has been reduced or removed. Particular emphasis has been placed on the development of autonomy following the abrupt withdrawal of estrogen. Substitution of the hormone at a reduced level, preventing tumor growth but not allowing regression, was found to delay or prevent autonomous change. The findings in this final paper of a series have led to a working theory of "hormonal carcinogenesis" and its possible control.

MATERIALS AND METHODS

Preceding papers have described the materials and methods, which are still in use (13, 14). In this paper, changes that may have occurred in the type of transplanted tumor due to various treatments have been "assayed" by transplantation to normal and EP-conditioned male rats. In such cases the normal animals have been observed for at least 9 months and, in most cases, for 1 year or more. Each tumor was measured twice weekly, although the actual measure-

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² A cancer unit supported by the National Cancer Institute of Canada.

Received November 25, 1975; accepted September 22, 1976.

³ The abbreviations used are: HD, hormone dependent; HS, hormone stimulated; EP, 10-mg pellet containing 90% estrone and 10% cholesterol; E³P, pellet similar to EP, but containing 90% estradiol.
ments have not been recorded but have been replaced by an indication of the extent of regression. Palpable tumors too small to be measured accurately weighed approximately 10 to 50 mg. Rats bearing an EP have been studied for periods often exceeding 15 months. Although body growth is almost stationary, toxic effects have not been a problem. Less than 1% of females had to be killed because of uterine infection or abscesses. In old rats, middle ear disease, lung disease, overgrowth of incisor teeth, and pituitary adenoma were the commonest causes for terminating an experiment. Breast tumors in particular may ulcerate through the skin and hemorrhage. Animals bearing a 20% EP showed no evidence of toxicity and few detectable gross biological changes.

Interpretation of Progression. A tumor has been described as having progressed only when its growth pattern had advanced from one of the types to another as previously described (14). Since progression is a gradual and continuous change; however, other minor advances have also been noted. These may occur within a single type and be reflected by a reduced requirement of estrogen for growth, so that a HD tumor showing hormonal progression might change from one not growing in a normal female to one which did so, although it had not progressed to the HS type capable of growing in an ovariectomized female or in an unconditioned male. Although the growth rates of most HD tumors increased in successive generations under a constant estrogen level, this might reflect a selection of more rapidly growing cells for transplantation and did not necessarily reflect an increase in progression with respect to hormone requirements. Gradual changes in spontaneous progression were also seen in successive generations of HS-type tumors whether they were maintained in EP or normal rats. In normal rats the growth rate was the only criterion of the degree of change until growth occurred equally rapidly in normal and conditioned animals, representing the autonomous state. Hormone-inhibited tumors also showed progression through the hormone-retarded stage to autonomy. The pattern of progression in such tumors was the opposite of that in HD and HS types and changed from one inhibited by estrogen to one on which the hormone finally had no influence. The HD and hormone-inhibited types of tumors were, therefore, both initially controlled by estrogen, but they showed progression as indicated by the fact that both eventually escaped from this estrogen control.

RESULTS

Presentation of Results. A schematic plan has been prepared in Chart 1 to show the general experimental design and the sequence of results to be presented. The tumors studied and the growth of their transplants under the influence of estrogen have been discussed in preceding papers (13, 14). Cells remaining dormant after transplantation have not been included in Chart 1 but would be represented by HD tumor cells transplanted to an unconditioned host and subsequently not growing unless the animal was treated at any time throughout its lifetime with estrogen. Chart 1 shows the typical picture of the prompt cessation of growth and regression in size of HD tumors following EP removal.

In many cases regression continued until the tumors were so small that accurate measurement was impossible. Other tumors showed partial degrees of regression which varied from very slight to almost complete. The number of tumors showing 50% regression in size has also been included in some tables. In some cases, to be discussed separately, tumors, particularly of a HS type, were less affected by EP removal; in some cases, growth continued but at a greatly reduced rate. Such cases are not outlined in Chart 1. Animals in which tumor regression was prolonged had a survival time beyond that which was compatible with a continued growth of the tumor. The viable cells remaining in a regressed tumor could be stimulated to regrowth by treatment as indicated by the arrows, and interest then focused on whether they showed a progression towards autonomy. The final section of the paper examines particularly those tumors that, after regression of variable duration, exhibited spontaneous regrowth that was associated with autonomous change.

Tumor Regression following EP Removal. The effects are shown of EP removal on the growth of more than 500 HD tumors originating in 9 different organs and transplanted to male and female rats. It also includes the effects on hormone-inhibited tumor growth caused by the insertion of an EP into the tumor-bearing animals (Table 1).

The results present an overall picture of extent and degree of regression that occurred in growing tumors when the estrogen pellet stimulus was removed surgically. Regression was noted in most tumors, although complete tumor regression was consistently less frequent in female than in male rats. Most mammary carcinoma transplants growing in males showed complete regression, but ovarian thecomas, although growing at a reduced rate, showed little regression after estrogen removal. In some cases hormone-inhibited tumors also showed a complete regression that followed the insertion of an EP.

Survival of Tumor-bearing Rats following EP Removal. Chart 1 shows that the EP stimulus was removed when tumors were growing rapidly. Control rats were killed shortly after this time because of the size of their tumors and the need to maintain them by transplantation. Experimental rats were eventually sacrificed either because of spontaneous regrowth of their tumors or, in cases where regression continued, because of unrelated causes. The length of survival of animals from the time of transplant is
shown in Table 2, as well as the average time (after transplant) and size of the tumor when the EP was removed. The increase in time of survival has been considered to be the difference between the actual survival from the time of the transplant of the experimental animal and the time when the EP was removed, irrespective of the actual age of the rat. Control animals would have died from excessively large tumors shortly after the time when the EP was removed. (Estrogen toxicity was not a factor in causing mortality.) EP removal in all tumor models resulted in survival periods of 2 to 3 times beyond those expected in animals with uninterrupted tumor growth. However, in some cases individual animals showed increases from 8 to 10 times. As noted, the average survival time includes some animals that had to be killed because of illness although no tumor was present. In the case of the mammary tumors, for example, 15% of the animals did not have tumors (85% of these were males) but were killed at an average time of 29 weeks after transplantation. HS tumors such as a carcinoma of the pancreas, although not regressing, showed a reduced growth rate and an increased survival time following estrogen removal. Even a very rapidly growing tumor such as the lymphoma also showed a slower growth rate in the absence of estrogen. Although estrogenization of rats bearing hormone-inhibited-type tumors led to tumor regression, the length of survival was not as impressive as it was for other types of tumors.

**Estrogen-induced Regrowth of Regressed Tumors.** Adrenal carcinomas that showed continuous regression following EP removal were studied to determine whether re-

### Table 1

Regression of growing transplanted tumors following EP removal

<table>
<thead>
<tr>
<th>No. of transplanted tumors</th>
<th>Type of tumor</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Transplants showing regression</td>
<td>Transplants showing regression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No. of rats</td>
<td>Partial (%)</td>
</tr>
<tr>
<td>13</td>
<td>Adrenal carcinoma</td>
<td>151</td>
<td>86</td>
</tr>
<tr>
<td>12</td>
<td>Breast carcinoma</td>
<td>59</td>
<td>91</td>
</tr>
<tr>
<td>25</td>
<td>Primary breast carcinoma</td>
<td>11</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>Salivary carcinoma</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ovarian thecoma</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>Leydig cell carcinoma</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>Cervical carcinoma</td>
<td>9</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>Uterine leiomyoma</td>
<td>8</td>
<td>87</td>
</tr>
<tr>
<td>1</td>
<td>Lymphoma*</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Anterior pituitary carcinoma</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>Anterior pituitary carcinoma</td>
<td>6</td>
<td>100</td>
</tr>
</tbody>
</table>

* The EP was removed when tumors weighed 10 to 20 g.
* 25 tumors were present in 14 rats.
* Regression in thecomas was very slight.
* Early HD tumor generations.

### Table 2

Survival of tumor-bearing rats following EP removal

<table>
<thead>
<tr>
<th>Transplanted tumors</th>
<th>No. of rats*</th>
<th>After transplant (av. wk)*</th>
<th>Tumor size (av. width + length cm)</th>
<th>Survival time after transplantation (av. wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal</td>
<td>177</td>
<td>12.9</td>
<td>6-37</td>
<td>5.1</td>
</tr>
<tr>
<td>Mammary</td>
<td>130</td>
<td>11.1</td>
<td>4-32</td>
<td>5.6</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>23</td>
<td>13.8</td>
<td>5-28</td>
<td>5.5</td>
</tr>
<tr>
<td>Ovary thecoma</td>
<td>17</td>
<td>9.7</td>
<td>2-22</td>
<td>5.2</td>
</tr>
<tr>
<td>Leydig cell</td>
<td>16</td>
<td>27.0</td>
<td>15-47</td>
<td>5.0</td>
</tr>
<tr>
<td>Uterus leiomyoma</td>
<td>15</td>
<td>24.5</td>
<td>15-34</td>
<td>5.0</td>
</tr>
<tr>
<td>Cervix</td>
<td>14</td>
<td>11.1</td>
<td>17-23</td>
<td>5.3</td>
</tr>
<tr>
<td>Anterior pituitary</td>
<td>12</td>
<td>15.6</td>
<td>10-30</td>
<td>4.8</td>
</tr>
<tr>
<td>HS tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>5</td>
<td>4.0</td>
<td>3-6</td>
<td>4.5</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4</td>
<td>10.5</td>
<td>6-14</td>
<td>5.1</td>
</tr>
<tr>
<td>Hormone-inhibited</td>
<td>14</td>
<td>23.0</td>
<td>13-37</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* Includes males and females.
* Mean and range rats were killed at approximately this time because of growing tumors, and this was considered to be the survival time of untreated rats.
growth could be induced by estrogen treatment or whether such regrowth was associated with progression to autonomy. The results are shown in Table 3. It may be seen that the reintroduction of an EP (or even a 10% EP or a 10% EP) was followed by a regrowth of regressed tumors even when regression had been present for as long as 39 weeks. When transplanted, the resulting tumors showed some evidence of progression, but only 3 of 26 exhibited the minor change of subsequently growing in unconditioned females (but not in males). In 13 rats, estrogen-induced regrowth regressed when the EP was again removed. With such a procedure, however, progression was more apparent, since regression was much less when the EP was removed for the 2nd time, although the final tumors remained HD on transplant. From all studies on a total of 110 adrenal tumors that regressed following EP removal, 91 exhibited renewed growth when they were repelleted so that a maximum of 17% might be considered as having no viable cells and as cured following EP removal. In some of these animals, however, renewed growth probably would have occurred after a longer time.

Completely regressed mammary carcinomas similarly were found to grow following estrogen treatment, and the resulting tumor also retained its HD property. Figs. 18 to 20 show such a sequence. The tumor shown in Fig. 19 weighed only 60 mg after 44 weeks of regression following estrogen removal. When transplanted, however, it grew rapidly as the anticipated HD adenocarcinoma (Fig. 18). When the same tumor transplant in another rat had regressed to 100 mg after 20 weeks, it was stimulated to regrow by estrogen, and after 4 weeks of growth to 5.0 g it also showed the picture of an adenocarcinoma that continued to be HD on transplant (Fig. 20). Primary mammary carcinomas in 12 male or female rats that followed an average estrogenization of 10 months (7 to 11 months) regressed after removal of the EP. When an EP was inserted after an average of 2 to 3 months of regression, regrowth of the primary tumor was noted in 9 of 11 animals within 2 months. Multiple tumors occurring in some rats, all of which regressed, did not all show regrowth in this time.

**Spontaneous Regrowth after Regression and Subsequent Progression of Transplanted Tumors.** After the withdrawal of estrogen and subsequent regression, many tumors eventually commenced spontaneous regrowth as indicated in Chart 1. Regrowth was sudden in many cases, leading to early death, whereas in others a more gradual regrowth occurred. In some animals, however, regression continued until the experiment had to be terminated, but the residual tissue grew when transplanted. In all cases the final tumors were transplanted to determine whether progression had occurred. Such findings have been detailed in Table 4 for 3 types of tumors: breast, adrenal carcinomas, and ovarian thecomas. Other types of tumors behaved similarly and have been grouped in 1 of the 3 types of response. In the case of breast carcinoma in females, 10 of 37 HD tumors (27%) showed only partial regression and a gradual regrowth after EP removal. Of these, 70% remained HD, 30% progressed to the HS type, but none became autonomous. In contrast, when regression was complete and followed by an abrupt regrowth, 85% of the tumors became autonomous. In 2 of 3 cases tumors which showed no measurable spontaneous growth apparently contained cells which on transplant were autonomous. In all studies, including those of tumors not transplanted, 13% had not shown spontaneous regrowth in male animals when the animals were killed after 28 weeks. Individual tumors, however, varied in this respect. In 1 tumor, 6 of 9 transplants did not regrow, whereas in 2 other cases all of 15 and 16 transplants showed spontaneous regrowth. Transplants of mammary carcinomas in male rats, which showed a spontaneous regrowth after regression, had in every case changed from HD tumors to become completely autonomous. Carcinomas of the salivary gland responded in a fashion similar to that of breast tumors. In 10 animals, 5 such carcinomas showed complete regression after EP removal, and abrupt spontaneous regrowths which were autonomous when transplanted (Figs. 12-14), whereas in 2 cases progression had occurred, but only to a HS type.

Adrenal carcinomas showed the same overall pattern as did mammary tumors, but the changes were more gradual and less dramatic, and many tumors did not exhibit regrowth after regression. Females particularly showed only partial regression, with their tumors remaining HD. The carcinomas of Leydig cells and cervix behaved like adrenal tumors, and autonomous changes were observed in 2 of 6 (Figs. 7 and 8) and 2 of 8 tumors (Figs. 9 to 11), respectively, all of which showed regrowth after regression. Uterine leiomyomas, despite very prolonged periods of regression, have been exceptional and have not shown spontaneous regrowth or autonomous change. Photomicrographs of regressed tumors of different organs showed curiously similar

**Table 3**

<insert table here>

* Minor progression shown on transplantation.
* EP removal repeated on same 13 rats shown on next line.
* Seven rats received a 10% EP, and 7 received a 10% EP.
Table 4
Spontaneous regrowth of regressed transplants following EP removal, and their subsequent progression

<table>
<thead>
<tr>
<th>No. of tumors</th>
<th>Type of tumors</th>
<th>Original tumor type</th>
<th>No. of rats</th>
<th>Final type %</th>
<th>Final type %</th>
<th>Final type %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 Breast</td>
<td>F 40 HD</td>
<td>10</td>
<td>HA^a</td>
<td>0%</td>
<td>27 HA</td>
<td>85.2</td>
</tr>
<tr>
<td></td>
<td>M 3</td>
<td>100</td>
<td>HD 70%</td>
<td>2 HA</td>
<td>100</td>
<td>7.4</td>
</tr>
<tr>
<td>12 Adrenal</td>
<td>F 57 HD</td>
<td>38</td>
<td>HA 5.2</td>
<td>4 HA</td>
<td>75</td>
<td>15 HA</td>
</tr>
<tr>
<td></td>
<td>M 39 HD</td>
<td>11</td>
<td>HA 9.0</td>
<td>15 HA</td>
<td>80.0</td>
<td>12 HA</td>
</tr>
<tr>
<td>4 Ovarian thecos</td>
<td>F 2 HD</td>
<td>2</td>
<td>HA 50.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M 10 HD</td>
<td>10</td>
<td>HA 30.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M 4 HS</td>
<td>4</td>
<td>No change</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a HA, hormone autonomous.
^b The final types as determined by transplantation have been shown only as HD or hormone autonomous. The number of HS type tumors may be calculated for the percentage of difference.
^c Eight tumors did not regress but showed no progression.
^d Ten tumors showed continuous growth after EP removal.

pictures of small nests of epithelial cells in masses of collagen-like material (Figs. 3, 8, 10, 13, 19, and 23) from which spontaneous autonomous growth presumably commences. Examples of the autonomous tumors developing are shown in Figs. 11, 14, and 26.

Ovarian thecos represented the extreme of requiring very low estrogen for growth over many transplant generations (such tumors normally would grow rapidly in females but not in ovariectomized animals). Regression after EP removal was insignificant, although some transitory slowing of growth and slight diminution in size were noted in one-half of the rats. The most marked regression encountered is shown in Fig. 6 and may be compared with a transplant that had remained dormant for 41 weeks (Fig. 5). Following removal of the EP, however, progression to autonomy was noted in some thecosmas despite the absence of regression. In fewer tests, a carcinoma of the pancreas, a lymphoma, and a liposarcoma have behaved in a fashion similar to that of the ovarian tumors.

The unique type of hormone-inhibited anterior pituitary tumor could also be made to regress, but in such a case this followed insertion of an EP. These relatively slow-growing tumors in male rats showed a prompt regression in the presence of estrogen; examples are shown in Chart 2. The histological changes following regression of the tumor of Rat 1 are shown in Figs. 15 and 16 and resemble those seen after EP removal in HD tumors. The spontaneous autonomous tumor regrowth of the transplant in Rat 2 and its histological appearance are also shown on Chart 2 and in Fig. 17.

The model of HD mammary carcinoma in male rats has been used extensively to induce autonomous change, as will be discussed later. Detailed experimental data obtained from a 10th-generation transplant may be seen in Chart 3. The variations shown in the growth rates of the separate tumors were not unusual. The histological change from the original HD transplant (Fig. 25) to the spontaneous autonomous regrowth of Tumor 1 is shown in Fig. 26. (The scirrhous type of carcinomatous change is commonly found in breast, but not in other tumors.)

sized pellet, but with a lesser estrone content, was substituted. The effects on subsequent tumor growth are shown in Table 5. Mammary carcinomas were studied extensively and showed the highest need of estrogen, since despite a 20% EP, 66% of tumors in females and 78.4% in males regressed. On the other hand, adrenal tumors continued growth in most animals even with a 10% EP (although this dose level initiates transplanted tumor growth very slowly). In most cases regression was more readily prevented by the same dose in females than in males. Salivary gland tumors, however, were exceptional, since in females they regressed despite a 20% EP, although they continued growth in similarly treated males. In fewer tests, carcinomas of the cervix and Leydig cell responded like those of the mammary gland. In 9 primary breast tumors that followed prolonged EP stimulation, the substitution of an E3P allowed continued growth, as did diethylstilbestrol (but not testosterone). Prolactin injections of 5 mg daily even in the presence of a 20% EP were associated with tumor regression. Similarly, other pituitary hormones have not supported growth when substituted for the EP.

**Progression of HD Tumors following the Substitution of Pellets of Lower Estrogen Content, after EP Removal.** With the use of the mammary tumor model in male rats, experiments were designed to control and minimize regression after EP removal by the substitution of pellets of a reduced estrone content similar to those in the experiments shown in Table 5, and then to determine whether the expected progression to autonomy had occurred. Intermittent removal and insertion of 20% to 25% estrone pellets allowed a reasonable control of tumor growth, and in such experiments in progress no tumor has shown evidence of an abrupt surge in growth which would suggest an autonomous change. Earlier attempts, however, did not succeed in preventing tumor regression adequately after EP removal, but they are included in Table 6.

In controls all 41 transplants from 8 different HD breast cancers that regressed following EP removal and then exhibited spontaneous regrowth became autonomous. The substitution of a 10% EP for the EP did not alter the response in 6 animals. However, when a 20 to 25% EP was substituted, although complete regression was not prevented and regrowth was not altered, only 30% of 10 tumors became autonomous. On the other hand, in 14 animals in which a 20% EP was more effective and allowed only a partial regression that was followed by regrowth, 14.3% became autonomous. Although they were HD on transplant, 2 of the tumors showed minor progression, since in the presence of a 20% EP they no longer regressed but exhibited slow growth. In 4 rats in which it was possible to achieve a more or less stationary growth pattern, all tumors remained HD. When the same procedure was used and a 20% EP was substituted, 7 salivary gland tumors showed regression followed by regrowth; of these, 5 remained HD and 2 showed progression, whereas 9 control tumors in rats without substituted estrogen all progressed to autonomy.

**Possible Tumor Reversion to a Less Autonomous State.** Successive early generation transplants of some tumors have indicated that the expected continuous progression to a more autonomous condition may not take place but that tumors had actually reverted to a less autonomous type. This change has been noted in 6 different mammary carcinomas, 6 adrenal carcinomas, and in a cervical carcinoma and an ovarian thecoma when transplants were maintained in EP hosts. Reversion to a HD state has occurred even in 3 tumors when transplants of the HS type from untreated animals were subsequently maintained in conditioned animals, although transplants to normal males continued to be

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**Table 5**

<table>
<thead>
<tr>
<th>90% EP replaced by %</th>
<th>Breast</th>
<th>Adrenal</th>
<th>Salivary gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP</td>
<td>Sex</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>30</td>
<td>F</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>6</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>20 × 2</td>
<td>M</td>
<td>10</td>
<td>20.0</td>
</tr>
<tr>
<td>25</td>
<td>M</td>
<td>7</td>
<td>18.6</td>
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<tr>
<td>20</td>
<td>F</td>
<td>11</td>
<td>66.6</td>
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<td>F</td>
<td>7</td>
<td>100.0</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

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*Chart 3. Typical growth response of 4 transplants, from the same HD mammary carcinoma, in each of 2 male rats. Growth occurred in 7 transplants, followed by regression after EP removal, and the spontaneous regrowth of 3 tumors (Tumors 1 to 3) which were autonomous on transplant.*
HS. The apparent highly malignant morphology of a tumor may also be dramatically modified by the hormone status of the host. The breast carcinoma (Fig. 25) noted in Chart 2, after the induced autonomous change, exhibited a scirrrous carcinoma actively invading the surrounding muscle (Fig. 26). This picture continued when the carcinoma was transplanted to unconditioned rats (Fig. 27). In contrast, transplants of the same tumor to an EP host grew more rapidly but reverted to the typical histological picture of an adenocarcinoma (Fig. 28), and subsequent transplanted tumors maintained in EP hosts continued to be HD.

**DISCUSSION**

Preceding papers have described many tumors of Nb rats that occurred either spontaneously or under the influence of estrogen and the subsequent hormone control of the growth of their transplants (13, 14). This study has focused on changes in growth and progression that follow the removal of the hormone stimulus from rats bearing estrogen-controlled growing tumors. Most tumors stopped growing following removal of the EP and either gradually regressed to a smaller size or became too small to measure. Under such conditions animals survived for longer periods than would have been expected had growth of the tumor continued and killed the animal. Primary mammary carcinomas that developed during EP treatment regressed in a similar fashion (9, 12). Cutts has also reported the regression of primary breast tumors following estrogen withdrawal and other ablative procedures (1-3). Although in the case of some tumors regression did not follow EP removal (e.g., carcinoma of the pancreas and a lymphoma), the tumors grew more slowly. Regression was usually less in female HD tumor-bearing hosts than in males, and this was considered to be related to ovarian hormone secretion. Regression was also produced in HI tumors, but in this case it followed the insertion of an EP. Since a small inoculum of HD tumor cells did not die but remained dormant indefinitely in the absence of the hormone (14), it is not known why most growing cells do not survive and why regression of tumors takes place after removal of the hormone. Presumably, such cells are those that, through selection by transplantation of rapidly growing HD tumors, have the highest requirement for estrogen and are most affected by its removal. The histological similarity of dormant and viable cells in regressed tumors are shown in Figs. 1 and 2 (adrenal) and Figs. 21 and 22 (breast).

Tumors of different organs that showed marked regression following estrogen removal, or hormone-inhibited tumors caused to regress by the hormone, all showed curiously similar histological pictures. Small nonpalpable clumps of epithelial cells persisted in masses of collagen-like stroma, as described in detail by others (2, 6, 16). Such cells remained viable and would respond at any time to become a growing tumor if stimulated by estrogen treatment of the host. The resulting tumor, when transplanted, remained HD and showed little progression from its original type. Tumors that were intermittently stimulated and then allowed to regress, however, frequently showed minor changes, indicating progression towards autonomy. In similar experiments not described in this paper, the growth of transplanted tumors was little affected in female rats that repeatedly bred and reared litters. In 75 rats bearing tumors from 15 adrenal or 12 breast HD carcinomas the physiological stimulation of repeated pregnancy did not cause
tumor growth. In 31 animals bearing HS tumors, approximately 15% showed an increase in tumor growth resulting from breeding. Growth and regression related to successive pregnancies was shown by 2 “pregnancy-dependent” breast carcinomas (Fig. 24), but a demonstrable increase in progression was noted in the subsequent transplants of tumors of 4 other rats.

Although progression has usually been found to be a slow and predictable change over many transplant generations, it would appear that hormonal manipulation that caused repeated fluctuations in tumor growth and regression tended to accelerate autonomous change. Conversely, the observations on reversion would support the suggestion that progression is minimized or reversed in cells growing in a constant hormonal environment. Whether this represents a reversion in cell type, or rather a cell selection under the particular experimental situation, is difficult to assess. Mammary tumor cells of different derivations may be present, as emphasized by Slemmer (15), and respond differently to hormones. As was shown, however, the hormonal status of the host may markedly alter the growth and the morphology of transplanted tumors.

In this paper the major emphasis has concerned the marked changes in progression of tumors that may occur following hormone manipulation within the life-span of the animal. Tumors of the many organs described, which regressed following estrogen withdrawal and which subsequently showed spontaneous regrowth, in most cases progressed to an autonomous type. This was particularly striking in transplants in male animals, suggesting that the normal hormones of the female modified in some way the autonomous change found in males. Although the extensive and rapid regression of breast and salivary gland tumors was striking, it was not a prerequisite for the development of autonomous change. Less reactive tumors such as those of the adrenal cortex or cervix, and even thecomas of the ovary which showed little regression after the EP removal, all showed progression to autonomy. The results, therefore, indicate that progression to autonomy is initiated by the withdrawal of the hormone on which the growth of the tumor is dependent and is not the result of regression per se. Our studies on mammary fibroadenomas reported 20 years ago (7) showed that hormone manipulation leading to a reduced tumor growth rate led to a more malignant and uncontrollable sarcomatous change and pointed to the conclusion that “benignancy would seem assured by the maintenance of cellular environmental conditions which allow progressive cellular growth” (8). In discussing this and other work, Foulds (4) concludes that “there is a disturbing possibility that therapy by suppressing or retarding growth may favor progression from the responsive to the irresponsive, independent state.”

In male rats bearing mammary tumor transplants, attempts were made to prevent the expected regression and autonomous change after removal of the EP by the substitution of those of a lesser estrone content. In such cases, although regression was not completely eliminated, many tumors did not progress to autonomy. Experiments in progress in which regression was minimized and tumor growth was compatible with the animal’s survival indicate a prevention of the development of autonomy. Metastasis to various organs was a frequent finding associated with adrenal carcinomas and will be reported as a separate study. Pertinent to this paper, however, was the unexpected behavior of liver metastases. It was noted repeatedly that, although the primary transplant in the neck showed typical regression following EP removal, the liver metastases continued to grow even in male rats (Figs. 3 and 4), causing death of the animal. Transplants of the liver metastases, however, were not autonomous and presented the enigma that in every case they had remained HD but apparently had retained the ability to continue growth in the liver in the absence of estrogen. The overall findings discussed in this study have been summarized in Chart 4. The top half of the chart indicates the different types of tumors that have been described and their gradual progression from the HD or hormone-inhibited type through HS or hormone-retarded types to eventual autonomy. The lower half shows how progression was induced by hormone manipulation, leading to rapid autonomous change, and how it may be prevented.

The important question may be asked whether the tumors that have been described can be considered as counterparts of those occurring in humans, particularly since the method of controlling progression to autonomy in rat breast carcinoma differs so radically from the currently accepted treatment of similar hormone-responsive tumors in humans. Tumors in this strain of rats have been found to occur spontaneously in many of the organs studied and morphologically resemble those found in man. The spontaneous tumor incidence was low but increased with advancing age. Most of these tumors were autonomous, but 6 spontaneous adrenal carcinomas, 4 breast carcinomas, and an endometrial carcinoma of the uterus, all in females, were typical HD tumors when transplanted. The incidence of all tumors could be increased by prolonged estrogenization in both sexes and, as more recently shown, in some organs by the combined action of estrogen and androgen (10). Tumor cells might remain dormant throughout life in unconditioned hosts but could be stimulated to grow at any time by estrogen. Some tumor models demonstrated striking

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**Chart 4.** Described findings in tumor progression supporting the theory of hormonal tumorigenesis.
regression following hormonal withdrawal, whereas others were little affected. As far as could be tested, primary tumors responded in a way identical to that of the models of their tumors maintained by transplantation. Such observations closely resemble those described for estrogen-influenced tumors in humans. Here both hormone-sensitive and refractory spontaneous tumors are found. The etiological factors are unknown, but hormones have been implicated. Tumors sensitive to estrogen may regress after endocrine organ ablation but recur either as estrogen-sensitive tumors or, usually, as autonomous growths after unpredictable periods of increased survival. Tumor nodules may remain dormant for protracted intervals and be estrogen sensitive when growth recurs. The similarity of breast carcinoma in humans and in rat models seems particularly striking, and the response to hormone treatment in the latter may suggest new approaches to therapy in man (11).

Many studies have concerned the etiology of solid tumors and will not be reviewed at this time, but the relevant contribution of Rous, Berenblum, Shubik, Greene, Bielschowsky, Prehn, Leighton, and others are discussed by Foulds (4) in relationship to his observations on tumor progression. The overall study of the behavior and the response to estrogen of the many types of solid tumors described suggests a new concept of carcinogenesis. Although it may have wider application to tumors related to growth-stimulating factors other than estrogens, it does not appear to involve forms of cancer induced by the direct action of various carcinogens. Estrogen treatment of normal animals is followed by development and cell hyperplasia, particularly in target organs (mammary tissue in males), eventually making a larger population of normal cells available for malignant change. It is theorized that all normal cells are in a continuous although undetectably slow and varied rate of progression, but during a life-span the capacity for limited proliferation under a constant stimulus does not exhibit apparent change. Progression may be defined as the tendency for a cell to escape from its inherent limited capacity for proliferation. It may be related to multiple cumulative minor mutations rather than to a sudden simple mutation, which has been suggested (5). An occasional cell, through random change, may develop a greater capacity to progress. This may not result in continuous growth per se, but in the presence of estrogen it exhibits a very slow, continuous, and limited proliferation. Through such a selection and stimulation of cells a recognizable carcinoma is produced and can be identified as estrogen dependent by transplantation. The initial rate of growth appears to be largely related to the dose level of the hormone and to the type of target tissue. Subsequent transplants may grow more rapidly through a selection of rapidly growing tumors for transplantation, but the rate of progression is independent and may remain very slow. However, the potential “cancer” cell may lie dormant and undetectable in its original host (or when transplanted) throughout the animal’s lifetime unless endogenous or exogenous hormone levels are increased to those that awaken cell growth. Estrogens, therefore, are not considered to be carcinogenic agents but rather act simply as an accelerator of the growth of cells that already show an exaggeration of the natural process of progression. They have been used in high dose levels only as an experimental tool to obtain HD cells for subsequent transplantation and study.

Progression independent of growth, however, may also be affected by hormones. It would appear that progression is particularly sensitive to changes in hormone levels in the animal but varies in different organs. Changes in progression were minimal in the presence of constant and continuous levels of hormone, irrespective of dosage. This might be interpreted as a form of homeostasis. Fluctuating levels in the hormonal milieu, however, led to more definite and rapid progression. These might be slight as in the case of repeated pregnancies or with brief intermittent experimental restimulation of tumors. Rapid progression within the life-span of the animal, however, may follow total withdrawal of the hormone stimulus, as was readily demonstrated in HD tumors composed of rapidly growing cells. In such instances large numbers of the most rapidly growing cells die in the absence of estrogen, leaving small nests of epithelial cells, presumably those most capable of survival without the hormone (but occasionally all cells may die). Progression continues so that after a prolonged interval a cell emerges at random which is capable of continuous growth in an unconditioned host. Repeated courses of brief stimulation and regression augment the selection process and result in a rapid progression to autonomy. This may explain the finding that many spontaneous tumors are autonomous when discovered. Cells of some organs, such as smooth muscle of the uterus, may possess an inborn capacity to resist progressive changes to even large variations in hormone level, whereas others are sensitive to changes of a small magnitude and rapidly progress to autonomy.

ACKNOWLEDGMENTS

Assistants and technical participants included Heather Watson, Susie Edra, Carrolle Spooner, Sue Slade, Cathy Young, Susaan Lomax, Vivian Young, Valerie Siemens, and Gwen Cornfield.

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Progression in Estrogen-responsive Tumors


All sections H & E, x 450.

Fig. 1. Adrenal cortex (Tumor 3), 35th generation in female. Ovarian metastasis of typical carcinoma. HD transplant (12 g) after 7 weeks.

Fig. 2. Adrenal cortex (Tumor 3), 22nd generation in female. Dormant carcinoma. HD transplant (26 mg) after 55 weeks.

Fig. 3. Adrenal cortex (Tumor 3), 26th generation in female. Carcinoma. Regressed HD transplant (from 14.0 to 0.2 g in 24 weeks) after removal of E1P.

Fig. 4. Adrenal cortex (Tumor 3), 29th generation in male. Thecoma. Dormant HD transplant (22 mg) after 41 weeks.

Fig. 5. Ovary (Tumor 5), 6th generation in male. Thecoma. Dormant HD transplant (3.3 to 2.2 g in 8 weeks) after EP removal.

Fig. 6. Ovary (Tumor 5), 6th generation in male. Thecoma. Regressed HD transplant (from 0.9 to 0.3 g in 34 weeks) after EP removal.

Fig. 7. Leydig cell (Tumor 1), 3rd generation in male, EP. Carcinoma. HD transplant (0.9 g) after 24 weeks.

Fig. 8. Leydig cell (Tumor 1), 3rd generation in male. Carcinoma. Regressed HD transplant (from 4.0 g to 72 mg in 35 weeks) after EP removal.

Fig. 9. Cervix (Tumor 1), 19th generation in female, EP. Carcinoma. HD transplant from tumor, Fig. 10. (1.5 g) after 10 weeks.

Fig. 10. Cervix (Tumor 1), 18th generation in female. Carcinoma. Regressed HD transplant from tumor, Fig. 11. (9.0 to 0.3 g) in 34 weeks after EP removal.

Fig. 11. Cervix (Tumor 1), 15th generation in female. Carcinoma. Autonomous, transplant from HD regressed transplant in female (from 7.0 to 0.1 g) in 36 weeks, but with spontaneous regrowth (to 4.7 g) by 46 weeks.

Fig. 12. Salivary gland (Tumor 2), 6th generation in male, EP. Carcinoma. HD transplant (2.6 g) after 6 weeks.

Fig. 13. Salivary gland (Tumor 2), 4th generation in male. Carcinoma. HD transplant (from 3 g to 28 mg) in 42 weeks after EP removal.

Fig. 14. Salivary gland (Tumor 2), 3rd generation in male. Carcinoma. Autonomous. Transplant from HD regressed transplant in male (from 6.0 to 2.6 g) in 8 weeks, but with spontaneous regrowth (to 39 g) by 14 weeks.

Fig. 15. Anterior pituitary (Tumor 4), 9th generation in male. Carcinoma. HI transplant from tumor, Fig. 16. (6.7 g) after 31 weeks.

Fig. 16. Anterior pituitary (Tumor 4), 8th generation in male. Carcinoma. Regressed HI transplant from tumor, Fig. 17. (14.0 to 1.2 g) in 24 weeks after insertion of EP (Rat 1, Chart 2).

Fig. 17. Anterior pituitary (Tumor 4), 6th generation in male. Carcinoma. Autonomous. Transplant from hormone-inhibited regressed transplant in male (from 2.5 to 0.1 g) in 16 weeks, but with spontaneous regrowth (to 10.1 g) by 31 weeks (Rat 2, Chart 2).

Fig. 18. Breast (Tumor 16), 5th generation in female, EP. Carcinoma. HD transplant (from tumor Fig. 19) (0.9 g) after 14 weeks.

Fig. 19. Breast (Tumor 16), 4th generation in male. Carcinoma. Regressed HD transplant from tumor Fig. 18. (14.0 to 60 mg) in 44 weeks after EP removal.

Fig. 20. Breast (Tumor 16), 4th generation in male. Carcinoma. HD transplant from HD regressed transplant in male (from 5 g to 0.1 g) in 20 weeks, but with induced regrowth by EP (to 4.6 g) in 4 weeks.

Fig. 21. Breast (Tumor 7), 12th generation in male. Dormant carcinoma. HD transplant (76 mg) after 29 weeks.

Fig. 22. Breast (Tumor 19), 6th generation in female, EP. Carcinoma. HD transplant (19.4 g) in 15 weeks.

Fig. 23. Breast (Tumor 19), 4th generation in female. Carcinoma. Regressed HD transplant from tumor Fig. 22. (11.0 g to 50 mg) in 56 weeks after EP removal.

Fig. 24. Breast (Tumor 15), 7th generation in female. Carcinoma. Regressed HD transplant from tumor Fig. 23. (12.0 to 3.8 g) in 4 weeks after weaning litter.

Fig. 25. Breast (Tumor 15), 10th generation in male. Carcinoma. HD transplant (44 g) after 8 weeks.

Fig. 26. Breast (Tumor 15), 11th generation in male (from tumor Fig. 25). Scirrhous carcinoma. Autonomous invasive transplant from HD regressed transplant in male (from 14 to 0.1 g) in 13 weeks, but with spontaneous regrowth (to 24.5 g) by 26 weeks (Rat 1, Chart 1).

Fig. 27. Breast (Tumor 15), 12th generation in male. Anaplastic carcinoma. Autonomous transplant (20 g) after 26 weeks (from tumor, Fig. 26).

Fig. 28. Breast (Tumor 15), 12th generation in male, EP. Adenocarcinoma. HS transplant (7 g) after 14 weeks (also from tumor, Fig. 26).
Progression in Estrogen-responsive Tumors
Hormonal Control of Growth and Progression in Tumors of Nb Rats and a Theory of Action

Robert L. Noble


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