Transplants were usually not sensitive to hormones, but an exception, the rats did not show evidence of androgenization. Estrogen, resulting in an incidence of 50% in 10 rats. In such a case, the rats did not grow in males but remained dormant and could be stimulated to grow after many months by estrogen treatment. The resulting tumors remained estrogen dependent, with one exception which was autonomous. Tumors regressed following estrogen removal but eventually showed a spontaneous regrowth which was then found to be autonomous. In the case of growing tumors, the reduction of estrogen to subthreshold doses for maximal growth allowed stationary or only slow growth of the tumors, but these remained estrogen dependent. Estrogen-dependent tumors responded to tamoxifen treatment by a cessation of tumor growth or regression despite continued estrogen treatment. Tumor regrowth eventually took place after prolonged tamoxifen treatment, but the tumors remained estrogen dependent when transplanted.

ABSTRACT

Carcinoma in the dorsal prostate of Nb rats was produced by prolonged treatment with androgen or androgen plus estrogen. The most effective combination was androgen followed by estrogen, resulting in an incidence of 50% in 10 rats. In such a case, the rats did not show evidence of androgenization. Transplants were usually not sensitive to hormones, but an estrogen-dependent tumor (Nb-2Pr-E) was successfully transplanted in estrogenized hosts. Cells of estrogen-dependent tumors did not grow in males but remained dormant and could be stimulated to grow after many months by estrogen treatment. The resulting tumors remained estrogen dependent, with one exception which was autonomous. Tumors regressed following estrogen removal but eventually showed a spontaneous regrowth which was then found to be autonomous. In the case of growing tumors, the reduction of estrogen to subthreshold doses for maximal growth allowed stationary or only slow growth of the tumors, but these remained estrogen dependent. Estrogen-dependent tumors responded to tamoxifen treatment by a cessation of tumor growth or regression despite continued estrogen treatment. Tumor regrowth eventually took place after prolonged tamoxifen treatment, but the tumors remained estrogen dependent when transplanted.

INTRODUCTION

The role of sex hormones in autogenous carcinogenesis and their subsequent control of tumor growth and progression have been studied in inbred Nb rats for some years. A wide variety of organs have shown a low spontaneous tumor incidence which became increased following prolonged treatment with estrogen. Many transplanted tumor lines were established which continued to be responsive to estrogens (8). It has become apparent more recently that the combined or sequential administration of antagonistic hormones such as estrone and testosterone may also produce tumors in some organs, but of a type different from those produced by a single hormone (11, 12). In the case of the breast, estrone treatment produced carcinoma only when commenced in immature rats. The resulting tumors were estrogen dependent when transplanted. A combined treatment of estrogen plus testosterone produced breast tumors in rats of any age which were characterized as black, hemorrhagic papillary carcinomas. Transplants could be obtained which were estrogen or androgen dependent (7). Carcinoma of the dorsal prostate was produced by androgen treatment alone or combined with estrogen. In both cases, the incidence was approximately 20%, but tumorigenesis was more rapid when both types of hormone were given (9). Initial androgen treatment followed by estrogen, however, has now been found to be the most effective way of causing cancer of the prostate, occurring in 5 of 10 rats. Spontaneous carcinoma of the prostate has rarely been noted in Nb rats and seldom reported in rats in general (4, 15, 16). Transplants of spontaneous tumors in other strains have had limiting parameters in studies attempting to relate them to the disease in humans, since their growth rate usually was little affected by hormone treatment. The growth of a transplanted prostate carcinoma of an Nb rat, however, was strongly influenced by the hormonal status of the host. This paper will add further observations on the production of adenocarcinoma of the prostate in Nb rats and will also describe transplant lines under estrogen control. Drago et al. (2) have also studied hormone dependence of the same Nb tumor lines which were sent to their laboratory. In addition, they have described the growth of these lines in athymic nude mice and the detailed histopathology of the Nb prostate tumors. A following paper (13) will describe the development of tumor lines from an estrogen-dependent tumor which required androgens for maximal growth.

MATERIALS AND METHODS

The production of carcinoma of the prostate in Nb rats has been described previously (9). Steroids (obtained from Sigma Chemical Company, St. Louis, Mo.) were administered in the form of compressed pellets. In order to maintain the most hormone-sensitive tumor lines, the animal with the most rapidly growing tumor of any treated group was used for transplantation. The antiestrogen tamoxifen (base; kindly supplied by Dr. W. Baumgarten, ICI Americas, Inc.) was dissolved in sesame oil at a concentration of 10 mg/ml. A dose of 5 or 1 mg was given weekly by s.c. injection, distal to the sites of the hormone pellet and the transplanted tumor tissue. The most hormone-sensitive transplant lines have been derived from prostatic carcinoma 52, and its origin was described previously (10). This tumor has now been designated Nb-2Pr to conform with designations of other laboratories using it. The affixes E, A, or Aut. are suggested to designate derived sublines which are influenced by estrogen, are influenced by androgen, or are autonomous. Tumor size was measured weekly, and the maximum length + width (cm) was summated.

RESULTS

Production of Primary Carcinoma of the Dorsal Prostate in Nb Rats. An updated summary of the means used to produce cancer of the prostate in Nb rats is shown in Table 1. This includes tumors previously tabulated and variations in duration of treatment, etc., to conform with the earlier publication (9). New observations on the effects of initial treatment with androgens followed by estrogen are also listed.
Occurrence of spontaneous carcinomas of prostate in normal rats: 776 males 45 to 56 weeks of age, 0 carcinoma; 409 males over 56 weeks of age, 2 carcinomas (0.48%).

Table 1

<table>
<thead>
<tr>
<th>Initial treatment</th>
<th>Subsequent treatment</th>
<th>Gross prostatic cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of pellet</td>
<td>No. of rats (wk)</td>
<td>Pellet</td>
</tr>
<tr>
<td>EPa</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td>TPP</td>
<td>104</td>
<td>None</td>
</tr>
<tr>
<td>TPP + EP</td>
<td>62</td>
<td>None</td>
</tr>
<tr>
<td>EP</td>
<td>25</td>
<td>(EP removed)</td>
</tr>
<tr>
<td>EP</td>
<td>33</td>
<td>TPP (EP removed)</td>
</tr>
<tr>
<td>EP</td>
<td>10</td>
<td>EP (TPP removed)</td>
</tr>
</tbody>
</table>

a Single 10-mg pellet (50 to 60% absorbed in 1 year).
b Dose of two or three 10-mg pellets repeated every 8 to 12 weeks (pellets completely absorbed in this time).

Although treatment with TPP,3 TPP plus EP, or EP followed by TPP produced tumors, the highest incidence was found when prolonged exposure to androgen was followed by estrogen. Following this sequence of treatment, the tumors were relatively small and were situated more centrally around the urethra, causing early signs of urinary obstruction. Histologically, all tumors were similar and predominantly glandular with varying amounts of scirrhous tissue; squamous cell metaplasia was minimal; the tumors have been described in detail (2, 9, 10). Many animals had metastases in the peritoneal cavity. Tumors which followed TPP or TPP plus EP treatment usually were large and were palpable in the lower abdomen with involvement of enlarged seminal vesicles and bladder. The effects of excessive androgen were obvious, with enlargement of the normal seminal vesicle and ventral prostate tissue. In contrast, animals which received TPP for a prolonged period followed by EP showed no evidence of the initial androgenization but some effects of estrogen. The testes were reduced in size and were large and were palpable in the lower abdomen with involvement of enlarged seminal vesicles and bladder. The effects of excessive androgen were obvious, with enlargement of the normal seminal vesicle and ventral prostate tissue. In contrast, animals which received TPP for a prolonged period followed by EP showed no evidence of the initial androgenization but some effects of estrogen. The testes were reduced in size and were large and were palpable in the lower abdomen with involvement of enlarged seminal vesicles and bladder.

Transplanted Primary Tumors of the Prostate. The transplants of primary tumor grew in over 98% of cases in Nb rats. Growth was usually autonomous and not influenced by hormone treatment of the host. Metastases in the lungs could be transplanted successfully. Tissue surrounding but distal to the grossly visible carcinomatous areas of the prostate or the apparently normal hyperplastic gland (from animals after prolonged treatment) has been transplanted. Growth rarely occurred and always resembled that in the tumorous area. Well-differentiated prostatic tissue was found to remain at the implant site after more than 1 year without showing evidence of growth. Transplants of 2 primary tumors have exhibited some growth differential in estrogenized and androgenized rats but have not developed the same degree of hormone responsiveness as shown by Tumor Nb-2Pr.

Growth of Estrogen-dependent Transplants of Nb-2Pr. The original established transplant lines of prostatic carcinoma Nb-2Pr were estrogen dependent. The growth of transplants in hosts of different sex and receiving varied treatment with sex hormones is shown in Chart 1. The results include all rats of 4 different subsines tested over transplant generations 6 to 26.

Tumors grew more rapidly in EP-treated male rats, whereas there was no growth in 12 normal males. In 10 other males (Group D), transplanted tumor cells were shown to remain viable for dormant periods of 7 to 20 weeks, since cell growth resumed in all cases within 6 to 7 weeks after the rats received an EP. The resulting tumors, when transplanted, remained hormone dependent with one exception. This became a rapidly growing autonomous tumor, as shown in Chart 1. This rat had received an EP after tumor cells had remained dormant for 19 weeks. Transplanted tumors grew rapidly in EP females, although less so than in EP males. In normal females, growth was slower. In the case of 2 males treated with EP plus TPP, tumor growth was very slow, whereas no growth took place in 6 males when treated with only TPP.

Tumor Regression after EP Removal and the Effects of Replacement Treatment with a Reduced Dosage of Estrogen. Estrogen-dependent tumors stopped growing or regressed in all male rats if the EP was removed. In the case of 4 animals, if the 90% EP was replaced by a 20% EP, only partial regression took place. Typical tumor growth curves are shown in Chart 2.

Removal of estrogen from 5 rats after 6 to 8 weeks of tumor growth may be seen to have effectively stopped subsequent tumor growth. Complete regression took place, and 3 tumors became too small to palpate (Group A). After 19 to 28 weeks of regression, however, spontaneous regrowth of all the tumors commenced. Transplantation of the tumors showed that all 5 had progressed from estrogen-dependent tumors to autonomous growths (A1). In 3 other rats, the EP was removed but immediately replaced by a 20% EP (Group B). The transplants stopped growing and partially regressed in size for 4 weeks, when growth then resumed at a slow rate. Tumors which did not regress completely due to replacement with the reduced dose of estrogen remained estrogen dependent when subsequently transplanted. In 1 animal, the substitution of a 30% EP for an EP interrupted tumor growth only for 1 week.

![Chart 1. Growth of estrogen-dependent transplants of prostate carcinoma in normal and treated hosts (transplant generations 6 to 17 of Nb-2Pr-E). Limits and mean tumor growth: A, 12 EP males; B, 14 EP females; B1, 8 EP females of slower growing line; C, 6 females; D, males. All received EP after 7 to 20 weeks, and subsequent growth is shown in D1 (not included in chart, 12 males with no transplant growth at mean of 39 weeks (range, 31 to 44 weeks); 6 TPP males with no transplant growth at mean of 24 weeks (range, 20 to 28 weeks); 2 EP plus TPP males with transplants weighing 1 and 1.8 g after 60 weeks).](image-url)
Tumor Regression following Treatment with Tamoxifen in EP-treated Rats. The antiestrogen tamoxifen was administered to rats bearing an EP and a growing transplanted estrogen-dependent prostatic tumor. The responses of 2 males and 4 females to weekly injections of the drug are shown in Chart 3. The growth of the tumors may be seen to have stopped rapidly after the start of tamoxifen treatment, even though estrogen treatment was not interrupted. An initial dose of 5 mg of tamoxifen could be reduced to an effective dose of 1 mg. The cessation of treatment was followed by continued regression for 10 weeks in one male (Chart 3A). A second course of an increased dose of tamoxifen again caused a cessation of tumor growth in this rat. Eventually, however, the control of the tumor by treatment with tamoxifen was reduced, and all tumors escaped to continue slow growth. In 4 female rats (Chart 3B), complete tumor regression was found in 3 animals treated with 5 mg of tamoxifen weekly. In one rat, a dose of 1 mg prevented growth. In 2 animals in which tumor growth resumed, removal of the EP caused a slight transitory reduction in tumor growth (not shown in Chart 3). Tumors in which growth resumed despite continued tamoxifen treatment (in 2 EP males and 1 EP female) were transplanted and showed no change from the original estrogen requirement for growth.

DISCUSSION

The production of gross adenocarcinoma in the dorsal lobe of the prostate in more than 40 Nb rats has involved prolonged treatment with testosterone propionate (other androgens have not been tested). If estrogen was administered simultaneously, prostatic tumors appeared more rapidly. Giving estrogen after androgen treatment was terminated doubled the incidence of tumors to 50% in 10 rats. The rats of this group, in contrast to others, although having grossly visible tumors of the prostate, did not show evidence of excessive androgenization. The observations establish that an androgen, testosterone, was involved in the methods described to produce cancer of the prostate in Nb rats. The ventral prostate was not involved. The prolonged administration of suitable doses of testosterone alone was carcinogenic, although metabolic conversion to small amounts of estrogen cannot be eliminated (9).

The results described were collected over a number of years and contain wide variations in the duration and types of treatments. Unfortunately, it was not feasible to design experiments using large groups of rats which would have strictly comparable doses of steroids and the same duration of treatment. It is possible that the use of pellets of the hormones was an essential factor in tumor production. Drago et al. (3) have recently produced prostatic cancer in Nb rats after only 45 days of treatment using hormones in Silastic implants. Androgens have not previously been seriously considered as having carcinogenic properties, although Kirkman and Algard (6) have reported extensive experiments on hamsters in which androgens played a positive role in tumorigenesis of some organs other than the prostate. Estrogen treatment of male rats consistently produced atrophy of the prostate and has not been associated with malignant change, although treated animals have seldom survived beyond 1 year because of pituitary involvement. Estrogen, however, when combined with androgen, may have a role in the etiology of prostatic disease. The transplant lines most sensitive to hormones were developed from Tumor Nb-2Pr (= 52 Pr). The primary tumor was found in a rat 30 weeks after removal of an EP. In this period, the secretion of endogenous androgens apparently recovered (the testes were of normal size) and probably was related to tumorigenesis (10). This finding was unique, and prostatic cancer was not found in 24 other rats similarly treated. This tumor (of the 40 different primary tumors tested) proved to be the most responsive to hormone manipulation.

The first 2 transplant generations of Nb-2Pr-E grew reluctantly in rats bearing EP plus TFP. Subsequent transplants grew readily but showed the unexpected property of being estrogen dependent rather than androgen dependent and would not grow in normal males. The tumor growing most rapidly in estrogenized hosts was always used to maintain transplant generations of the estrogen-dependent tumor lines. Estrogen-dependent cells transplanted into males remained viable but dormant and could be stimulated to grow by estrogen treatment after many months of dormancy. Tumors which developed from dormant cells of the prostate under such circumstances usually remained estrogen dependent when transplanted, as found in similar studies of other estrogen-dependent tumors such as carcinoma of the breast, salivary gland, cervix, etc. (4). In a single rat, however, the growth which was...
initiated by estrogen in dormant prostate carcinoma cells proved to be autonomous. Two other examples have been noted in over 100 similar experiments using tumor cells of other organs. It appears, therefore, that in approximately 3% of such cases dormant cells may progress from hormone dependency to autonomy but not commence growth unless stimulated by an increased hormone level of the host. Kirkman and Algard (6) have also studied the activation of dormant cells in a few transplants of hormone-dependent tumor models in hamsters. A change to autonomy, however, was not noted.

Transplants of prostate tumors growing in estrogenized male hosts stopped growing and regressed following removal of estrogen. The eventual spontaneous regrowth of the tumors always indicated a change to autonomy in a fashion similar to that described for estrogen-dependent tumors of other organs (5). On the other hand, the substitution of a 20% EP as partial replacement treatment after EP removal reduced the growth rate and extent of tumor regression. Transplants of these tumors, however, remained estrogen dependent. More extensive similar experiments on tumors of the breast have shown that the autonomous change which always follows complete hormonal withdrawal in males can be delayed or prevented by treatment with doses of estrogen that are subthreshold for growth (1, 8). Tamoxifen, an antiestrogen, had a striking effect in inhibiting tumor growth, particularly so since this took place in rats which continued to receive an amount of estrogen greatly in excess of that required to support tumor growth. (A 30% EP has been found adequate to allow tumor growth.) Although the tumor regression produced by tamoxifen was prolonged (for 10 weeks in 1 rat), all tumors eventually showed regrowth. Such tumors when transplanted had retained their original dependence on estrogen and had not become autonomous. It is possible that tamoxifen in the doses used may not completely antagonize the high dose levels of estrogen but may be sufficiently effective to cause tumor regression, a situation analogous to removal of the EP and replacement with subthreshold doses of estrogen. Tumor regrowth in both settings was found to have retained hormone dependency. Tumors which ultimately escaped the effects of tamoxifen may reflect a resistance to the drug by the host or tumor cells. The preliminary results with tamoxifen suggest that treatment with this drug in the presence of estrogen may achieve prolonged regression of estrogen-dependent tumors of the prostate but not cause an acceleration of tumor progression to autonomy in contrast to that which follows surgical removal of the EP. The historical approach to the control of hormone-dependent tumors in humans has been to make every effort to remove completely the offending hormone by ablative surgery. Although prolonged regressions may be achieved, the ultimate tumor regrowth is autonomous. The control of tumor growth in Nb rats by a reduction of the hormone to allow only a submaximal rate of growth or, similarly, the use of tamoxifen prolongs or prevents the development of autonomous changes in estrogen-dependent carcinoma of the prostate.

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REFERENCES

Production of Nb Rat Carcinoma of the Dorsal Prostate and Response of Estrogen-dependent Transplants to Sex Hormones and Tamoxifen

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