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A Classification of Transplantable Tumors in Nb Rats Controlled by Estrogen from Dormancy to Autonomy

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SUMMARY

Transplantable tumor lines were previously established from a variety of estrogen-induced tumors in Nb rats, including tumors of the adrenal, cervix, salivary gland, and pancreas, a lymphoma, and a liposarcoma. Spontaneous tumors, however, were found to arise in untreated females and showed the same characteristics. Tumor growth was dependent upon or influenced by estrogen when assessed in estrogenized and unconditioned hosts. Intermittent estrogenization was effective, but tumor growth took place more slowly. The type of response observed led to a new classification of five types of hormone-responsive tumors including tumors inhibited by estrogen. Estrogen-dependent tumor cells might remain dormant indefinitely and not grow in unconditioned animals until stimulated to grow by estrogen. The growth rate of hormone-dependent adrenal carcinomas was related to the amount of estrogen. Tumor growth started more rapidly in the presence of low estrogen dose levels in old rats used as hosts than it did in young rats. Breast carcinomas required the largest amount of estrogen for growth, whereas ovarian thecomas would grow in normal females but not in males. The growth rate in conditioned hosts of most transplanted tumors (some have maintained hormone dependency over 10 years) increased over successive generations. Progression, however, towards a more autonomous state after repeated transplantations was remarkably slow, and a sudden change to autonomy was rarely noted. In contrast, transplants of 9,12-dimethylbenz(a)anthracene-induced mammary carcinomas progressed rapidly to autonomy. Fould's concept of progression (2, 3) has been discussed but the described classification of tumors under hormone influence apparently allows a more detailed analysis of definition of different types of progression.

INTRODUCTION

A varied spectrum of transplantable tumors arising after prolonged estrogenization in NB rats has been described (4) and forms the basis for the present study. Tumors were found in 14 organs, many of which are not considered to be under estrogen control, but they could be transplanted successfully only into hormone-conditioned hosts.

This paper continues these observations and shows that such transplants may remain dormant in the absence of estrogen or grow proportionately to the amount of estrogen given to the host. The growth behavior of transplanted tumors to estrogenized and unconditioned rats has led to a general classification of 5 types which include all tumors under estrogen influence. With this classification, it has been possible to follow and define conditions under which progression towards autonomous growth takes place over repeated transplant generations. The observations are discussed in relation to Fould's concept of tumor progression.

MATERIALS AND METHODS

The methods used have been described in a previous paper (4). Transplanted tumor lines of the most rapidly growing tumors were maintained in hosts estrogenized by 90% estrone pellets, and every 3rd or 4th generation was usually transplanted to groups of 2 to 3 unconditioned females or males. The former allowed a normal ovarian function to affect tumor growth, whereas males or ovariectomized females were considered to be unconditioned. Pellets of other concentrations of estrone were made using additional cholesterol as a diluent. Ten and 20% estrone pellets usually had no effect on body growth of testis weight in males, whereas 30% pellets were effective, releasing approximately one-fifth the amount of estrone as did at 90% EP.[3] For some experiments 90% testosterone propionate pellets were prepared in the same way as were estrogen pellets. All established tumor lines transplanted and grew successfully in over 95% of rats. Occasionally, transplants were made from tumor cell homogenates. These were prepared by gentle homogenizing with the addition of a minimum amount of Hanks' solution and then filtered through 3 layers of gauze. The resultant suspension could be administered i.v. through a 26-gauge needle without toxic reaction. Caliper measurements of s.c. tumors were recorded as the maximum width and breadth in cm. The sum indicated tumor size, and the equivalent tumor weight was obtained from graphs constructed from a large series of measurements and weights of dissected tumors obtained at autopsy. Estrogen-inhibited tumor lines were maintained by transplantation into normal male rats. DMBA in sesame oil was administrated by stomach tube.

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[3] The abbreviations used are: EP, estrone pellet; DMBA, 9,12-dimethylbenz(a)anthracene; HD, hormone dependent; HS, hormone stimulated; HR, hormone retarded.

RESULTS AND COMMENTS

Factors Affecting the Initiation and Growth of Transplanted Tumors

Time of Conditioning. As a routine an EP was inserted at the same time that the tumor was transplanted. For comparison, transplants of adrenal carcinomas were made to 4 groups of 3 rats that had received an EP 4 to 6 months previously. The initiation and growth of the transplants did not differ significantly from controls pelleted at the time of transplantation. Conversely, transplants did not grow in rats previously estrogenized but with pellets removed.

Continuous *versus* Intermittent Estrogenization. Groups of 3 male rats received simultaneously an adrenal tumor transplant and an EP. Others were treated in the same way, except that the EP was surgically removed for 2 and 5 days, respectively, every week. The continuously treated animals developed 5-g tumors in 34 days. Those treated for 5 days a week required 41 days to develop the same size tumors, and those receiving estrogen for only 2 days required 55 days, a delay of 61%. Repeated operations in a control EP group did not delay tumor development. Intermittent pelleting with a high dose of estrogen did not prevent tumor growth but delayed it. A similar study using female animals and pelleting intermittently for 5, 4, and 2 days/week was performed for 4 weeks, and then the pellets were left undisturbed. The tumor transplants of all animals grew at rates not significantly different from the group which received continuous estrogenization. The difference in response of male and female animals to intermittent pelleting may result from ovarian stimulation in the females.

Dormant Cells. In a preliminary experiment using a HD adrenal carcinoma, the effects of delayed pelleting for 1 and 3 weeks after tumor transplantation were determined in groups of 3 rats. Tumor growth commenced after a delay of 3 and 10 days, respectively, when compared with rats pelleted at the time of transplantation. Since tumor cells apparently survived for 3 weeks in an unconditioned host, the time of pelleting was extended in other experiments (Table 1).

Adrenal tumor transplants of 13 different tumors remained dormant and did not grow in 46 rats (35 females and 11 males) but were stimulated to grow when an EP was inserted from 3 to 13 months after transplantation. In 2 cases, a 20% EP also initiated growth after 20 weeks of dormancy. In each case a control rat did not exhibit any tumor growth when killed at the same time or later than its pelleted counterpart. Similarly, tumor cells injected into 1-day-old rats remained dormant until stimulated by estrogen to grow. Although tumor cells remained dormant for 9 months or more, they commenced growth promptly after

Table 1

Stimulation of growth by estrogen of dormant transplants

The table includes 72 tumor transplants; 4 to 11 transplants were made from the adrenal tumors, and 1 to 8 transplants were made from the other tumors.

Type	No. of tumors	Dormant period (av. wk)	Results of EP stimulation: EP stimulation (av. wk)	Results of EP stimulation: Resulting tumor growth (av. g/4 wk)
Adrenal carcinoma[a]	3	11	13	3.6 {13.2 / 0.04}
	4	15 {19 / 12}	20	4.6 {15.6 / 0.4}
	6	27 {30 / 20}	16	7.6 {18.0 / 0.4}
	4	36 {37 / 35}	15	0.8 {2.8 / 0.04}
	4	46 {48 / 43}	13	0.4 {1.4 / 0.04}
	3	53 {57 / 54}	10	0.4 {1.0 / 0.12}
Breast carcinoma	4	25 {31 / 18}	15	3.6 {8.0 / 0.16}
Uterus leiomyoma	2	32 {36 / 28}	37	9.5 {18.8 / 0.28}
Ovary thecoma	3	23 {38 / 10}	9	6.0 {15.6 / 0.6}
Salivary gland carcinoma	2	28 {36 / 20}	22	5.0 {7.5 / 2.5}
Cervix carcinoma	1	21 {26 / 13}	11	3.8 {6.4 / 2.0}
Anterior pituitary carcinoma	1	44	17	0.5
Leydig cell carcinoma	1	37	4	4.3
Hormone inhibited		EP removed after		
Anterior pituitary carcinoma	3	25 {33 / 8}	11	1.3 {2.3 / 0.5}

[a] A total of 13 different tumors.

pelleting; subsequent growth, however, continued slowly, so that the tumors rarely attained a large size. In 17 cases tumors that developed (even after 10 months of dormancy) were transplanted and were found to have retained hormone dependency, growing only in conditioned hosts.

HD tumors of other organs showed the same property. Transplants of 4 different mammary tumors were stimulated to grow, even after periods of dormancy of up to 31 weeks. Similar findings are shown for tumors of the uterus, ovary, salivary gland, cervix, pituitary, and Leydig cells of the testis. In all cases control animals did not show spontaneous tumor growth. Five tumors of 4 different organs that were stimulated to grow were transplanted and retained hormone-dependent properties. In addition, 3 pituitary tumors that were inhibited by estrogen for 8 to 33 weeks commenced growth after the EP was removed (although 8 tumors failed to grow). Histological sections of clumps of dormant cells did not indicate any evidence of cell multiplication or of an infiltrative cellular reaction.

Amount of Estrogen. Data are presented on the effects of estrone pellets of varied concentrations on the growth of a HD adrenal tumor. This tumor was selected since slow growth occurred in most normal female rats without treatment, but not in males or ovariectomized females. Chart 1 summarizes tests on female animals 3 to 6 weeks of age.

Pellets containing 30 to 90% estrone were equally effective in initiating and stimulating rapid growth (Chart 1, *Area A*). If 2 or 3 90% EP were used simultaneously, no difference in the tumor growth rate was noted. When pellets contained only 20 or 10% of estrone, however, the start of tumor growth was increasingly delayed and tumor growth was correspondingly slow (Chart 1, *Areas B and C*) Tumors in 2 of 7 rats with 20% pellets grew more rapidly, and 2 animals with a 10% EP did not develop tumors. In the case of untreated animals, tumor growth occurred even more slowly in 26 of 31 rats, but only after a protracted period (Chart 1, *Area D*). In 5 cases tumor growth was more rapid, similar to animals bearing a 10% EP. The slow-growing tumors that developed in many of the various groups of rats were transplanted and in every case retained hormone dependency. When one compared the effectiveness of 10 and 20% EP in similar experiments but with male rats, it was found that growth was more limited and did not occur in the life-span of the animal with a 10% EP, although a 20% EP caused slow tumor growth in some instances.

In the case of estrogen-dependent tumors of other organs, the evidence at present suggests that those of the salivary gland, cervix, uterus, and Leydig cells responded to the same estrogen levels as did arenal carcinomas. Tumors of the pituitary, pancreas, and particularly the ovary appeared to require less estrogen for growth and usually grew in normal female animals. Carcinomas of the breast required the greatest amount of estrogen, and growth did not occur in 5 of 6 different tumors even in the presence of a 20% EP.

Age of Host. It has been frequently noted that transplants of dependent adrenal tumors might grow in unconditioned old female rats, but very slowly in young females. When the

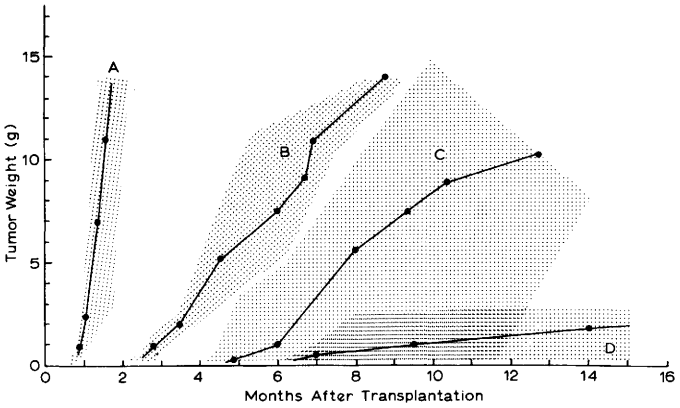

Chart 1. Limits and average tumor growth in young female rats treated with different levels of estrogen. *Area A*, 24 rats with 30 to 90% EP; *area B*, average 5 of 9 rats with 20% EP; *area C*, average 24 of 24 rats with 10% EP; *area D*, average 26 of 31 normal females.

same adrenal tumor was transplanted simulatenously to 15 pairs of young (3 to 5 weeks) and old (6 to 12.5 months) females at an average age of 9.8 months, the older partners showed tumor growth at an average of 6.8 months (2.3 to 11.0 months) sooner that did the younger animals. The tumor did not grow in either young or very old unconditioned male rats. Similarly, tumor growth was more rapid in old females when compared with young animals, conditioned by a 20 or 10% EP. With a 30% EP, growth was at the same rapid rate in rats of any age. Tumor growth was measured in a similar manner as indicated for young rats shown in Chart 1. In 6 of 8 old rats with a 20% EP, tumor growth was more rapid and fell within the limits shown in Chart 1, *Areas A* and *B*. Similarly, with a 10% EP, the growth patterns of 6 of 9 rats fell in *Area B* (for a 20% EP in young rats) and the others in *Area C*. In 23 old untreated rats 11 tumors grew at a rate within the limits of *Area B* and 12 grew within the limits of *Area C*.

Sex. The relatively greater requirement for estrogen of male rats to allow tumor growth comparable with females was attributed to androgen-estrogen antagonism and could also be demonstrated with higher doses of estrogen. In 2 male EP rats an adrenal tumor transplant averaged 19 g in 4.5 months. In 3 EP males, which also received 2 90% testosterone propionate pellets (which were replaced once), tumors in 12 months weighed only 16 mg. No growth occurred in normal males in 13 months. This and other experiments indicate that, in male rats or in rats treated with testosterone, the effectiveness of estrone in inducing tumors or in stimulating their growth was reduced. In only 1 of 75 adrenal tumors could a direct inhibiting effect of androgen on tumor growth be demonstrated. This tumor did not grow in normal male rats but did so in castrates, and such growth could be inhibited by androgen treatment.

Initiation *versus* Growth of Transplant. The prolonged time before adrenal tumor transplants became palpable in rats with low doses of estrogen may be seen in Chart 1. In contrast, 12 transplants of 5 different adrenal carcinomas (from 6- to 45-generation transplants) in male rats had the EP removed when the tumors averaged 10 g (by calcula-

tion), and a 20% EP was substituted. In every case tumor growth continued uninterrupted and 5 weeks later averaged 15 g in weight. In 6 cases even a substituted 10% EP was followed by growth, but at a slower rate. In other experiments 2 salivary gland carcinomas, a carcinoma of the cervix and a leiomyoma of the uterus, showed the same response as adrenal carcinomas. Breast carcinomas were exceptional and showed a greater requirement for estrogen. In a comparable experiment with 20% EP in 16 transplants of 9 different tumors (from 10- to 25-generation transplants), the tumor size averaged 14 g but regressed to 3.2 g in 7 weeks, despite estrogenization, with only a single exception.

Classification of Transplanted Tumors by Their Growth Response to Estrogen

From the study of all tumors throughout their transplanted lifetime, it has been possible to formulate an all-embracing classification of types of tumors. To establish the category of a tumor, the tumor was transplanted to unconditioned and conditioned young animals and its growth was noted. Conditioning was by a 90% EP.

Type I: HD. Transplanted tumor cells survived but did not grow in the absence of adequate doses of estrogen. The rapidity of growth after estrogenization was proportional to the dose.

Type II: HS. Transplanted tumor cells always grew more rapidly in the presence of estrogen, but slower growth occurred in its absence.

Type III: Hormone Autonomous. Transplanted tumor cells grew at an equally rapid rate in the absence or presence of estrogen.

Type IV: HR. Transplanted tumor cells always grew more slowly in the presence of estrogen, but faster growth occurred in its absence.

Type V: Hormone Inhibited. Transplanted tumor cells survived but did not grow in the presence of suppressive doses of estrogen.

In this assay, animals were observed over their life-span to determine whether tumor growth occurred with or without estrogen and the rate at which growth took place. For practical purposes, the duration of the assay may be shortened, as in the initial paper (4). However, with many HS tumors growth may be extremely slow in unconditioned rats. In a recent test, an adrenal carcinoma grew to an average size of 10 g in 2 male EP rats in 4 months. In unconditioned males, however, the tumors averaged only 160 mg at postmortem 1 year after transplant. If the test had been concluded earlier, at 8 to 9 months, the tumor would have been classed as Type I, as no significant growth would have been found in the unconditioned animals. Since HS (and HR) tumors grew in unconditioned as well as in estrogenized rats, repeated transplants from conditioned or unconditioned animals were feasible. In comparative studies, even when transplants were made from unconditioned hosts, the HS status was usually maintained for many generations, although with increasing growth rates. Transplants of the most rapidly growing tumors from conditioned rats bearing HS tumors, on the other hand, frequently led to a return to the HD status, presumably through an exclusive selection for transplantation of HD cells growing more rapidly under the influence of the EP.

Progression Occurring in Tumors during Consecutive Transplant Generations

Rate of Growth. Since most tumors were routinely transplanted when the most rapidly growing one of a group reached 10 to 20 g in a conditioned host, the intervals between transplants served as a rough indication of the rate of growth of the tumor. Such values have been presented in Chart 2 and the tumors divided into slower and faster growing groups.

A rapid increase in the rate of growth of all transplants occurred in the 1st few generations. After 10 generations the growth rate in most tumors continued to increase but much more slowly. The average growth rate shown for groups of adrenal and breast tumor transplants continued to increase gradually. In a few tumors, such as 6 sublines of the Leydig cell carcinoma and hormone-inhibited pituitary tumors, the growth rate of successive transplants has changed very little.

Changes in Type. Many of the older tumors maintained by transplants from EP hosts for many years have been reviewed to determine what spontaneous progressive changes in type towards autonomy may have occurred. Data have been summarized in Table 2.

Most tumors have retained their original hormonal status

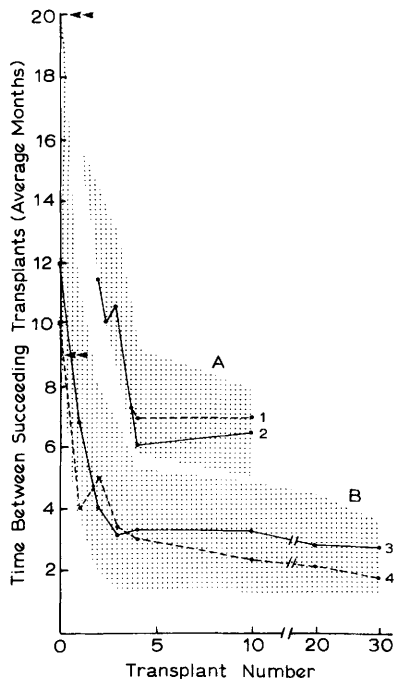

Chart 2. Time between transplant generations of slower and faster growing HD tumors. *Arrows*, range of ages of rats bearing all primary tumors. *Area A*, extremes of time between transplants of slower growing tumors (*Curve 1*) and Leydig cell carcinoma (*Curve 2*); average of 2 estrogen-inhibited pituitary carcinomas. *Area B*, extremes of time between transplants of faster growing HD tumor (*Curve 3*); average of 14 adrenal carcinomas (*Curve 4*); average of 8 breast carcinomas.

Table 2

Growth and spontaneous changes in type of repeatedly transplanted tumor lines

Tumor origin	Generations (av. no.)	No. of tumors	No. of total transplants[a]	Initial type	Observed changes in types: No. of tumors	Total no. of transplants	No. of generations	Final type
Adrenal cortex[b]	19 {47, 10}	22	210	HD	4	4	4–35	HS (F only)[c]
					2	2	10–19	HS
	12 {24, 7}	7	48	HS	Unchanged			
Breast	15 {26, 8}	14	172	HD	3	8	4–19	Hormone autonomous
					1	2	6 7	HS
Cervix	28	1	49	HD	1	1	11	HS (F only)
Uterus (leiomyoma)	11	1	10	HD	1	1	6	HS (F only)
Leydig cell	11	1	39	HD	Unchanged			
Salivary gland	17 {20, 14}	2	43	HD	Unchanged			
Lymphoma	36	1	94	HS	Unchanged			
Liposarcoma	7	1	8	HD	1	All	6	HS
Ovary (thecoma)	30 {41, 14}	2	109	HD	2	2	19–28	HS
Pancreas	8	1	10	HD	Unchanged			
Thymus carcinoma	11	1	4	HD	1	All	7	HS
Vagina fibrosarcoma	5	1	3	HS	Unchanged			
Orbit fibroadenoma	7	1	4	HD	1	All	6	HS
Anterior pituitary	6 {8, 3}	8	48	HD	7	All	8	HS
					1	1	8	Hormone autonomous
	16 {21, 12}	5	80	HS	Unchanged			
	21 {31, 11}	2	92	Hormone inhibited	1	4	13 19	HR

[a] Number of transplants to groups of 2 to 4 unconditioned rats in which changes would have been apparent.
[b] Includes transplanted line of an ovarian metastasis.
[c] F, female.

over many generations and some tumors, such as adrenal and hormone-inhibited tumors of the pituitary, have now been maintained for 10 years. Similarly, even tumors of the partially autonomous HS category have not changed in type when transplanted from EP hosts (adrenal tumors and a lymphoma) in 3 years. In relatively few instances, a sudden change to autonomy was noted, usually only in a single animal of a group bearing the same transplant. In Table 2 the total number of tumor transplants are listed (each usually represented 2 to 4 animals) in which any change towards autonomy would have been detected. The most extreme change from dependency to autonomy occurred almost exclusively in breast carcinomas. The resulting autonomous tumors grew rapidly but showed an altered morphology to a scirrhous type of growth containing few recognizable epithelial elements. Carcinomas of the anterior pituitary were exceptional in that all rapidly changed from HD to HS type and in 1 case to an autonomous tumor. Once the tumor became HS, however, it apparently remained so for many generations. Hormone-inhibited tumors either remained unchanged or gradually became more autonomous, changing to the HR type. Although an abrupt change to autonomy seldom took place, a slow, gradual progression was found in many tumors. This consisted of increasing growth rates and a lesser requirement for estrogen, as shown by most tumors originally not growing in unconditioned females but eventually doing so; many, however, did not progress to the HS stage of growing slowly in males. HS tumors, when transplanted repeatedly from unconditioned hosts, showed a more consistent and predictable although gradual progression of growth, which became more rapid in unconditioned animals.

DMBA-induced Breast Tumors. A single comparative study of mammary tumors induced by DMBA was conducted on 40 females weighing an average of 138 g (135 to 140 g) which received a single p.o. dose of 15 mg of the carcinogen in oil. One-half of the rats received an EP 10 days later. Multiple mammary carcinomas arose at the same time in all animals of both groups surviving 7 months. Transplants of 5 tumors arising in the estrogenized rats failed to grow in groups of 4 EP animals. In contrast, 6 tumors induced with DMBA alone transplanted readily to all rats and 4 were maintained by continuous transplants. Of these, 3 were autonomous in the 2nd generation (adenocar-

cinomas). One tumor was initially HD, but after 13 generations (in 38 months) all transplants had progressed to autonomy.

DISCUSSION

This paper is a continuation of a more detailed study of a variety of transplantable HD tumors, induced by estrogen, described previously (4). The tumors arose in a bizarre spectrum of 14 organs, including the adrenal cortex, salivary glands, cervix, ovary, pancreas, and a lymphoma and liposarcoma. The unusually successful establishment of continuing HD models was probably related to the selection of the most rapidly growing dependent tumor cells from transplants in heavily estrogenized hosts. Hormone dependency of most tumor transplants has lasted indefinitely over the 10 years since the study commenced.

The amounts of estrogen used in the experiments, a 90% EP, provoked a rapid growth of susceptible cells resulting in primary tumors, but once these were transplanted, they could then be maintained by much lower levels of estrogen. Growth rates were the same when conditioning was by pellets containing from 90 to 30% estrone. Even intermittent pelleting allowed growth, but at a slower rate. Multiple 90% pellets did not inhibit tumor growth. With lower concentrations of estrone, however, the growth rate of adrenal tumors was reduced proportionately. When HD adrenal, mammary, and other tumor cells were transplanted to unconditioned male or female animals, the grafts remained viable but dormant and did not grow until they were stimulated to do so by the insertion of an EP, even after periods of 14 months. Similarly, tumor cells inhibited by an EP remained dormant and commenced growth only after the EP was removed. Animals bearing dormant cells were indistinguishable from normals and presumably would survive a normal life-span without tumor growth; their tumor-producing potential could only be ascertained by the effect of estrogenization. Fisher and Fisher (1) have found that small inocula of 50 to 250 sarcoma cells apparently could survive but remain dormant in rats until triggered into growth by surgical operation. In adrenal tumor cells, however, larger inocula of at least 10 M did not grow in the absence of estrogen. The dormant state of the endocrine tumor cells would appear closely to resemble the situation of some tumor metastasis in humans which may commence growth many years after the removal of the primary lesion.

From a study of many hundreds of transplants of the various tumors described in which growth was influenced by estrogen, it has been possible to suggest a new classification. The types of response have been determined by the ability of transplants to grow in conditioned hosts, in contrast to normal or hormone-deprived animals. This quantitative type of assay of the amount of estrogen required to affect tumor growth (either directly or indirectly) allowed a comparison of tumors of different organs and a study of changes occurring over successive transplant generations. Mammary carcinomas apparently required the largest amounts of estrogen for growth, but some tumors, such as ovarian thecomas, were so sensitive that it might be questioned that even "autonomous growth" may be a reflection of immeasurably minute amounts of estrogen present in unconditioned controls. An unexpected finding was the ability of transplants of some adrenal tumors to commence growth much sooner in old unconditioned females than in young ones, suggesting a lower resistance of some type in the aged animals.

The concept of progression of various tumor characteristics leading to autonomous uncontrolled growth is well established now since the classical observations by Foulds (2, 3) on mammary tumors in mice. The results described of progressive changes over extended transplant generations of tumors originating in estrogenized hosts are obviously examples of the phenomena described by Foulds, but now it seems possible to define definite stages of progression more precisely. However, HD tumor cells in unconditioned animals may remain dormant and not show progressive growth in the rat's life-span. As was shown, all 46 HD tumors of 10 different organs transplanted over many generations to over 2000 rats exhibited an increase in growth rate, although this occurred very slowly in some tumors. Since the most rapidly growing tumors were always transplanted from EP rats, this could represent a process of selection rather than progression. Remarkably few tumors showed the expected sudden change from dependency to autonomy and this was confined almost exclusively to breast carcinomas, the type of tumor requiring the largest amounts of estrogen for growth. Most transplanted tumors reported by others in rats if initially under hormone influence have rapidly become autonomous, which could be due to the fact that initial transplants contain a mixed population of cells of differing hormone dependency (although pituitary tumors may be exceptional and show rapid progression). In the method described, a "cloning" of the most rapidly growing tumor cells in estrogenized animals in initial generations has been a prerequisite to establish HD cell lines. Transplants of tumors induced by other means such as DMBA may, however, rapidly progress to autonomy.

Hormone-inhibited tumors, although not studied as extensively, also showed the same type of progressive changes as do HD tumors, but the growth response to EP was reversed. Cells remained dormant in EP animals but grew if the pellet was removed. Transplanted tumors (hormone inhibited) initially inhibited by estrogen progressed, so that slow growth occurred in its presence (HR tumors). Progression in such cases similarly led to a more autonomous growth which took place under the influence of less estrogen.

Progression of HD cell lines maintained in EP hosts was very gradual and was usually seen only as a change to growth becoming possible in unconditioned females (but not in males). Only rarely did progression to the HS type occur with transplants growing slowly in males. Some transplants, however, over successive generations from EP rats showed growth in unconditioned males and a gradually increasing growth rate. For descriptive purposes, such progression in estrogenized hosts has been termed "hormonal progression." This represents the most gradual and minimal change that has been found in all tumors progressing towards

autonomy. It would appear, however, to be most readily demonstrated in tumors requiring the largest amounts of estrogen for growth.

Once a tumor became a HS type, it was possible to maintain transplants for either estrogenized or unconditioned males. In the case of the former, hormonal progression either continued or in many cases the transplants reverted to a HD type, presumably by cell selection. Transplants from unconditioned hosts may show a gradual increase in growth rate over 5 to 15 generations before being classed as autonomous. Such a change has been termed "spontaneous progression" and although representing a more predictable and rapid type of progression, still remains a gradual process. Spontaneous progression would appear to exemplify Foulds' definition of progression as a "stepwise irreversible qualitative change towards autonomy, which occurred in the absence of the hormone." To complete the study of progression, a subsequent paper will describe "induced progression," a predictable change in a single generation following manipulation of hormone levels in hosts bearing any of the described types of HD or hormone-inhibited tumors. In these studies we have found that, when a large tumor mass is caused to regress by the removal or addition of hormone, autonomous change occurs. Studies in progress indicate that this type of change may be prevented by not allowing complete regression of the tumor.

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