The Transplantability and Growth of Urethan-induced Pulmonary Adenomas of the Mouse*

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It has been observed that implants of small primary tumors are less transplantable and grow more slowly after "taking" than those of larger ones. Browning (5) transplanted spontaneous mammary tumors from strain C3H mice isologously to the eye and observed that many of the 1–2.5-mm. tumors were not transplantable. However, grafts from all the larger tumors grew following implantation. With increasing age and progressive enlargement of the primary tumors from which grafts were prepared, the incidence of "takes" and the extent of tumor growth in the anterior chamber increased, and the time required to half fill the chamber decreased. Martinez *et al.* (9) transplanted spontaneous mammary tumors of mice subcutaneously and observed fewer "takes," and these after a longer time interval, when implants were prepared from smaller as compared with larger tumors. In addition to these experiments with mammary tumors, a preliminary observation[1] on the transplantability of primary lung tumors of the mouse suggested that the incidence of "takes" was lower for grafts prepared from small as compared with large tumors. In the present study, induced primary lung tumors of different sizes were again transplanted to confirm this initial observation, to obtain information on the time required for the transplants to "take," and to follow their subsequent growth.

It is well known that some animal tumors, e.g., of the skin, breast, ovary, thyroid, pituitary, and bladder, grow in hosts treated with hormones but not in untreated hosts. These tumors have been referred to as dependent or conditioned in contrast to others that require no hormonal stimulation for growth (8). The conclusion by a number of investigators that pulmonary adenomas of the mouse are relatively benign or progress through a benign stage on their way to malignancy (6, 7, 10–13) suggested to this investigator that these tumors, too, might be dependent during one phase of their development. In view of this possibility and the known enhancing effect of hormonal treatment in the transplantation of some dependent tumors, some of the mice in the present experiment were treated with estrogen in an attempt to increase the transplantability of lung tumor grafts and/or subsequent tumor growth.

MATERIALS AND METHODS

A 10 per cent solution of urethan in distilled water was injected intraperitoneally into male CAF_1/JAX hybrid mice, 3 times weekly for 6 weeks, each mouse receiving 0.01 ml. of solution/gm body weight. The mice were sacrificed 1 year after the first injection. Their lungs were removed and rinsed briefly in sterile physiologic salt solution, and the tumors were excised under a dissecting microscope, care being taken to free them as completely as possible from surrounding lung tissues. The isolated tumors were placed into four separate petri dishes according to tumor size, as follows: 0.5–1 mm., 1–2 mm., 2–3 mm., and >3 mm. A piece of tumor approximately 1 mm. in size was transplanted by trocar subcutaneously in the pubic region to 2–3-month-old male CAF_1 hybrid mice. In total, 179 tumors from nineteen donors were transplanted, each mouse receiving one transplant. Only 156 mice are included in the data; the remainder died early or could not be studied because of post mortem changes. The 156 mice were divided into two groups, 83 that were treated with estrogen and 73 untreated controls. Estrogen treatment consisted of α-estradiol benzoate[2] which was injected subcutaneously in the interscapular region—15 μg/injection. The mice received one injection 1 day prior to tumor transplantation and thereafter one injection weekly for

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the next 3 months. The mice were inspected weekly, and when tumors were present the two largest diameters were recorded. Animals were sacrificed after an observation period of 4 months from the time of implantation and all visible tumors removed. Each tumor was again measured and the data included in Table 2 under "tumors at autopsy." Latent period is defined arbitrarily as the time in days for a graft to reach a diameter of approximately 2 mm. In those instances in which no tumors were palpable at time of autopsy, the subcutaneous tissues at the site of transplantation were searched, and questionable masses were excised for microscopic examination. The presence or absence of tumor tissue in each specimen was confirmed histologically.

At the end of the experiment the disposition of each graft in the 0.5–1 mm. to >3 mm. primary groups was recorded under three headings: (*a*) established—those grafts with at least one diameter measuring 2 mm. or more; (*b*) persistent—those grafts in which the largest diameter measured <2 mm.; and (*c*) non-"takes"—grafts that showed no microscopic evidence of pulmonary adenoma, or grafts that were not recovered. The totals for established and persistent grafts were combined in calculating incidence of "takes."

TABLE 1

TRANSPLANTABILITY AND GROWTH OF PRIMARY PULMONARY ADENOMAS IN STRAIN CAF_1/JAX HYBRID MALE MICE

	Primary tumor (largest diameter)			
	0.5–1 mm.	1–2 mm.	2–3 mm.	>3 mm.
Total number implants	43	38	34	41
Incidence of "takes": number (per cent)*	21 (49)	27 (71)	32 (94)	41 (100)
Disposition of implants:				
Established: number (per cent)	9 (21)	19 (50)	27 (79)	41 (100)
Persistent: " " "	12 (28)	8 (21)	5 (15)	
Non-"takes" " " "	22 (51)	11 (29)	2 (6)	
Latent period, av. days	84	70	64	59
Tumor diameters at autopsy, av. mm.	2.6×2.0	3.6×2.7	6.4×5.1	9.6×8.1
Observation period, all mice, av. days	120	122	122	116

* Includes implants that became established and those that persisted.

RESULTS AND DISCUSSION

The incidence of "takes" for the transplants from the different primary tumor groups and the growth of the "takes" are given in Table 1. Treatment with α-estradiol benzoate had little or no influence on the incidence of "takes," latent period, or tumor diameter at autopsy for the 0.5–1-mm. to >3 mm. primary tumors that were used in transplantation. Consequently, the data for the estrogen-treated and untreated mice were combined. Examination of these combined results shows that, with increase in diameter of the tumor from which grafts were prepared, (*a*) the incidence of "takes" increased, e.g., 21 out of 43 grafts (49 per cent) "took" in the 0.5–1-mm. primary tumor group as compared with 41 out of 41 (100 per cent) in the >3-mm. primary tumor group; (*b*) the number of established tumors increased from 21 per cent for the 0.5–1-mm. primary tumor group to 100 per cent for the >3-mm. tumor group; (*c*) latent period decreased, e.g., it required an average of 59 days for grafts from the >3-mm. primary tumor group to reach a diameter of 2 mm., as compared with an average of 84 days for grafts from the 0.5–1-mm. tumor group; and (*d*) tumor diameter at autopsy was increased—the two largest diameters in the 0.5–1-mm. tumor group averaged 2.6 mm. × 2.0 mm., while in the >3-mm. group the two diameters averaged 9.6 mm. × 8.1 mm. after observation periods of 120 and 116 days, respectively. Thus, the transplantation of grafts derived from the larger primary lung tumors resulted in a greater number of "takes," a shorter latent period, and tumors with larger diameters at the end of the experiment than when grafts from the smaller lung tumors were transplanted, although in a number of instances exceptions were observed to this relationship (see donor numbers 9, 18, 25, and 36 in Table 2).

In evaluating the results summarized in Table 1, the possibility must be considered that technical difficulties, in part, may have been a contributing factor. Although primary tumors were excised and grafts prepared with care, it is still possible that the grafts derived from the smaller tumors contained relatively more nontumorous to tumorous tissue than those derived from the larger tumors; also, that any mechanical injury introduced during excision of tumors and in preparation of grafts may have affected the smaller tumors to a greater extent than the larger ones. It is recognized, in addition, that the grafts transferred from the 0.5–1-mm. primary tumor group were smaller in size than those prepared from the larger primary tu-

mors. Although these technical difficulties may well have contributed to the final results for the 0.5–1-mm. primary tumor group, it is less likely that they significantly influenced the behavior of those transplants derived from the larger primary tumors (Table 1).

The mice in the present experiment were all given the same treatment with urethan, with the result that numerous lung tumors of different sizes were induced. All the tumors were harvested at the end of 1 year in preparation for transplantation. There is no way of knowing whether the tumors seen at autopsy were all initiated at approximately the same or at different times and whether they differed in rate of development. The fact that variations in tumor diameter were observed at the time they were harvested is probable evidence that differences existed in both rate of development and in growth potential. It would appear from this that, if primary tumors of a particular size, e.g., 0.5–1 mm. in diameter, were harvested and grafts transplanted at 4 months after the start of urethanization rather than at 12 months, as in the present investigation, the incidence of "takes," latent period, and subsequent tumor growth might have differed significantly for these two groups. Because induced lung tumors of a particular size may differ in rate of development and in growth potential depending upon the time that the tumors are excised for study, one cannot state what relationship the results in the present investigation bear to the general problem of primary lung tumor development *in situ*.

A general increase in tumor growth rate has been observed when lung tumors are transplanted serially (2, 3). This also was observed in the present investigation, since many of the grafts which had been prepared from 2–3-mm. and >3-mm. primary tumors and which were transplanted subcutaneously attained diameters as large as 12–22 mm. during a 4-month period of observation (Table 2). If, instead of being excised at the end of 1 year, the primary tumors had been permitted to grow *in situ* for an additional 4 months, it is unlikely that they would have enlarged to the same extent as did the grafts following transplantation, although it is recognized that an occasional one might have done so. Allen (1) induced lung tumors in mice with injections of urethan and observed after as long as 15 months that some of the tumors occupied a considerable portion of the lungs. Since multiple primary tumors doubtless were induced (1), it is possible that fusion of adjacent nodules contributed to total tumor mass in this experiment. The factors responsible for the apparent increase in growth rate following tumor transplantation in the present experiment are not known. One may speculate that the increased rate resulted from: (*a*) a process of selection; assuming the grafts were composed of tumor cells of varied growth potential, one might expect that those with more rather than less potential would survive the rigors of transplantation; or (*b*) a greater availability of nutrients or enhancing substances, or a

TABLE 2

LATENT PERIOD AND SUBSEQUENT GROWTH OF "TAKES" FOLLOWING TRANSPLANTATION OF PRIMARY LUNG TUMORS INTO STRAIN CAF_1/JAX HYBRID MALE MICE

Includes primary tumors whose grafts reached a diameter of 6 mm. or more during the experiment

Primary tumors		Tumor "takes"	Tumors at autopsy		Total observation
Tumor donor (no.)	Tumor diameter (mm.)	Latent period (days)	Two largest diameters (mm.)	With sarcomatous areas	period (days)
1	3	55	15× 8	–	126
	3	62	9× 9	–	
	3	69	11× 9	–	
2	1–2	55	8× 5	–	126
	2–3	62	8× 5	–	
	3	62	8× 6	–	
	3	62	7× 6	–	
3	3	51	10×10	+	115
	3	58	7× 6	–	
4	3	51	6× 4	–	122
	3	86	12×11	+	
7	1–2	69	6× 5	–	126
	2–3	69	8× 4	–	
	3	62	16×12	–	
9	2–3	51	16×14	+	115
	3	44	11×11	–	
	3	51	10× 9	–	
	3	58	9× 7	–	
13	2–3	49	7× 7	–	113
	3	42	22×18	–	63
	3	42	15×13	+	84
	3	42	12×12	+	113
	3	49	15×14	+	99
	3	49	10× 7	–	60
18	2–3	28	10× 6	–	124
	3	63	7× 7	–	
19	3	49	8× 7	–	124
	3	71	6× 6	–	
25	2–3	42	12×10	+	124
	2–3	71	6× 5	–	
	3	63	10×10	–	
30	3	28	14×11	+	124
	3	28	7× 7	–	
	3	56	11×10	–	
	3	56	10×10	–	
36	2–3	51	20×11	–	122
	2–3	51	19×15	–	122
	2–3	65	7× 7	–	122
	3	51	18×18	+	72
	3	51	15×10	–	122
	3	51	13×10	–	122
	3	58	10×10	+	122
	3	58	10× 9	–	122
	3	58	10× 6	+	122

release from growth-inhibitory factors in the mice bearing the transplants, as compared with those bearing the primary tumors.

Sarcomatous areas of varying extent have been observed in pulmonary adenomas of the mouse following serial transplantation but not in the primary tumors (1–4, 14). A number of primary tumors were examined in the present experiment, and no sarcomatous areas were observed. Sarcomatous areas were noted, however, among some of the tumors that developed following transplantation. Although serial sections were not prepared, an examination, generally, of several slides per tumor showed that these areas were associated with the larger, rather than the smaller, subcutaneous tumors (Table 2). This may be explained if, as expected, sarcomatous cells proliferated at a more rapid rate than pulmonary adenoma cells.

SUMMARY

Pulmonary adenomas induced with urethan in strain CAF_1 hybrid mice were excised after 1 year and sorted into four groups according to tumor diameter as follows: 0.5–1 mm., 1–2 mm., 2–3 mm. and >3 mm. Grafts approximately 1 mm. in size prepared from these tumors were transplanted subcutaneously into estrogen-treated and untreated mice and the animals sacrificed 4 months thereafter.

It was observed that hormonal treatment had no influence on incidence of takes, latent period, or subsequent growth of grafts regardless of the size of the primary tumor from which each graft was prepared. On the average, grafts from the larger primary tumors yielded a higher incidence of takes, required less time to become established, and grew to a larger size than did grafts derived from the smaller primary tumors. The possible limitations of technic and the relationship of these results to the development of primary lung tumors *in situ* have been discussed.

Tumor growth among the transplants appeared to have increased over that anticipated for the primary tumors *in situ*. Sarcomatous areas were observed among some of the grafts that grew to a relatively large size, but not among the primary tumors.

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