agreement with the idea that the surviving chimeras were immunologically reactive, since both host- and graft-type hemagglutinins were found in their sera 3-4 weeks following treatment. Generally, subnormal hematocrit and body weight values coincided with the emergence of antibody titers.

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Tumor Induction in Hypophyseal Grafts in Radiothyroidectomized Mice; Hypothalamico-hypophyseal Relationships.* (28731)

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Secretory tumors of the anterior pituitary gland can be induced in laboratory rodents by a variety of methods(1,2). Thyrotropin-secreting tumors (TtT) develop in mice given thyroid-destroying doses of I^{131}; tumors (MtT) of the mammotropin-secreting cells develop in both rats and mice subjected to chronic estrogen treatment and in grafts of normal pituitary tissue in estrogenized rats; pituitary tumors of a variety of secretory cell types develop in mice after exposure to ionizing radiation; and transplantation of mouse pituitary tissue to extracranial sites has recently been shown to be tumorigenic in intact females and estrogen-treated males. The initial events and factors involved in the neoplastic transformation have not, however, been fully clarified. For example, the importance of the radiation exposure of the anterior pituitary gland during radiothyroidectomy to the development of TtT has been disputed(3,4).

The experiments described here were initiated in an attempt to clarify: a) the role of pituitary irradiation in TtT induction by I^{131}, and b) the importance of an immediate circulatory connection between the pituitary and hypothalamus in tumorigenesis.

Materials and methods. Female LAF_1 and male C57Bl/6 mice were received† at 6 to 8 weeks of age. Those to be radiothyroidectomized were fed a low iodine diet‡ and distilled water from the day of receipt. Radiothyroidectomy was performed 10 to 21 days later by an intraperitoneal injection of 60-65 μc I^{131}. Grafts were made 14 to 33 days after I^{131} treatment. Pituitary glands from mice of the same age and sex as the recipients were transplanted beneath the left kidney capsules with a 14-gauge trochar dampened with isotonic nutrient medium. Thirty to thirty-one days prior to grafting some of the male mice were castrated.

The animals were inspected frequently and those *in extremis* or with external evidence of tumors were sacrificed. Animals found soon after death were also autopsied. Surviving female mice (Exp. 1) were sacrificed 600 days after grafting; remaining males of

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† LAF_1 mice were from Jackson Memorial Laboratories, Bar Harbor, Maine, or from Cumberland View Farms, Clinton, Tenn. C57Bl mice were from Jackson Laboratories.

‡ Nutritional Biochemicals, Cleveland, Ohio.

TABLE I. Pituitary Grafts in Radiothyroidectomized and Intact LAF_1 Female Mice (Exp 1). (See also Fig. 1 & 2.)

Group	Treatment: Donor	Treatment: Host	No. mice grafted	Mice autopsied*: No.	Mice autopsied*: %†	Mice autopsied*: Mean survival, days ± S.D.
A	intact	intact	14	10	71	514 ± 64
B	thyrex	"	13	7	54	505 ± 41
	intact hosts combined:		27	17	63	510 ± 54
C	intact	thyrex	15	9	60	534 ± 43
D	thyrex	"	14	10	71	539 ± 58
	thyrex hosts combined:		29	19	66	537 ± 50

* Only those mice surviving 420-600 days after grafting are included. † Of those grafted.

Groups E and F (Exp. 2) were killed 684 days after grafting; and males of groups A-D (Exp. 2) were killed only *in extremis* (longest survival 736 days). At autopsy most pituitary glands *in situ* and pituitary grafts of sufficient size were removed and weighed to the nearest 0.5 mg on a torque balance. The tumors used for subsequent transplantation were weighed by difference in sterile aluminum foil containers dampened with nutrient medium. For histological examination, samples were fixed in 10% neutral formalin or AFA, paraffin embedded, sectioned, and stained with hematoxylin and eosin by standard procedures. Grafts were considered positive only if they were grossly tumorous (*i.e.*, weighed more than 8 mg) or if there was histologically demonstrable pituitary tissue at the transplantation site.

Several of the tumors occurring in grafts and in host pituitary glands *in situ* in Exp. 1 were further tested for host specificity by intramuscular transplantation in the thighs of small numbers of young adult LAF_1 female mice which were intact, gonadectomized or radiothyroidectomized (Table II). Some assay mice were grafted with tumorous host pituitary tissue in the right thigh, and with tumor originating in the subcapsular graft from the same host in the left thigh. Tumors that appeared were measured in 2 surface dimensions with calipers at monthly intervals. In doubly transplanted mice, when one grafted tumor grew to about 1 cm diameter before the other graft was palpable, the tumor was surgically resected to keep the mouse alive. Mice found *in extremis* after 300 days were sacrificed and surviving animals were killed 730-740 days after grafting. Most of the tumors were weighed at autopsy. In some cases, however, animals with large tumors died and were cannibalized before removal from the cage. In other cases, small tumors found at autopsy were measured with a mm rule and used for histological preparation or transplantation. In these cases, tumor weights were estimated from the rule or the most recent caliper measurements for summary in Fig. 3 and 4.

Results and discussion. Experiment 1. The first grossly enlarged subcapsular graft was found in an LAF_1 female mouse that died 420 days after transplantation. Therefore, only those mice (54-71% of total, Table I) autopsied after 420 days are included in the tabulations.

Fourteen of 17 pituitary grafts beneath the kidney capsules of intact female hosts were larger than the host pituitary glands *in situ*, and 10 weighed 8 mg or more (Fig. 1). All 19 subcapsular grafts in thyroidectomized hosts weighed 8 mg or more (Fig. 2). The largest tumors in grafts in both groups were found 500 days or more after transplantation. Radiothyroidectomy of the donors of the pituitary tissue did not markedly modify the tumorous response of the grafts in either type of host.

Sixteen of the 19 thyroidectomized host pituitary glands *in situ* were tumorous, weighing 8 or more mg (Fig. 2). All intact host pituitary glands were, however, of normal size (Fig. 1).

Recent reports(6,11) indicate that tumor formation occurs in grafts of anterior pituitary tissue in several strains of otherwise un-

TABLE II. Results of Assay Passages (First Intramuscular Grafts) of Pituitary Tumors Induced in Exp 1. (See also Fig. 3 & 4.)

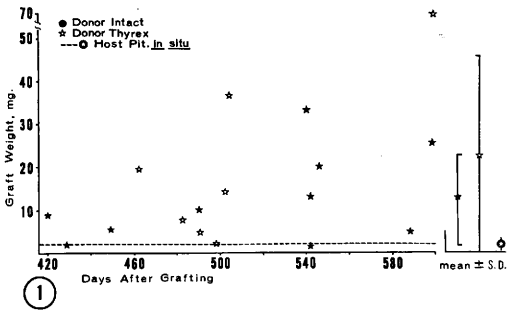
Original group	Tumor strains: No. grafted	Tumor strains: No. grew	Latency,* days ± S.D.	Duration,† days ± S.D.
Subcapsular grafts				
A	5	4	552 ± 132	639 ± 120
B	3	3	521 ± 164	559 ± 133
C	8	6	566 ± 120	550 ± 117
D	5	3	517 ± 50	591 ± 130
total:	21	16 (76%)		
Pituitary tumors *in situ*				
C	8	8	464 ± 145	519 ± 144
D	5	4	390 ± 136	480 ± 109
total:	13	12 (92%)		

* Time from transplantation until tumors were palpable in recipient mice in which tumors developed.

† Time from transplantation until death or sacrifice in "negative" recipients surviving the minimum tumor age (338 days for recipients of subcapsular tumors; 300 days for recipients of primary tumors).

treated female mice. In rats, electron microscopic studies have shown that such grafts contain poorly granulated acidophils(7) similar to those observed in estrogen-induced MtT(8). Gonadotropic, thyrotropic and adrenotropic functions are abolished or greatly reduced in ectopic grafts of normal pituitary tissue(1,9,10) but the mammotropic function persists and results in mammary hyperplasia and an increased incidence of mammary carcinoma(11,12). Mammary glands of *all* of the host mice in Exp. 1 were grossly hyperplastic at autopsy.

The presence of well-developed mammary glands in a mouse bearing a pituitary tumor cannot, however, be taken as proof that the tumor is an MtT. Mammary glands of female mice with intact ovaries and grafted with functional TtT frequently are well developed(13). Apparently, the ovaries of such mice are stimulated by a gonadotropic side-effect of the hormonal products of the TtT. The stimulated ovaries in turn release estrogens which cause mammotropic release by the host pituitary gland *in situ*(13). Several tumorous grafts from Exp. 1 were thus assayed for host specificity by further transplantation (Table II, Fig. 3). Several tumors originating in thyroidectomized host pituitary glands *in situ* were similarly assayed for comparison. On the basis of previous results(1), TtT would be expected to grow preferentially in thyroid-deficient hosts, whereas autonomous MtT would be expected to grow preferentially in intact females.



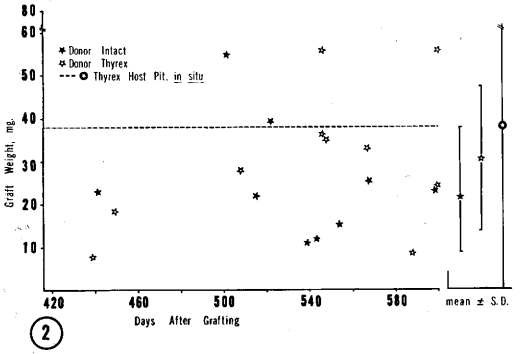
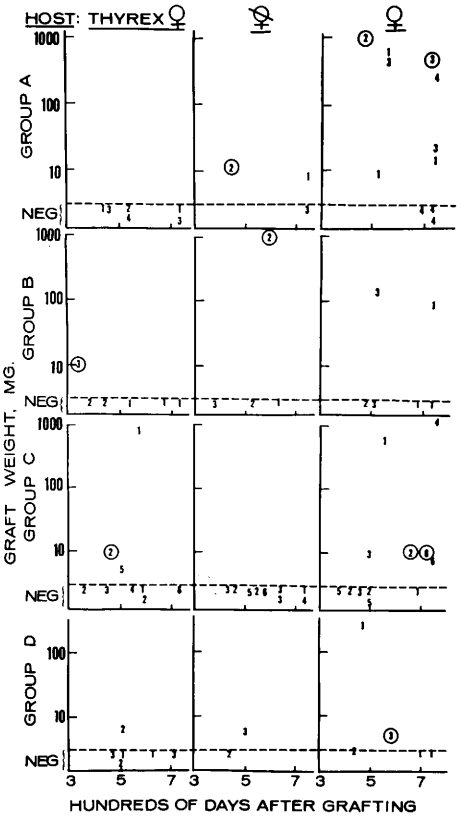


FIG. 1. Comparison of final graft size, time of death and host pituitary weight in intact female LAF_1 mice that received subcapsular pituitary grafts from intact or thyroidectomized donors. See also Table I.

FIG. 2. Comparison of final graft size, time of death and host pituitary weight in thyroidectomized female LAF_1 mice that received subcapsular grafts from intact or thyroidectomized donors. See also Table I.



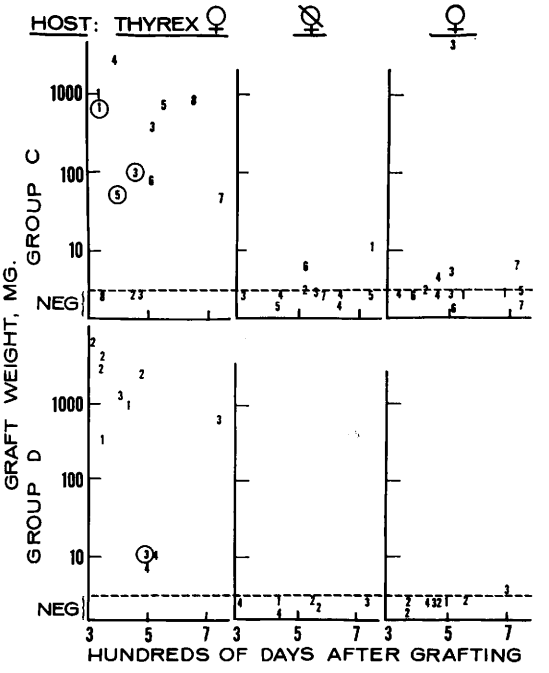


FIG. 3. Fate of intramuscular transplants of tumors that arose in subcapsular pituitary grafts in intact (groups A and B) or thyroidectomized (groups C and D) LAF_1 female mice in Exp. 1. Numbers in the bodies of the graphs indicate the individual tumor strains within a given group and are placed to indicate the weight of the graft and the time of sacrifice of the host. Circled numbers indicate tumor graft weights estimated from linear dimensions. "Neg" indicates that the grafts were not found at autopsy (see also Table II).

FIG. 4. Fate of intramuscular transplants of tumors that arose in the pituitary glands *in situ* in the thyroidectomized female LAF_1 host mice in Exp. 1. Symbols as in Fig. 3. (See also Table II.)

Tumors that originated in subcapsular grafts from *all* groups in Exp. 1 "took" most frequently after intramuscular transplantation in intact females (Fig. 3). Mammary glands of the intact host mice were usually markedly stimulated, and thyroid glands were of normal size in both intact and gonadectomized hosts with tumors, except in the case noted below.

In contrast, tumors originating in the thyroidectomized host pituitary glands *in situ* grew after intramuscular transplantation in highest frequency and with the shortest latency in thyroidectomized mice (Table III, Fig. 4). Eight of the 10 grafts found in animals with intact thyroid glands weighed 10 mg or less and the thyroid glands of all these mice were grossly enlarged. One tumor strain (No. 3, group C, Fig. 4) was, however, fully autonomous, becoming palpable by 390 days after grafting in some intact hosts. Thyroid glands of hosts of this strain were greatly enlarged and both mammary glands and gonads were stimulated. In one case, since tumors arising from both the host pituitary gland and the subcapsular graft co-existed at time of autopsy, the exact source of the thyroid stimulation was not known.

TABLE III. Subcapsular Pituitary Grafts Identified at Autopsy and Weights of Host Pituitary Glands *in situ* in C57Bl Male Mice (Exp 2).

Group	Donor	Host	Autop/gr*	Grafts ident.† No. (%)	Pituitary wt, mg ± S.D.	Duration, days ± S.D.‡
A	intact	intact	8/15	6 (75)	1.3 ± .2	589 ±100
B	thyrex	"	10/15	9 (90)	1.5 ± .3	667 ± 62
		intact hosts combined:	18/30 (60%)	15 (83)	1.4 ± .3	636 ± 76
C	intact	thyrex	11/17	8 (73)	82 ±52	471 ± 66
D	thyrex	"	11/16	10 (91)	107 ±56	534 ± 60
		thyrex hosts combined:	22/33 (67%)	18 (82)	96 ±53	506 ± 61
E	intact	castrate	19/24	16 (84)		648 ± 72
F	thyrex	"	17/20	13 (76)		623 ± 70
		castrate hosts combined:	36/44 (82%)	29 (81)		637 ± 70
		all hosts combined:	76/107(71%)	62 (82)		599 ± 68

* Mice autopsied 390 or more days after grafting over No. of mice receiving grafts.
† No. of grafts identified at autopsy with percent of total number autopsied in parentheses. Only those grafts demonstrated in histological sections are included.
‡ Time from grafting until death or sacrifice of animals with identified grafts.

These data indicate that the tumors arising in thyroidectomized host pituitary glands *in situ* were functional TtT, and with one exception, were thyroid-deficiency dependent, in agreement with results of others(1). Tumors arising in subcapsular grafts were apparently composed, however, of autonomous mammotropic cells (Mt). If cells of other functional types were present, they were not of sufficient number or functional capacity to cause changes that were obvious on gross autopsy in thyroids, gonads, or adrenal glands.

Experiment 2. The development of MtT in subcapsular grafts of pituitary tissue in intact female mice is believed to be related to an endocrine imbalance between the host pituitary gland and the ovaries(2,6,11) and susceptibility to tumor formation is apparently genetically controlled(6). Ectopic pituitary grafts infrequently became tumorous in C57Bl female mice, and such tumors were not observed in grafts in C57Bl males(6). Mice of the C57Bl strain are, however, susceptible to induction of TtT by radiothyroidectomy. In Exp. 2, the fate of subcapsular pituitary grafts was thus investigated in intact, or in gonadectomized or thyroidectomized male C57Bl hosts.

No tumors were found in the intact or gonadectomized mice in which pituitary tissue was histologically demonstrable at the graft site 390 or more days after transplantation (Table III). One of 18 grafts in a thyroidectomized mouse (group C, Table III) weighed 5.5 mg 546 days after grafting and was used in entirety for transplantation. No tumors were apparent in the recipients 256 days after passage and the nature of the transplanted tissue is not currently known. All other grafts to thyroidectomized animals were minute, requiring histological confirmation of viable pituitary tissue. In contrast, the pituitary glands *in situ* were grossly tumorous in all 18 of the radiothyroidectomized hosts (Table III).

Conclusions. The principal findings of the experiments reported were: 1) Pituitary grafts beneath the kidney capsules of both intact and radiothyroidectomized female LAF_1 mice gave rise to neoplasms that did not behave as typical thyrotropic tumors when further transplanted. Rather, they resembled mammotropic tumors in host specificity and hormonal effects. Radiothyroidectomy of the original pituitary donors did not markedly affect the induction of such tumors.

2) The thyroid hormone deficiency result-

ing from radiothyroidectomy was not of itself sufficient to induce formation of thyrotropic tumors in pituitary tissue in grafts remote from the hypothalamus in C57Bl male mice. This is in marked contrast to observations on induction of mammotropic pituitary tumors in rats by estrogen(5).

3) In agreement with previous work of others(1), the majority of the tumors developing in thyroidectomized host pituitaries *in situ* were initially dependent on a deficiency of thyroid hormone, *i.e.*, they were transplantable only to hypothyroid hosts. Those tumors that grew on grafting in intact mice (*i.e.*, were autonomous) were thyrotropic in effect.

The tumors that develop in mouse pituitaries *in situ* following radiothyroidectomy have been shown to originate from the cells that are believed to secrete thyrotropin under normal circumstances(1,2). Furthermore, the thyrotropic action of ectopic pituitary grafts in hypophysectomized mice is detectable, though considerably less than that of the gland *in situ*(9). It thus seems probable that at least some of the cells from which TtT develop survive transplantation procedures of the type employed in these studies.

Evidence recently reviewed by Greer(9) and Blanquet(10) indicates that the thyrotropic cells (Tt) of the normal anterior pituitary gland are controlled both directly by blood levels of thyroid hormone, and indirectly by a neurohumoral factor originating in the anterior hypothalamus. Release of the latter is in turn under the control of the circulating thyroid hormone levels. The direct effect appears to be immediate, whereas several hours are required for the hypothalamus to respond to altered blood levels of thyroid hormone(10). In the light of the current understanding of this dual control mechanism, two postulates on the nature of the primary change in the transformation of normal Tt into dependent tumor Tt are suggested:

1) Dependent neoplastic Tt have lost the requirement for the hypothalamic stimulating factor which is essential for proliferation of normal Tt. Dependent Tt are, however, apparently still subject to direct suppression by increased thyroid hormone levels.

2) Alternatively, dependent tumor Tt could be exquisitely sensitive to the hypothalamic factor. In this case, proliferation in the thyroidectomized animals would occur in response to the minute quantities of the hypothalamic factor in the blood that reached transplantation sites far from the hypothalamus. This possibility is suggested by results with MtT. In the latter(5) progressively smaller doses of estrogen were required to stimulate proliferation during progression of the mammotropes from normal through dependent to autonomous.

A third possibility, that the cells from which dependent TtT arise do not survive the pituitary transplantation procedure employed, seems unlikely as indicated above. If true, however, normal Tt must be considerably more sensitive to the trauma of transplantation than are the Tt of dependent tumors.

The current data are consistent with any of these postulates, but shed no further light on the role of pituitary irradiation by I^{131} in the dependent tumor transformation.

Summary. Pituitary glands from intact or radiothyroidectomized mice were grafted beneath the kidney capsules of similar intact and radiothyroidectomized recipients in all 4 donor-host combinations in mice of both sexes and in castrated males. Tumors arose in high frequency in such grafts in intact and in thyroidectomized female LAF_1 mice irrespective of the pretreatment of the donors. Transplantation assays indicated that the tumors arising in such grafts were most probably not thyrotropic in nature, but rather were mammotropic, regardless of the treatment of the first host. In contrast, the tumors arising in the thyroidectomized host pituitaries *in situ* were typically thyrotropic in behavior. No tumors were found in subcapsular pituitary grafts in intact or gonadectomized C57Bl/6 male mice, and a small mass (5.5 mg) of as yet unknown character was found at the graft site in but one of 18 thyroidectomized males. The pituitary glands *in situ* of all of the thyroidectomized males were, however, tumorous. It is tentatively concluded that immediate circulatory connection with the hypothalamus is important in,

and probably essential to, the induction of dependent thyrotropic tumors. The possibility that the cells capable of thyrotropic tumor formation did not survive the transplantation seems much less likely. The transformation of a normal thyrotropic cell into a dependent tumor cell thus appears to involve a change in the requirement for, or response to, hypothalamic factors. The role of hypophyseal irradiation in induction of thyrotropic piutitary tumors by radiothyroidectomy with I^{131} was not further clarified.

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Electrical Activity in Sympathetic Nerves of Immunologically Sympathectomized Rats.* (28732)

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Mouse salivary gland contains a factor which promotes the growth of the sympathetic nervous system(1). The antiserum to this nerve growth factor produces extensive and permanent destruction of sympathetic ganglion cells when the material is administered to newborn animals(2,3). Although the damage to the sympathetic nerves produced by the antiserum in rats is virtually complete, a small number of distinct ganglion cells and fibers remain. A recent study in this laboratory(4) demonstrated that in rats treated with the anti-nerve growth factor the residual sympathetic fibers were not functional. This was shown in the perfused hindquarters of treated rats where both direct and reflex activation of the sympathetic chains failed to elicit a vasoconstrictor response. The purpose of the present investigation was to determine if immunization with the anti-nerve growth factor alters the nature of the electrical activity emanating from the sympathetic tracts.

Methods. Experiments were performed on 4 control and 4 immunized rats drawn from litters raised in the laboratory. The immunization was carried out by treating newborn rats with daily subcutaneous injections of a bovine antiserum developed to mouse salivary gland nerve growth factor.† The antiserum with a concentration of 68,000 units/ml was administered subcutaneously for 14 days at a dose of 1% of body weight. Nerve potentials were recorded from the sympathetic chains when the animals were 2 months old.

Preparation of the rats was performed under deep ether anesthesia. The animal was tracheotomized and a catheter was inserted

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