Inhibition of the *in Vitro* Synthesis of Pituitary Prolactin and Growth Hormone by Mouse Pituitary Isografts[1] (34470)

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Transplanted hormone-producing pituitary tumors have been reported to decrease the host's pituitary gland size and decrease its hormone content (1, 2). Recently we have found that the *in vitro* synthesis of prolactin and growth hormone is decreased in pituitary glands from rats bearing these tumors (3). Boot *et al.* (4) demonstrated that transplantation of mouse pituitary glands under the kidney capsule secrete large quantities of prolactin and eventually become neoplastic. Only very small amounts of other hormones are produced by these transplants. The object of the present work was to ascertain if the prolactin secreted by the mouse pituitary transplant influenced hormone production by the host's pituitary gland.

Methods. Dr. L. M. Root provided the female (♀C57BL × ♂CBA)F_1 mice which at 6 weeks of age were implanted under the kidney capsule with pituitary glands from isologous hosts. The mice were decapitated at varying periods of time up to 74 weeks, and the weighed, pooled pituitary glands were incubated in 1.2 ml of a solution containing one part tissue culture Medium 199 and nine parts Krebs-Ringer-bicarbonate buffer of pH 7.4. A yeast-protein hydrolyzate (12.5 μg) containing 12.5 μCi ^{14}C was added to the flasks which were open to the atmosphere for 4 hr in a shaking incubator bath at 37°. At the termination of incubation the pituitary glands were homogenized in 0.2 ml of distilled water in a glass homogenizer fitted with a Teflon pestle. The mixture was frozen and thawed three times before subjecting triplicate 40-μl samples of the protein solutions to polyacrylamide gel electrophoresis used by Bloemendal *et al.* (5). A 7.5% acrylamide gel buffered at pH 8.9 with 0.083 *M* Tris was used as the separating gel. This technique permits excellent resolution of the growth hormone from other pituitary constituents; however, the prolactin and albumin bands are in close proximity to each other. Since the albumin is not synthesized in the pituitary it contains little radioactive amino acid. Therefore, when studying the synthesis of prolactin, both the albumin and prolactin bands were cut from the columns, combined, and dissolved in H_2O_2. Lewis *et al.* (6) and Yanai *et al.* (7) have previously described the electrophoretic identification of prolactin and growth hormone in mouse pituitary glands. The growth hormone band was also dissolved in H_2O_2. Aliquots (0.5-ml) of the dissolved proteins were added to 12.5 ml of counting mixture and the radioactivity determined by liquid-scintillation techniques.

Results. The results presented in Table I show that the implantation of isologous pituitary glands into mice does not cause a decrease in the size of the host's pituitary gland and thus differ from results obtained in rats (8). This result was obtained even in mice bearing mammary tumors, thus indicating that prolactin was produced by the transplants.

The incorporation of the radioactive amino acids into prolactin and growth hormone of the normal mouse pituitary gland was demonstrated to proceed at a brisk rate (Fig. 1). Mice bearing pituitary gland transplants for 6 weeks incorporated slightly less isotope

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TABLE I. Effect of Pituitary Isografts on the *in Vitro* Incorporation of Radioactive Amino Acids into Mouse Pituitary Growth Hormone.

Flask no.	Duration of pituitary gland implantation (weeks)	Weight of isografts (mg)	No. mice	Total pituitary weight (mg)	cpm/mg Pituitary
1	0	—	8	17.8	12,100
2	6	<2	10	22.1	5780
3	23	5	10	21.1	3500
4	48	30	10	27.2	2400
5	74	200	10[a]	22.8	7420

[a] Five of these mice had mammary tumors.

into the host's pituitary prolactin, but 23 weeks after transplant of isografts, the host's pituitary gland synthesized only about 40% that found in normal mice. A progressive decrease in the ability of the pituitary gland to synthesize prolactin was observed in mice bearing isografts for 48 weeks and at 74 weeks reached a level equal to 10% that found in controls.

Growth hormone was synthesized approximately twice as rapidly as prolactin in normal nongrafted mice. After transplantation of pituitary isografts, the ability of the host's pituitary gland to synthesize growth hormone was significantly reduced. A maximum decrease in growth hormone synthesis was observed in mice bearing isografts for 48 weeks, but after 78 weeks of implantation an unexplained reversal of this trend was observed. It should be noted, however, that at this prolonged time five of the ten mice bearing transplants had measurable mammary tumors. It is not known if any relationship exists between these events.

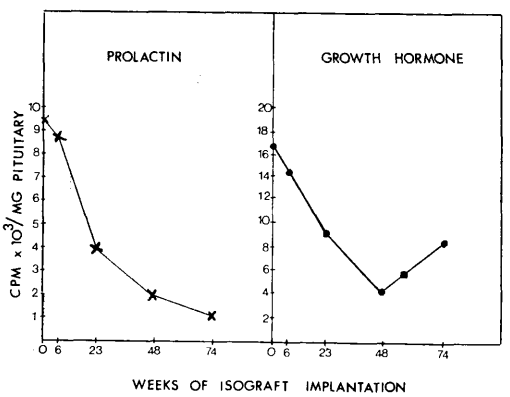

FIG. 1. Effect of pituitary isografts on prolactin and growth hormone synthesis in the host's pituitary gland.

Discussion. The mechanisms which regulate the pituitary gland synthesis and release of hormones are under intensive investigation. We have previously suggested that since neither prolactin nor growth hormone is known to stimulate the release of any substance from a specific target organ, which is capable of subsequently inhibiting pituitary gland function, it may be that the control of these pituitary gland hormones is governed by their own serum concentration (1, 3). Thus, an elevated serum concentration of prolactin or growth hormone may negatively affect hormone synthesis in the pituitary gland. In these earlier experiments, pituitary tumors which secrete prolactin and growth hormone were used and pituitary glands from rats bearing these tumors synthesized only small amounts of prolactin and growth hormone *in vitro* (3).

The purpose of the works reported herein was to ascertain if mice bearing isografts of pituitary gland which produced prolactin had the same decrease in hormone production found in rats bearing transplanted pituitary tumors.

Kwa and Verhofstad (9) showed that pituitary isografts produce a large increase in the plasma prolactin concentration. Boot *et al.* (4) have studied the properties of pituitary isografts in mice and showed that prolactin produced pseudopregnancy in the host. To us, the data suggest that the isografts decreased estrogen production by the ovary. The prolactin content of the pituitary gland is known to be increased by estrogen, but

growth hormone synthesis is not influenced by physiological amounts of the steroid (3). In rats bearing hormone-secreting pituitary tumors, the suppression of the pituitary gland prolactin synthesis was restored to normal by estradiol, but the suppression of growth hormone was again not affected by the estrogen (10). Thus the decrease in growth hormone synthesis observed in mice with pituitary isografts is probably independent of ovarian influences. Welsch *et al.* (8) have presented good evidence that the hormones produced by the pituitary isograft act effectively in ovariectomized rats to decrease pituitary gland prolactin concentration. Prolactin produced by these rat pituitary isografts was found to decrease the host's pituitary prolactin content in intact and ovariectomized rats.

The premise for the discussion so far has been that pituitary isografts produce only prolactin and no other pituitary hormone. If the concept that feedback inhibition of the pituitary gland hormones is mediated by the plasma concentration of the hormone, we must infer that the receptor which is affected by the plasma prolactin concentration produces a decrease in both prolactin and growth hormone synthesis, or invoke the observation of Swelheim and Wolthuis (11) and Gittes and Kastin (12) who showed that pituitary gland transplants produce growth hormone.

The results presented here show that mice bearing isologous pituitary gland transplants that cause a decrease in the host's pituitary gland prolactin and growth hormone synthesis may be caused by the excess prolactin produced by the graft and mediated through a nonspecific feedback inhibition at the hypothalamo-pituitary level or by growth hormone secreted by the pituitary isografts which causes a specific decrease in prolactin and growth hormone synthesis.

Summary. Pituitary glands from mice bearing pituitary isografts in the renal capsule for 6–74 weeks were incubated with ^{14}C-labeled amino acids, and the incorporation into prolactin and growth hormone was determined. Mice with isografts for 23 weeks synthesized 60% less prolactin and growth hormone than did normal intact mice. Implantation of isografts for periods up to 74 weeks further decreased the ability of the pituitary gland to synthesize the prolactin hormone; however, at 74 weeks, the extent of this inhibition in growth hormone synthesis was somewhat decreased. It is proposed that the decrease in prolactin and growth hormone synthesis is mediated by hormone(s) secreted by the tumor acting at the hypothalamo-pituitary level through an autofeedback process.

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