1. Ingle, D. J., Endocrinology, 1958, v62, 78.

2. ———, Diabetes, 1965, v14, 93.

3. Ingle, D. J., Griffith, J. Q., Jr., Surgery of the Rat, in The Rat in Laboratory Investigation, Chap. 16, J. B. Lippincott Co., Philadelphia, 1952, pp. 446-47.

4. Ingle, D. J., Altamero, T. L., Jr., Flores, V., Cancer Research, 1956, v16, 437.

Received June 11, 1965. P.S.E.B.M., 1965, v120.

Neoplastic Changes in Pituitary Intrarenal Isografts in Mice. (30494)

NECHAMA HARAN-GHERA (Introduced by P. Shubik)
Department of Experimental Biology, Weizmann Institute of Science, Rehovoth, Israel

One of the effective means of tumor induction in the pituitary gland is by sustained interference with the physiological homeostatic equilibrium of that organ—*e.g.*, (a) induction of functioning thyrotropic pituitary tumors *in situ* by destruction of the thyroid gland or by blocking its normal functioning (1), and (b) induction of various types of tumors in isografts of pituitary gland, *i.e.*, after transplantation at sites remote from hypothalamic influence(2,3,4). It so happens that such isografts continue secreting large amounts of prolactin(5), though no appreciable amounts of other tropic hormones; and as a consequence, mammary glands undergo early hyperplastic changes and later, neoplastic changes(2). Regarding the characterization of types of neoplasms produced in the pituitary gland, methods have been developed (originally in connection with pituitary tumor induced by ionizing radiation) by bioassay techniques for distinguishing thyrotropic, mammotropic and adrenotropic tumors (6).

In our studies on the role of hormones in mammary tumor development(7,8), the technique of pituitary intrarenal isografts was used as a means for endogenous, continuous prolactin secretion. Many of these pituitary isografts became transformed after a long latent period into pituitary tumors.

The aim of the present study was to analyze the type of these pituitary tumors by transplantation bioassays in isologous hosts.

Materials and methods. Mice of the genetically uniform (C57L × A/He)F_1 strain inbred in our laboratory were used in these experiments at about 6-8 weeks of age. They were fed Purina Laboratory Chow and water *ad libitum*. Female mice received isografts of a single pituitary gland beneath the left kidney capsule through an 18-gauge hypodermic needle fitted with a metal plunger. The pituitary gland donor mice of 2 age groups—60-day and 300-day-old were of the same sex as the recipients. One small group of male mice received a pituitary renal implant from 60-day-old female donors.

Eight of the primary pituitary tumors were bioassayed by retransplantation of tumor tissue beneath the kidney capsule of normal hosts.

Results. The pituitary intrarenal graft tumor incidences in the different treated groups are summarized in Table I. Of the pituitaries grafted beneath the kidney capsule of female hosts, 60-80% showed progressive growth, suggesting neoplastic transformation. The female mice bearing pituitary graft tumors also showed marked mammary gland hyperplasia, while all the other organs appeared normal. The intact pituitaries of these mice were also normal. In a small group of 6 males grafted intrarenally, 2 of the grafts (from female donors) developed into tumors. These males showed grossly very enlarged seminal vesicles, while the other

TABLE I. Pituitary Tumor Development in Intrarenal Isologous Pituitary Grafts.

Age of pituitary donor (days)	Mice bearing tumors /survivors	% Pituitary tumors	Avg age of tumor-bearing mice (days)
60	24/30 ♀	80	600
60	2/6 ♂	33	665
300	13/22 ♀	60	420

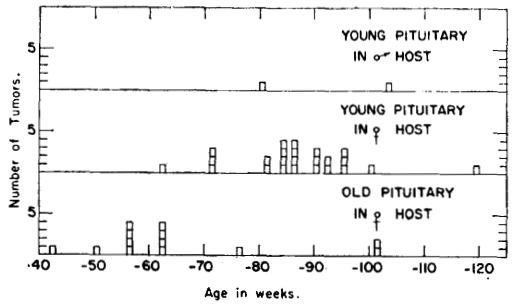

FIG. 1. Age of pituitary tumors occurring in intrarenal pituitary isografts taken from young or adult donors.

organs appeared normal. Histologically, all the tumors in the pituitary isografts were chromophobe adenomas. The tumor cells were fairly uniform in size and shape, sometimes invading the kidney tissue. It was interesting to note a difference in the latency of tumor development related to the age of the pituitary donor. As seen in Fig. 1, tumor development occurred later in pituitary isografts from young donors, the average latent period being 600 days as against 420 days in pituitary isografts from old donors.

Seven primary pituitary tumors occurring in females and a primary tumor from a male host were retransplanted intrarenally in male and female mice. All of these grafts grew progressively within 150-200 days, and proved to be mammotropic pituitary tumors with a somatotropic effect. The female host bearing the tumor graft had, in every case, a very marked mammary gland stimulation, predominantly alveolar hyperplasia with some stimulated ducts distended with milky secretion, the mammary gland stimulation thus resembling that of pregnancy. The ovaries were of normal size. The granulosa follicles were small; some luteinizing follicles were present; and a marked stromal luteinization was observed. The uterine horns were normal. The somatotropic effect was expressed in weight increase of 20-40% in the liver, spleen and kidney. Tumor-bearing males did not show any mammary gland stimulation, but did show a somatotropic effect. The remarkable features in them were the enlarged seminal vesicles. All the other sex organs were normal.

Several of these pituitary tumors have been maintained, and their third passage is being carried out at present, all of them showing the characteristic mammotropic-somatotropic pituitary tumor pattern.

Discussion. It is generally recognized that endocrine tumors fail to respond properly to the normal homeostatic regulators. Accordingly, 3 types of tumors have been noted: 1) hormone-dependent, growth depending on a certain hormonal imbalance; 2) hormone-responsive; 3) autonomous—once the tumor is established it will grow in any environment, including the normal host.

Two types of mammotropic pituitary tumors were induced in mice and rats: dependent tumors induced by large doses of estrogens, and autonomous tumors induced by ionizing radiation(9). In the present experiments another method is described for induction of autonomous transplantable mammotropic pituitary tumors. These tumors were induced in intrarenal pituitary isologous grafts by unrestrained proliferation of cells through severance of the hypothalamic pituitary interrelationship. The cells giving rise to these tumors were probably the mammotropes connected with prolactin secretion. Prolactin usually elicits both a mammotropic effect, expressed in marked mammary gland stimulation, and a luteotropic effect, demonstrated in the continuous dioestrous cycle in vaginal smears of female hosts, and enlargement of the accessory sex organs such as the seminal vesicles in male mice. The transplantable tumors, judged by their gross effect on male and female hosts, secreted prolactin and some growth-hormone, and showed similar characteristic anatomic and histologic patterns to those described by Furth *et al*(10) for radiation-induced autonomous mammotropic pituitary tumors. This is another example of the induction of an endocrine tumor due to prolonged homeostatic disturbance, enhancing tumor growth until an autonomous tumor is established.

The time required for the grafted tumors to become palpable was short—150-200 days—with the intrarenal transplantation technique. This method, probably due to good blood supply to the graft, seems to be more effective than the subcutaneous method

(10), in which palpable tumors were obtained in the first passage after 280-430 days.

Summary. Isologous pituitary glands transplanted beneath the kidney capsule of normal hosts were transformed after a long latent period into pituitary tumors. Transplantation bioassays of these tumors showed that they were all mammotropic pituitary tumors exerting some somatotropic effects also.

1. Gorbman, A., Edelman, A., Proc. Soc. Exp. Biol. and Med., 1952, v81, 348.

2. Mühlbock, O., Boot, L. M., Cancer Res., 1959, v19, 402.

3. Bardin, W., Liebelt, A. G., Proc. Am. Assn. Can. Res., 1960, v3, 7.

4. Heston, W. E., J. Nat. Cancer Inst., 1964, v32, 947.

5. Everett, J. W., Endocrinology, 1954, v54, 685.

6. Furth, J., Recent Progr. Hormone Res., 1955, v11, 221.

7. Haran-Ghera, N., Acta Inter. Con. Cancer, 1962, v18, 207.

8. ———, ibid., 1963, v19, 765.

9. Furth, J., Kim, U., Clifton, K. H., Nat. Cancer Inst. Monogr., 1960, No. 2, 149.

10. Furth, J., Gadsen, E. L., Clifton, K. H., Anderson, E., Cancer Res., 1956, v16, 600.

Received June 14, 1965. P.S.E.B.M., 1965, v120.

Modification of the Action of Pilocarpine by Adrenergic Blocking Agents.* (30495)

C. A. SCHNEYER

Department of Physiology and Biophysics, University of Alabama Medical Center, Birmingham

Pilocarpine has long been used as a substitute for parasympathetic nerve stimulation, especially of the salivary glands. Recently it was shown that, in the rat, the protein composition (total proteins and amylase) of pilocarpine-evoked parotid saliva differs markedly from that of saliva elaborated in response to stimulation of the parasympathetic nerve(1). On the other hand, amylase or total-protein levels of pilocarpine-evoked saliva are markedly similar to those levels observed when sympathomimetic stimulation of the gland is employed(2). These facts suggest that pilocarpine may be acting on non-cholinergic sites as well as cholinergic sites. Accordingly in the present investigations, the 2 adrenergic blocking agents, 1-isopropylamino-3-(1-naphthyloxy)-2-propanol hydrochloride (Inderal),† which blocks adrenergic beta-receptors(3), and phenoxybenzamine (dibenzyline), which blocks adrenergic alpha receptors(4), were used in conjunction with pilocarpine in an attempt to delineate the mechanism of pilocarpine action.

Materials and methods. Adult male Long-Evans rats were fasted (water *ad lib*) for 24 hours prior to experimentation. Rats were anesthetized with Nembutal and the auriculotemporal nerve and the duct from the parotid gland were exposed. The parotid duct was cut and saliva was collected by micro-pipette applied to the duct orifice. Flow of saliva was evoked by electrical stimulation with supramaximal shocks (Harvard stimulator, Model 935B) applied to the auriculo-temporal nerve, or by intraperitoneal injection of supramaximal doses of pilocarpine (1 mg/100 g body weight). When the adrenergic blocking agents, dibenzyline and Inderal were used, they were administered by intraperitoneal injection prior to nerve stimulation or pilocarpine administration. When used in conjunction with pilocarpine, at least equi-molar doses of the blocking agents were injected, and frequently the molar doses of Inderal and dibenzyline were 2 to 4 times those of pilocarpine. Amylase activity of saliva was determined by the method of Myers, Free and Rosinski(5,2), and expressed as milligrams of reducing substance (as glucose) per mg of secretion, formed in a 15-minute digestion

* Supported in part by a grant from USPHS (DE-01674).

† I wish to thank Dr. A. Sahagian-Edwards of Ayerst Laboratories, New York, for generously supplying the Inderal for these investigations.