nyl *N*-methylcarbamate, phenyl *N*-methylcarbamate, and erythrocyte acetylcholinesterase, but they did not use the kinetics and experimental treatment employed in our work.

Evaluation of the k_{2c} and K_I values of other carbamates by the procedures used with eserine (*3*) may be possible provided that the limited solubilities exhibited by many permit values of $[I]$ approaching K_I to be realized.

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Pituitary Tumors in Mice after Prolonged Feeding of Synthetic Progestins

Abstract. *An enhanced development of pituitary tumors was observed in virgin female mice of the C57 Leaden strain following repeated oral administration of synthetic progestins. This finding appears to parallel the co-carcinogenic enhancement of mammary tumor development elicited in C3H mice treated repeatedly with progesterone.*

The observation of spontaneous pituitary tumors among inbred laboratory mice is relatively rare, although tumors, classed as chromophobe adenomas of the anterior pituitary, have been found in retired female breeders (*1–4*). Their occurrence in untreated mice two or more years old has led some experimental investigators to designate these pituitary tumors as a disease of senescence (*2*). Experimentally, the induction of tumors in the anterior pituitary follows long periods of hormonal imbalance, induced by ablation of a secretory target organ of the pituitary, by disruption of hypothalamic control of the pituitary, or by prolonged treatment with estrogen. Techniques for tumor induction include surgical, chemical, or irradiation-induced thyroidectomy, irradiation of the head or whole body, gonadectomy soon after birth, and repeated injections of estrogen (*2–4*). As for the latter, the induction of chromophobe adenomas of the adenohypophysis was first reported in 1936, following prolonged stimulation of mice with oestrin (*1*). More recent studies of pituitary tumor genesis induced by estrogen have been reviewed by Furth and Clifton (*2*) and Russfield (*3*). Progestins, by contrast, have not been associated with the induction of these tumors in the earlier literature. This report describes the development of pituitary tumors in mice fed orally-effective synthetic progestins.

Exogenous progesterone is a potent co-carcinogen for the chemical and viral induction of mammary tumors when administered repeatedly to C3H virgin female mice in order to keep them in an anovulatory state (*5*). Because of an appreciable incidence of spontaneous mammary tumors in untreated C3H females (*6*), a study was planned to test for effects of an orally active progestational agent in a mouse that does not develop spontaneous mammary tumors. The present study was made with virgin female C57 Leaden (C57L) mice. The animals, housed in groups of six per plastic cage, had unlimited access to Purina mouse chow and tap water for the duration of the experiment. For the phase of the study now reported, two commercially available synthetic progestins ("I," a mixture of approximately 50 parts Norethynodrel to 1 part Mestranol, and "II," a mixture of 50 parts Norethindrone to 1 part Ethynylestradiol) were obtained without binder and dissolved individually in peanut oil to obtain concentrations of 7 μg and 70 μg of I or II per 0.05 ml of solvent. Commencing when they were 13 weeks old, four groups of mice (24 mice per group) designated for bioassay of these solutions were fed 0.05 ml of one of the mixtures by gavage once a day, 5 days a week. Two control groups of mice were fed an equal volume of saline or peanut oil used as a solvent. A routine necropsy examination was made of all mice found dead during the course of the study. At first this did not include examination of the head in the absence of gross abnormalities. All tissues with gross pathologic changes were fixed in Bouin's solution for later histologic examination.

During the 81st to 84th week of the experiment four of ten mice found dead or moribund on routine examination in the 70 μg-I group had enlarged clitoral glands and grossly distended mammary ducts filled with a grayish-white secretion. When another mouse in the same group died, the head was examined to ascertain whether the activated mammary glands might be due to the presence of a pituitary tumor secreting prolactin. A soft, hyperemic, spheroid pituitary tumor (6 mm in diameter) was found; it had compressed, but apparently had not infiltrated, the surrounding brain. Examination (between the 84th and 89th week of the experiment) of the six remaining mice in the 70 μg-I group disclosed that they all had similar tumors, as did some mice of the other three groups given progestin (Table 1).

Seven of 15 surviving control animals were killed concurrently for comparison with those fed the progestin mixtures. None had pituitary tumors or mammary ducts distended by secretion. The eight surviving controls were killed after the 90th week, ending the experiment. In two of these mice early neoplastic foci were found. In one mouse treated with saline, a dark red, cyst-like area less than 1 mm in diameter was found in the anterior pituitary; a similar 1-mm area was found in one mouse treated with peanut oil (Table 1).

There were no gross changes in the mammary glands, adrenals, or thyroids of the two controls to suggest excessive secretion of any pituitary hormone. By contrast, the mammotropic nature of the pituitary tumors in the groups treated with progestin was apparent grossly, as evidenced by a secretory state of the mammary glands in most of the hosts of the pituitary tumor. The mammary glands that were not grossly secretory in a few hosts were moderately prominent, although ductal distention was not unusual. Despite the mammotropic effect of these pituitary tumors, none of the mice had mammary cancers.

Grossly, the mammotropic pituitary

tumors were 2 to 7 mm in diameter, dark red, soft, and hemorrhagic. The largest growths practically obliterated the normal pituitary architecture, while the smaller ones consisted of a neoplastic focus in one or both lateral portions of the adenohypophysis. Congested, dilated blood sinuses and cysts filled with blood were common in the neoplastic foci. The growths were not encapsulated. Direct invasion of the pars intermedia, neurohypophysis, and the perineural space of the small cranial nerves bordering the largest tumors indicated that the growths were locally malignant. Mitotic figures were common; however, there were no signs of metastasis.

Interesting but less striking than the pituitary tumors were hepatomas in 10 of the 96 animals treated with progestin; there were no hepatomas among 48 controls examined during the same period of time (*7*).

The induction of pituitary tumors subsequent to prolonged feeding of a progestational agent had not been anticipated on initiation of the study; hence, these reported findings are based on too few animals to provide conclusive inferences. The rarity of these tumors in mice bred at random is suggested by the finding of one spontaneous pituitary adenoma in more than 11,000 autopsies of Slye's stock mice (*8*). Adenohypophysial adenomas in less than 1 to 4 percent of control female LAF_1 mice 28 months or older were reported by Gardner, Upton *et al.*, Furth, Clifton, Russfield, and others (*1–4, 9, 10*). By contrast with the rarity of pituitary adenomas in virgin female mice, Heston and Russell estimated the incidence of their spontaneous occurrence among C57L and C57 Black retired female breeders to be from 10 to 33 percent (*10*). The heightened incidence observed by them in old, bred females of the C57 strains may therefore be a natural manifestation of pituitary tumorigenesis enhanced by endogenous progestin, which, in our virgin females, was duplicated and augmented by prolonged administration of a synthetic progestin.

The neoplastic pituitary reaction elicited by the two progestational mixtures bioassayed may signify a C57 strain-specific reaction to progestins as a class, rather than a type of neoplastic reaction that one might expect from mice given a carcinogen. With few exceptions (*3, 11*), current indications of strain specificity in the neoplastic reaction of the C57 pituitary include the rare occurrence of such tumors except in retired C57 breeders, and our inability to induce these tumors in virgin C3H/He females by oral administration of the same progestins (*7*). Until additional data are obtained, progestin enhancement of a rare spontaneous neoplastic phenomenon cannot be equated with progestin carcinogenicity.

Table 1. Pituitary tumors in C57L ♀'s after prolonged feeding of syntheic progestins.

Treatment, by gavage 5 times/wk	No. with tumors/ No. alive, after 84 wk of exposure*	Week of necropsy during which tumors were observed	Approximate size of tumors (mm^3)
70 μg-I†	7/7	84–89	2–7
7 μg-I†	6/11	84–89	1–5
70 μg-II‡	5/8	84–89	2–4
7 μg-II‡	7/15	84–89	2–6
Control: 0.05 ml peanut oil	1/8	90	1
Control: 0.5 ml saline	1/7	90	<1

* 24 mice per group at the beginning of the experiment. † 50 parts Norethynodrel : 1 part Mestranol, in 0.05 ml peanut oil. ‡50 parts Norethinodrone: 1 part Ethynylestradiol, in 0.05 ml peanut oil.

The term spontaneous pituitary tumor implies that an unidentified carcinogenic factor is affecting the test animals under conditions sufficient for tumor development in the longest-living and hardiest few. Speculatively, and in the absence of more definitive data suggesting carcinogenicity, enhanced development of pituitary tumors in C57L females fed an exogenous progestin may represent a co-carcinogenic phenomenon. In that sense, an enhanced development of pituitary tumors in C57L mice treated with synthetic progestins appears to parallel the co-carcinogenic enhancement of mammary tumor development previously elicited with exogenous progesterone in mice that carry the Bittner virus (*5*).

In the C3H female treated with progesterone, as in the C57L female treated with synthetic progestin, the co-carcinogenic role of the progestational agent is suggested by an enhanced incidence, earlier onset, and more rapid development of tumors that are histologically identical to those that would have developed in an older and lesser number of exposed animals in the absence of progestin. Conversely, the enhanced development of spontaneous tumors in mice that would not have developed such tumors during their normal life time (but did after treatment with progestogens) may demonstrate that prolonged treatment with a co-carcinogen or tumorigenic-enhancing agent may be as critical as repeated threshold exposures to a carcinogen.

As an alternate possibility, an orally effective progestin in a biologically susceptible host may act as a direct mammotropic cell stimulant, or as a repressor of homeostatic mechanisms inhibiting mitotic and secretory activity of the pituitary mammotrophes. The role of exogenous progestin in the induction of pituitary tumors might then be by one of two basic pathways: (i) normal pituitary mammotrophes, proliferating because of excessive, persistent stimulation of their reproductive capacity (due to exogenous progestin) become, over a period of time, mammotropic pituitary tumor cells; or (ii) mammotrophes minus extracellular inhibition of their reproductive capacity (due to partial inhibition of the hypothalamus by progestin) become, over a period of time, a mammotropic pituitary tumor. These possibilities have been reviewed previously as parts of the unitary concept that cancer is a terminal stage of normal cell proliferation under inadequate homeostatic restraint (*12*).

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