

TABLE I. Response of Young Dogs to Semliki Forest Virus (Mouse-Adapted).

Route of inoculation	Exposed	Showing symptoms	Min to max* incubation period, days
Intracerebral	2	2	9-10
Intranasal	2	0	0
Intradermal	2	0	0
Intracardial	2	0	0
Intraperitoneal	1	1	7-8

* Incubation period = Time between exposure and onset of symptoms.

and diluted to a 10% suspension with physiological saline. The suspensions were then centrifuged for 5 minutes in a horizontal centrifuge at 2000 r.p.m. The supernatant from each suspension was injected intracerebrally into 6 unvaccinated mice and into 6 mice which previously had been immunized with Semliki Forest virus. After 3-4 days the unvaccinated mice showed symptoms of central nervous system involvement, while the immunized mice showed no nervous symptoms

and were discarded after a 21-day observation period.

Summary. 1. A strain of Semliki Forest virus passaged intracerebrally in Swiss albino mice has been successfully transmitted intraperitoneally and intracerebrally to mongrel puppies. 2. Puppies exposed by intracerebral, intranasal, intradermal, intraperitoneal, and intracardial routes to Bwamba Fever virus and puppies exposed by intranasal, intradermal, and intracardial routes to Semliki Forest virus showed clinically no signs of nervous symptoms, and mice injected intracerebrally with brain suspensions from these puppies showed no evidence of Bwamba Fever or Semliki Forest disease.

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Production of Pituitary Tumors in Mice by Chronic Administration of a Thiouracil Derivative.* (20204)

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In the course of an investigation of the long term effects of the administration of large doses of I^{131} to mice, Gorbman(1) observed the development of tumors of the anterior lobe of the pituitary gland in those animals who survive complete destruction of the thyroid gland. Many of these pituitary tumors were very large with weights more than 100 times the normal pituitary weight. This observation was soon confirmed by subsequent studies from his laboratory(2) and by others (3,4). Two different opinions as to the mechanism resulting in the formation of these adenomas of the pituitary have been ex-

pressed. Goldberg and Chaikoff(5) found that U.S.P. thyroid added to the mice's diet in small amounts prevented the formation of pituitary adenomas following radiation thyroidectomy and concluded that the enlargement of the pituitary gland induced in the mouse by I^{131} injections results from hypothyroidism alone. Gorbman(2) also found that thyroxin injections reduced the incidence and size of the pituitary tumors expected to develop after I^{131} and that implants of normal thyroid gland which remained viable were effective in preventing formation of pituitary tumors. However, more recently Gorbman has postulated that it is not the hypothyroid state alone which causes the development of the tumors but that a factor related to the ionizing radiation itself may be an influence aggravating or synergizing the thyroidectomy

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in its tumorigenic action upon the pituitary gland. He bases this opinion on the fact that in later experiments, mice which had been pretreated with a low-iodine diet and in which he was able to accomplish complete or almost complete destruction of the thyroid gland with 30 μ c of I^{131} did not develop pituitary tumors; while in contrast, mice on regular diets in whom a comparable destruction of the thyroid gland was caused by 200 μ c of I^{131} did develop pituitary tumors. In another experiment(6) mice on the low-iodine diet were radio-thyroidectomized with 30 μ c of I^{131} and were then exposed to 545 R of whole body x-irradiation or were given an additional 170 μ c of I^{131} . These animals did develop pituitary tumors.

If it is the hypothyroid state alone which causes development of these pituitary tumors, either total thyroidectomy or administration of one of the antithyroid drugs should be effective in their production. Seifter *et al.*(7) report greatly enlarged pituitaries in 2 of 3 rats killed after 2 years. One of these in a selenium-treated rat was a chromophobe adenoma, the other in a thiouracil-treated rat was a chromophobe carcinoma. Whether these tumors resulted from administration of the antithyroid drugs is open to question because a fair number of pituitary adenomas develop spontaneously in old rats. Dalton, Morris, and Dubnik(8) reporting on the long term administration of thiouracil to mice describe 2 cases on the drug for more than 464 days in which the pituitaries had an adenoma-like appearance.

Materials and methods. In the course of other experiments carried out in this laboratory, a number of male A-strain mice, 4 to 6 weeks old, were placed on a diet consisting of 0.8% propylthiouracil[§] in Purina fox chow with meat meal. These mice were maintained on this diet until the last 4 were sacrificed after an interval of 534 days. In addition another group of mice of the same age and strain was set up. Each mouse of this group had a surgical thyroidectomy. This thyroidectomy was done with the aid of a dissecting microscope and at the time of surgery it was

thought that all thyroid tissue had been removed.

Results. At autopsy 2 of the 4 mice on propylthiouracil had massive tumors of the pituitary gland. In one mouse the gland was about 5 mm in diameter and in the other it was approximately 3 mm in diameter. The larger of the 2 glands was hemorrhagic. In the third mouse the gland was 2 to 3 times normal size. There was no marked enlargement of the pituitary in the fourth mouse. Microscopic examination of the pituitary glands of the first two mice showed multiple adenomata of the anterior lobe composed of large chromophobe cells with large nuclei. Several large adenomata made up of chromophobe cells were also seen in the gland of the third mouse. In the fourth mouse, the anterior lobe of the pituitary appeared normal except for the fact that the chromophobe cells seemed to be diffusely enlarged and increased in number.

All 4 mice on propylthiouracil had markedly enlarged thyroid glands—the glands being about 10 times their normal size. Microscopic findings in all were similar. In some areas the glands had an alveolar structure with the follicles composed of tall cells and containing little colloid. In other areas the alveolar pattern was lost and the cells were arranged in solid cords and sheets. In all four mice there was unmistakable evidence of invasion of veins by thyroid tissue. In addition, there was invasion of the strap muscles in one mouse and of lymphatics in another. The lungs of 2 of the animals (the 2 with the smaller pituitary glands) contained multiple, small, glistening, pale pink nodules on gross examination. Microscopic examination revealed these nodules to be composed of thyroid tissue. On the basis of this evidence, a pathologic diagnosis of carcinoma of the thyroid was made in all four animals. Carcinoma of the thyroid resulting from long term administration of thiouracil derivatives has also been reported by Hall and Bielschowsky(9) in the rat and by Morris, Dalton and Green(10) in the mouse.

The pituitary glands of all of the surgically thyroidectomized mice were normal. However, residual functioning thyroid gland tissue

[§] Obtained through the generosity of the Lederle Laboratories.

was found in each animal when microscopic sections of the laryngeal region were examined.

Discussion. This material is presented at this time because of the difference of opinion which has arisen regarding the pathogenesis of the pituitary tumors which follow radioiodine thyroidectomy. It seems apparent from the fact that pituitary tumors arise in mice from the hypothyroid state caused by the antithyroid drugs as well as the athyroid state caused by adequate doses of I^{131} , that the lack of circulating thyroid hormone need be the only factor present to result in the production of chromophobe tumors of the anterior lobe of the hypophysis. Surgical thyroidectomy in our hands was unsuccessful. The hypertrophy of the residual thyroid tissue remaining after operation seemed to leave the animals in a normal euthyroid state.

Summary. 1. Data are presented on 4 mice treated for 18 months with a diet to which propylthiouracil had been added. 2. Three

of 4 mice had chromophobe adenomata of the anterior lobe of the pituitary gland. 3. All 4 mice had carcinomas of the thyroid gland.

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Fractionation of the Swine Follicle Stimulating Hormone. (20205)

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A number of investigators have fractionated pituitary glands for gonadotropins. Van Dyke *et al.*, (1) reported the isolation of a swine follicle stimulating hormone (FSH) which was 80-85% "pure" by electrophoresis. Greep, Van Dyke and Chow (2) earlier published a method for obtaining FSH from swine pituitaries. Li, Simpson and Evans (3) succeeded in isolating an electrophoretically homogenous FSH preparation from sheep pituitaries which was essentially free of other pituitary hormones. The authors have investigated a number of methods to accomplish the concentration of FSH from the residues obtained as a result of the acid acetone extraction of whole swine pituitaries. It was found that two alcohol fractionations of these residues give an FSH with a high biological activity which is only slightly contaminated with

lutinizing hormone (LH) and thyrotropin (TSH).

Experimental. Frozen whole hog pituitaries are comminuted and extracted by a slightly modified Lyons (4) procedure. The 70-80% acid acetone insoluble fraction contains FSH with small amounts of LH and TSH.

Step I. One kg of the wet acetone insoluble fraction (30-40% solids) is suspended in 10 liters of water and rapidly neutralized with 1N NaOH to pH 8.5-9.0. After stirring for an hour at room temperature the pH is again checked and readjusted to 8.5. The mixture is then placed in a refrigerator (0° to 5°C) overnight. Slow agitation is accomplished by an electric stirrer with a rheostat. After standing overnight the extract is centrifuged and the residue discarded. Tests have shown that more than 80% of the gonadotropin is