

mum effects of tetraethylammonium indicated that tetraethylammonium had no demonstrable effect on the action of epinephrine at the effector cells of the nictitating membrane.

Results. Tracings illustrating a typical experiment in Fig. 1 show that tetraethylammonium compound blocks the release of epinephrine from the adrenal medulla.

In all of the 6 cats used in this study the nictitating membrane was stimulated to contract as a result of electrical stimulation of the splanchnic nerve. Repetition of stimuli at intervals over a period of 30 minutes to one hour produced similar contractions. Retraction usually persisted for several minutes.

After administration of tetraethylammonium bromide, stimulation of the splanchnic nerve was not followed by retraction of the membrane. Injection of 3 mg/kg abolished retraction in most of the animals but 10 mg/kg abolished it in all animals. However, intravenous injection of .001 mg/kg of epinephrine hydrochloride at the peak of the tetraethylammonium effect produced marked retraction of the nictitating membrane.

Summary. 1. Tetraethylammonium bromide blocks the release of epinephrine from the adrenal medulla.

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17147. Tumorous Growths in the Pituitary and Trachea Following Radio-toxic Dosages of I^{131} .*

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The possible occurrence of thyroidal tumors after partial beta-ray destruction of the thyroid gland has prompted the investigation of long-term effects of high dosages of I^{131} . No thyroidal growths have been observed in our experiments with mice, but tumors of the trachea and hypophysis have been found. This report deals with the incidence of such tumors and provides a description of the growths as well as the conditions under which they are obtained. A detailed characterization of the cytological properties of the hypophyseal tumors is being prepared separately.

Methods and materials. Most of the work included in this report was done with adult mice of the C_{57}^+ strain. Smaller numbers of the A ,[†] I^+ and $CFW^{\ddagger}$ strains also were em-

ployed. Although more than 500 animals have been utilized in different phases of this study, the present report concerns 158 mice observed for a period of time sufficient to show the metaplastic and atypical growth changes, as well as 34 untreated control mice. Purina Laboratory Chow and water comprised the exclusive *ad libitum* diet of the mice, and they were kept at controlled temperature ($72^{\circ} \pm 2^{\circ}F$), humidity 65% ($\pm 5\%$), and illumination (12 hours per day).

Radioactive iodine was given subcutaneously as NaI without carrier, in neutral aqueous solution in dosages of 10, 50, 80, 100, 160, 200, 300, 400, 500 and 1000 microcuries.[§] At least 10 animals were included in each dosage group for the C_{57} and CFW strains, and in most cases, especially with the 100, 200 and 300 microcurie dosages, there were 40 or more animals.

Calibration and measurement of the dosages was done with a Geiger counter and standards

[§] 100 μc in normal adults produces almost complete, and 200 μc complete, thyroidal destruction.¹

¹ Gorbman, A., *PROC. SOC. EXP. BIOL. AND MED.*, 1947, **66**, 212.

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[†] Breeding stock of these strains originally obtained from colony of L. C. Strong, Yale University.

[‡] Purchased from Carworth Farms, New City, N. Y.

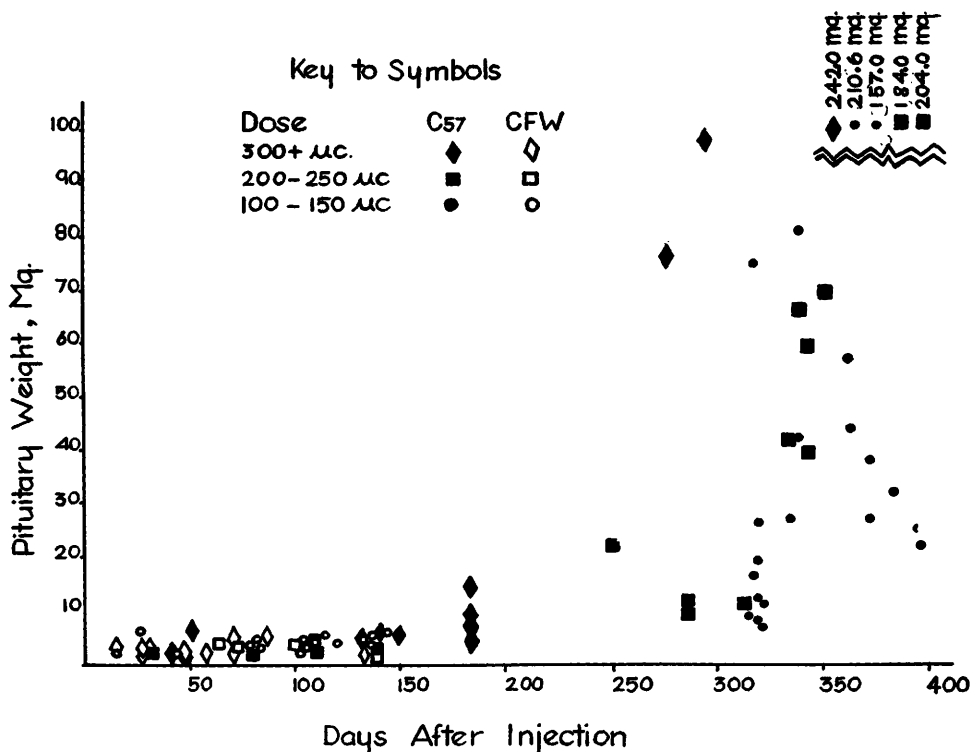


FIG. 1.
Weights of pituitary glands after injection of I^{131} .

which consistently have given a millicurie unit with about 90% of the activity indicated by measurements of the Oak Ridge National Laboratory. Measurement of I^{131} uptake in several separate series of mice injected under these conditions showed that only 5% to 10% of the administered I^{131} is stored in the thyroid, reaching this maximum 12 to 14 hours after injection. The pituitary in our observations never contained I^{131} in concentrations significantly higher than the blood.

Animals were sacrificed in groups at intervals, or singly at irregular times if moribund. Pituitaries were fixed in 10% formalin and weighed from 70% alcohol. Tracheas were sectioned serially for microscopic examination.

Observations. The 10 μ C and 50 μ C dosages were without effect upon the pituitary, as judged by weight. The 80 μ C dose was similarly ineffective in producing tumorous pituitaries, although it did elicit a 3-fold or 4-fold enlargement of the gland in animals which were of small weight at the time of

injection. The 80 μ C dose also produced metaplastic changes in the tracheal epithelium. Dosages of 400 μ C or more almost always resulted in death at an interval too early for development of hypophyseal tumors. For these reasons detailed discussion of results is confined to animals given less than 400 μ C of I^{131} .

Pituitary gland. Fig. 1 indicates the general pattern of results obtained. It seems clear that soon after injection of 100 μ C to 325 μ C quantities of radioactive iodide, and up to 150 days thereafter, there was mild enlargement of the hypophysis. The average control pituitary weight in this interval was 1.2 mg. The average of I^{131} -injected animals was 3.34 mg. Between 150 and 250 days rapid increments in pituitary weights occurred in mice given adequate I^{131} dosage. As indicated in Fig. 1, after 250 days very few pituitaries under 10 mg could be found. The average control pituitary of older mice weighed 2.1 mg. The average of all the I^{131} mice

was 34.7 mg. The data indicate a tendency for larger tumors of earlier occurrence after the largest (300+ μ c) dose. The 3 largest tumors weighed 242, 204 and 210 mg, and followed 325 and 200 and 100 μ c dosages, respectively. Excluding 5 extremely large glands of over 150 mg, the average weights of pituitaries 250 days or more following injection, were 27.5 mg for the 100-160 μ c dosage group, 39.7 mg for the 220-225 μ c group, and 87.3 mg for the 300+ μ c group.

The glands weighing more than 20 mg characteristically showed hemorrhagic areas, particularly on the dorsal side near the infundibular stalk. Gross examination of serial sections of the tumors proved that they are limited to the anterior lobe.

Pituitaries weighing more than 30 mg frequently produced blindness and motor disturbances in the mice. Such glands lost the smooth outlines of smaller tumors and frequently were lobed. In most instances a hollow was formed on the ventral side of the brain by displacement of its parts. The larger tumors, by pressure against the brain, actually caused various degrees of separation of the parietal and occipital bones. The resulting distortion in the shape of the head made it possible to identify, from external examination, those animals with tumorous pituitaries. All of the hypophyseal tumors appeared to exert their influence upon neighboring tissue by pressure alone. In no instance was there evidence of erosion or invasion of brain or bone or other tissue by growing hypophyseal masses.

Trachea. In contrast with most other tissues, the tracheal epithelium appeared extremely sensitive to the accumulated radiant energy in the thyroid gland, and cellular responses were visible as early as one day following I^{131} injection. Early responses were all of the degenerative type, and their degree and rate of development varied with the size of the I^{131} dose.

Pycnosis of nuclei, loss of cilia, and finally death and desquamation of the cells was the usual order of changes observed after dosages of 80 μ c or more. Even with the lowest effective dose such changes were completed

within a week, and regeneration begun at that interval. The 80 μ c dose produced minimal lesions; the 1000 μ c dose produced within 48 hours complete desquamation of that tracheal epithelium closest to the thyroid. In cases of large scale loss of the tracheal epithelium accumulations of macrophages appeared within several days and were seen to contain cellular debris. After the 80-160 μ c doses, tracheal epithelial regeneration was, as a rule, of the normal type and occurred from the normal epithelium surrounding the lesions. After doses of more than 200 μ c tracheal regeneration was, as a rule, with a metaplastic epithelium of variable form and only rarely entirely normal. Sometimes it consisted of cuboidal ciliated or unciliated cells. Most often, especially with the higher doses, it was simple or stratified squamous in type. Some of the stratified squamous tracheal epithelia in CFW mice were extremely active mitotically.

Changes in the fibrous tunica propria and in the tracheal cartilages also occurred, but only with doses in excess of 300 μ c. Temporary loss of basophilia in the cartilage often was observed soon after injection, and death of many chondrocytes was usual. Such changes often were followed by a hyaline thickening of the perichondrium which in some instances became as thick as the entire cartilage and in some instances strongly resembled bone. Thickening of the fibrous tracheal tunica propria was usual after the higher doses in animals sacrificed about six to ten months after injection. In 5 extreme cases the fibrous thickenings projected into the tracheal lumen as small tumors almost completely obstructing it. Such animals before autopsy had a labored wheezing type of breathing.

Discussion. The mechanism by which tumorous growth in pituitaries was elicited in our experiments does not seem to be deducible from the data at our disposal. Administration of goitrogens leads to mild enlargement of the pituitary gland.² In this laboratory (unpublished data) a 0.1% thiouracil diet fed to mice for almost 400 days yielded

² Higgins, G. M., *Am. J. Med. Sci.*, 1945, **210**, 347.

pituitary weights as high as 8.2 mg, and averaging 4.4 mg, compared to the control weight in old mice of 2.0 mg.

It does not seem likely, therefore, that the radiation thyroidectomy experienced by our animals is the principal factor to be considered, though it conceivably might play a synergistic role in combination with some other influence.

It has been well established^{3,4} that chronic administration of estrogens to rodents results in tumorous enlargements of the pituitary. It is tempting to note that many of our long-term C₅₇ females were in extended periods of vaginal cornification, and had enlarged mammary glands and uteri, as well as loosening of the pubic symphysis, when sacrificed 300 or more days after treatment. On the other hand, male C₅₇ mice were found to have some of the largest hypophyseal tumors in the series and yet displayed none of the usual signs of estrogenization. At the present time, attempts are being made at experimental analysis of this situation, but a really rational approach to the problem does not seem to be indicated by the available data.

It would seem that the destruction of tracheal epithelium in our mice is a direct result of the ionizing beta radiation emanating from the thyroid gland. The regenerated epithelia, though usually metaplastic never showed neoplastic tendencies, excepting possibly those cases of stratified squamous epithelia which were mitotically extremely active. Though apparently benign, the tracheal

tumors found in our mice were, or were about to be, fatal through tracheal obstruction and hindrance to respiration.

It should be emphasized that the range of beta particles of I¹³¹ in tissue is usually considered to be approximately 2 mm.⁵ The thyroid capsule alone is as thick as this around most human thyroids, and hence beta irradiation of thyroid-contiguous tracheal structures appears unlikely. Nevertheless, attention has been drawn⁶ to the relatively frequent occurrence of signs of tracheal injury in patients undergoing I¹³¹ therapy for hyperthyroidism. The possibility of fibrous tracheal proliferation in humans following I¹³¹ treatment of the thyroid remains to be considered.

Summary. Following doses of radioactive iodine sufficient to cause destruction of most or all of the thyroid, tumorous enlargements were found in the pituitary glands and tracheas of mice. Hypophyseal growths as large as 240 milligrams (control, 2.1 mg) were found. Less than 10% of animals sacrificed more than 250 days after I¹³¹ administration had pituitaries smaller than 10 mg in weight.

Tracheal tumors of the fibrous tunica propria, obstructing the tracheal lumen, were of lower incidence (4%) but appeared earlier. Fibrous thickenings of the tunica propria, and metaplastic regeneration of tracheal epithelium were more common.

³ Cramer, W., and Horning, E. S., *Lancet*, 1936, **230**, 1056.

⁴ Wolfe, J. M., and Wright, A. W., *Endocrinology*, 1938, **23**, 200.

⁵ Marinelli, L. F., Quimby, E. H., and Hine, G. J., *Am. J. Roentgenol. and Rad. Therap.*, 1948, **59**, 260.

⁶ Lukens, R. M., *Ann. Otol. Rhinol. and Laryngol.*, 1948, **57**, 633.

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