I. Immunol., 1940, v61, 89.

8. Long, C. N. H., Katzin, B., and Fry, E. G., *Endocrinology*, 1940, v26, 309.

9. Hoberman, H. D., *Yale J. Biol. and Med.*, 1950, v22, 341.

10. Engel, F. L., in (17).

11. Marshall, L. M., and Friedberg, F., *Endocrinology*, 1951, v48, 113.

12. Fischel, E. E., *Josiah Macy, Jr. Foundation Conference on the Connective Tissue*, C. Ragan, Editor, 3rd meeting, Feb. 14, 15, 1952, New York. See also in (17).

13. Fagraeus, A., and Berglund, K., *Nordisk Med.*, 1952, in press and personal communication.

14. Moeschlin, S., Baguena, R., and Baguena, J., *Bull. de l'Acad. Suisse des Sciences Med.*, 1952, v8, 153.

15. Kabat, E. A., Wolf, A., and Bezer, A. E., *J. Exp. Med.*, 1948, v88, 417.

16. Kass, E. H., and Kendrick, M. I., *Fed. Proc.*, 1952, v11, 472.

17. Shwartzman, G., editor, *Symposium on the Effect of ACTH and Cortisone upon Infection and Resistance.* Section on Microbiology, N. Y. Acad. Med., 1952.

18. Ragan, C., (in 17).

19. Vaughan, J. H., Bayles, T. B., and Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 274.

20. Stoerk, H. C., *Ann. N. Y. Acad. Sciences*, 1950, v52, 1302.

21. Dixon, F. J., Talmadge, D. W., and Maurer, P. H., *J. Immunol.*, 1952, v68, 693.

22. Dougherty, T. F., Chase, J. H., and White, A., PROC. SOC. EXP. BIOL. AND MED., 1945, v58, 135.

23. Eisen, H. N., Mayer, M. M., Moore, D. H., Tarr, R., and Stoerk, H. C., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 301.

24. de Vries, J. A., *J. Immunol.*, 1950, v65, 1.

25. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, v62, 697.

26. Kabat, E. A., and Heidelberger, M., *J. Exp. Med.*, 1937, v66, 229.

27. Van der Scheer, J., Bohnel, E., Clarke, F. H., and Wyckoff, R. W. G., *J. Immunol.*, 1942, v44, 165.

28. Landsteiner, Karl. *The Specificity of Serological Reactions*, 1946, Harvard University Press, Cambridge.

29. Ramon, G., *Rev. D'Immunol.*, 1937, v3, 193; 1938, v4, 5.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

Role of Ionizing Radiation in Eliciting Tumors of the Pituitary Gland in Mice.* (19873)

AUBREY GORBMAN AND ABRAHAM EDELMANN.

From Brookhaven National Laboratory, Upton, Long Island, and Columbia University, New York City.

The finding that large fatal growths of the pituitary gland form in mice about a year after injection of thyroid-destroying doses of radioiodine, I^{131} (1), has been verified in several laboratories. These growths, as much as 200 times as large as the normal gland, are composed almost entirely of chromophobic cells, and their development is neither favored nor retarded by gonadectomy (4). Furth *et al.* (3,5) have found that transplants of the pituitary growths form large tumorous masses and metastasize in radiothyroidectomized hosts, and that later transplant generations grow even in normal hosts. More recently it has been shown that injection of thyroxine (2,6) or thyroid gland implants (6) inhibit the development of the tumors, suggesting that they are stimulated by the hypothyroidism induced by the radiothyroidectomy. However, radiothyroidectomy alone is insufficient to produce the tumors, since they do not develop in mice whose thyroids were destroyed by *small* quantities of I^{131} while they were being fed a low-iodine diet (6). Thyroids of mice fed such diets absorb a much larger proportion of the administered I^{131}, making it possible to destroy the thyroid glands with much smaller quantities of I^{131}. This suggests that the radiant energy in high doses of I^{131} may be a factor in precipitating the

* Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

TABLE I. Relationship Between Dose of I^{131}, Whole Body X-Irradiation, and Incidence of Late Hypophyseal Tumors.

No. of mice	Diet	Dose of I^{131}, μc	Dose of x-ray	Age at autopsy (mo)	Wt of pituitaries, mg — In detail	Wt of pituitaries, mg — Avg	Wt of pituitaries, mg — Median
25	Purina chow	0	0	14.2	1, 1, 1, 1.1, 1.1, 1.2, 1.3, 1.3, 1.4, 1.4, 1.4, 1.5, 1.5, 1.5, 1.5, 1.5, 1.5, 1.6, 1.6, 1.7, 1.7, 1.7, 1.7, 1.9, 2.1	1.46	1.5
21	Purina chow	200	0	13 –14.4	1.1, 1.7, 3.5, 8.4, 11.8, 22.2, 22.5, 23.5, 28.6, 30.6, 31, 51, 66.1, 81, 90.2, 100, 110, 112.5 Unweighed: 1 "small tumor," 2 "large tumors"	44.2	29.6
11	Purina chow	0	545r	13.6–14.7	.75, 1, 1.4, 1.6, 1.6, 1.7, 1.8, 1.9, 1.9, 2.2, 2.2	1.65	1.65
11	Low-iodine	30 + 170	0	13.6–15.3	9.7, 10, 21, 52, 99.2, 118, 157.1, 254.2 Unweighed: 1 "small tumor," 2 "large tumors"	103.3	34
10	Low-iodine	30	545r	13 –14.4	2, 12, 19, 24.4, 28, 41.9, 56.4, 80.2, 84.2, 151	49.9	35

tumorous development of the pituitary.

To test this possibility 77-day-old C_{57} male mice were placed on the Remington low-iodine diet for a period of one month before giving them 30 μc of I^{131}. This treatment has been found(6) to destroy or almost destroy (with no regeneration) the thyroids of C_{57} mice, but it is not followed by growth of hypophyseal tumors. Some of these animals (Table I) received a whole-body exposure of 545 r of whole body x-radiation (250 kvp: 30 ma 0.25 mm Cu, 1 mm Al; h.v.l. 0.9 mm Cu) 7 days after the I^{131} treatment. Others were given a high dose (170 μc) of I^{131} after the return to the normal Purina Lab Chow diet. As controls some mice, on a normal diet, were given the tumor eliciting dose of 200 μc of I^{131}, and others were given only the 545 r x-ray treatment. The organization of the investigation, the different types of control experiments, and the results are presented in detail in the table.

It seems clear that x-radiation is able to supply the necessary tumorigenic influence for the pituitary gland in mice whose thyroids have been destroyed by small amounts of I^{131}. The radiothyroidectomy or x-ray alone does not elicit hypophyseal tumors. It may be concluded that in the earlier experiments on production of hypophyseal tumors with large amounts of I^{131}, the endocrine (thyroidectomy), and radiant factors may have operated either additively or synergically in their tumorigenic action.

These experiments suggest that radiothyroidectomy induces physiological changes in the pituitary which sensitize the pituitary to the tumorigenic properties of the x-irradiation. It also is of interest that by this method of treatment, *i.e.* 30 μc I^{131} given to mice on a low-iodine diet, and followed by whole-body x-irradiation, tumors may be produced in a specific locus. To our knowledge similar instances of specific localization of a tumor following whole body x-irradiation have not been reported before.

Summary. 1. Pituitary tumors do not form after radiothyroidectomy of C_{57} mice by small

amounts of I^{131}. 2. Pituitary tumors result when such radiothyroidectomy is followed by whole-body x-irradiation, or by larger amounts of I^{131}. 3. Similar doses of x-rays alone do not lead to growth of the tumors. It appears that ionizing radiation induces these atypical growths in pituitaries physiologically altered by radiothyroidectomy.

1. Gorbman, A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 237.

2. Goldberg, R. C., and Chaikoff, I. L., *Endocrinology*, 1951, v48, 1.

3. Furth, J., and Burnett, W. T., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 222.

4. Gorbman, A., *J. Clin. Endocrinol.*, 1950, v10, 1177.

5. Furth, J., Gadsden, E. L., and Burnett, W. T., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 4.

6. Gorbman, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 538.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

A Mechanism of Cholesterol Deposition on Arterial Walls. (19874)

JOHAN BJORKSTEN. (Introduced by Edwin L. Gustus.)

From Bjorksten Research Foundation, Madison, Wis.

It is established that cholesterol deposition takes place in the arterial walls of animals (1,2) and man(3,4) and that such deposition is a forerunner of arteriosclerosis and atherosclerosis(5-7). The cause of this cholesterol deposition has not been known(8).

In view of the fact that at least some correlation exists between progressing age and cholesterol deposition in arteries, and in view of the working hypothesis that the fundamental aging reaction is a protein cross-linkage or tanning reaction(9,10) it seemed pertinent to determine whether known cross-linking agents for protein would render arterial wall surfaces selectively adsorptive to cholesterol.

Methods. To this end fresh hog aortas* were slit open. Round test pieces of 20 mm diameter were punched out by means of a cork borer. These test pieces were placed in water solutions of a cross-linking or tanning agent, and control pieces from the same aorta were placed in water. After an interval as shown below, all of these test pieces were immersed in a cholesterol suspension prepared as follows: Fifty g of cholesterol were milled on a rubber mill with submicron particle size acetylene carbon. The object of this was to color the cholesterol particles so that they could be followed visually and photographed. The rubber mill effects a very intimate mixture and complete wetting of the carbon particles by cholesterol. This was verified by microscopic examination. On subsequent dispersion in water, it was found that the carbon particles remain inside the cholesterol particles, and thus are believed not to affect the surface behavior of the cholesterol particles. The cholesterol thus pigmented was added to 300 g of a 4% solution of carboxy methyl cellulose in water, dispersed 10 minutes in a Waring Blendor, and diluted with an additional 200 cc of water. The resultant cholesterol dispersion is quite stable, and can be stored at least for several weeks, though it may be advisable to shake it before use to ensure uniformity.

The aortas were kept immersed in this cholesterol suspension for the periods of time shown below, whereupon they were placed in a 4 inches high by 3⅜ inches wide cylindrical jar containing 100 ml of clean tap water and washed twice by shaking for ½ minute in a shaking machine, ⅛ inch stroke, 450 cycles per minute. The areas which had been in contact with the cross-linking solutions showed heavy cholesterol deposits, while the other areas failed to adsorb cholesterol, and after washing as just stated, were completely free from any traces of this substance. Black

* I am indebted to Oscar Meyer Co. for their cooperation in supplying the aortas fresh from the packing line.