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Received Sept. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

Correlation Between Mitotic Activity and Transplantability of Pituitary Tumors Following Radiothyroidectomy* (33771)

B. MESSIER (Introduced by G. Jasmin)

Département d'Anatomie, Université de Montreal, and Institut du Cancer de Montréal, Hôpital Notre-Dame, Montréal, Québec, Canada

Profound cellular changes occur in the anterior pituitary of mice following the injection of a thyroid-destructive dose of ^{131}I (1, 2). Thyrotropic tumors invariably appear several months after the radiothyroidectomy (3) and these may be successfully transplanted to isologous hosts.

In spite of the early and rapid increase in the size of the thyrotrope population after radiothyroidectomy (4), transplantability is not attained before several months. It seemed worthwhile to investigate if a parallelism existed between cell proliferation and acquisition of transplantability.

Materials and Methods. Male and female LAF_1 mice were radiothyroidectomized by intraperitoneal injection of 200 μCi of ^{131}I to each animal. Four, 8 and 12 months later, a group of 8 radiothyroidectomized mice of each sex was treated as follows. One hr prior to sacrifice, each animal received an intraperitoneal injection of thymidine-^3H (1 μCi/g of body wt.). After death, the pituitary was divided approximately in two equal parts. One part was placed in Bouin-Hollande solution to which 5% chrome alum was added just prior to fixation. The other half was again cut in two parts. One part was implanted subcutaneously in the lower back of an isologous female recipient and the other part was similarly implanted in a male recipient. The recipient animals were about 3 months of age and had been radiothyroidectomized 1 month previously.

The pituitary halves fixed in the modified Bouin-Hollande solution were processed for radioautography (5). The frequency of radioactive cells was established on a large size population on account of their scarcity in some group of animals. The following counting procedure was adopted. For each animal, a mean number of all types of anterior pituitary parenchymal cells was established in 50 random fields delineated by a ruled square on an ocular disc. With this value on hand, several hundred fields could be scanned for labeled cells without the tedious task of counting the total number of cells in each field. This procedure allowed us to survey complete sections, representing a population of several thousand anterior pituitary cells per animal. The frequency of labeled cells was then uniformly expressed per 10,000 pituitary cells.

* Supported by a grant from the National Cancer Institute of Canada.

TABLE I. Transplantability of Primary Pituitary Tumors at Two Different Intervals after Radiothyroidectomy.

Pituitary donor Group	Sex	Recipients	Latency (months)	No. of "takes"
Rte[a] 8 months (II)	♀	5 ♂	16	0/5
		8 ♀	12	1/8
	♂	8 ♂	10	2/8
		7 ♀	12	1/7
Rte 12 months (III)	♀	8 ♂	8	2/8
		6 ♀	6	4/6
	♂	8 ♂	6	4/8
		5 ♀	7	4/5

[a] Rte = radiothyroidectomized.

Results. A. Transplanted pituitaries. Intercurrent disease caused a premature death of all animals grafted with pituitaries from donors radiothyroidectomized 4 months previously (I). Such death occurred 2 months after pituitary transplantation, at which time no tumor growth could be observed.

With the female donor mice, radiothyroidectomized 8 months previously (II), only 1 grafted animal out of 13 (5♂, 8♀) developed a palpable tumor (Table I). This tumor appeared 12 months after transplant and grew very slowly for 4 months. With the male donor mice, also radiothyroidectomized 8 months before (II), 3 grafted animals out of 15 (8♂, 7♀) developed a tumor. The first of these three tumors appeared 10 months after transplant and grew at a moderate rate.

Pituitaries of female donor mice, radiothyroidectomized 12 months previously (III), yielded tumors in 6 of the 14 (8♂, 6♀) grafted recipients. The first tumor appeared 6 months after transplant. A somewhat higher frequency of grafted tumors was observed in male donor mice. Indeed, 8 of the 13 (8♂, 5♀) grafted mice developed a tumor, the first one appearing 6 months after transplant. In general, grafted tumors originating from pituitaries of animals radiothyroidectomized 12 months before grew more rapidly and to a larger size. The histological characteristics of both the donor pituitaries and the transplanted tumors were similar to those reported by Halmi and Gude (1) and Furth (6).

B. Thymidine-^3H uptake. Anterior pituitaries of intact animals contained relatively few cells incorporating thymidine-^3H. Only 3.4 labeled cells were found per 10,000 cells (Table II). This value rose significantly to 19.6 in animals sacrificed 4 months after radiothyroidectomy. The frequency was essentially the same after 8 months (18.4) but reached a new significant peak of 56.0 radioactive cells after 12 months. No particular distribution pattern of the radioactive cells could be detected.

Discussion. Malignancy is a broad term to designate a spectrum of biological properties such as unrestrained growth, altered differentiation, metastasis (or transplantability). The relative importance of each of these properties may vary in the course of the life history of a neoplasm. Thus, the present study attempts to evaluate if a parallelism exists between cell proliferation and acquisition of transplantability.

Much of the previous work with thyrotropic tumors deals with transplanted lines (7, 8) in which the acquired property of increased cell proliferation is well established. A study

TABLE II. Frequency of Labeled Anterior Pituitary Cells in Radiothyroidectomized Mice.

Treatment	No. of animals	Total cells counted (thousands)	Labeled pituitary cells	Labeled cells/10^4 pituitary cells
Intact	8	310	87	3.4 ± 4.2[a]
Rte[b] 4 months	8	143	262	19.6 ± 10.7[c]
Rte 8 months	7	159	297	18.4 ± 8.0[c]
Rte 12 months	8	156	820	56.0 ± 20.0[cd]

[a] SD of the mean.

[b] Rte = radiothyroidectomized.

[c] Significantly different from intact controls.

[d] Significantly different from two first experimental groups.

of earlier events in the chronology of developing tumors is that of Kwa (9) who provides valuable data on the progressive increase of the pituitary weight following radiothyroidectomy. However, the transplantability of the tumorous pituitaries was not assayed. More recently, Clifton (10) used thymidine-^{3}H uptake to study cell proliferation kinetics during the induction of thyrotropic tumors. Here also no correlation was made between cellular proliferation and transplantability.

The quantitative results on thymidine-^{3}H uptake reported in the present work cannot be compared with those of Clifton on account of basic differences in the labeling procedures. Indeed, our pulse labeling and early sacrifice (1 hr) provide a more short-term picture of the proliferative capacity of the pituitary cells.

The sudden rise (Table II) in the frequency of labeled cells at 4 months is not paralleled by successful transplantation (as based on previous unpublished data). Possibly the newly-formed cells are not sufficiently altered to lead to the formation of a transplanted tumor. Conversely, transplantation of pituitaries from animals radiothyroidectomized for 8 months yields some tumors (Table I), although no significant change in the frequency of radioactive cells is observed. It is only 12 months after destruction of the thyroid that pituitaries exhibit both an increase in cell proliferation and a higher yield of transplanted tumors. Thus, it may be concluded that several months after radiothyroidectomy must elapse before a clear-cut relationship appears between increased cellular proliferation and successful transplantation.

No effort was made to determine the nature of the proliferating cells. Several authors (1, 11, 12), however, have convincingly demonstrated that a specific cell type (the thyrotrope) was involved in the tumorous transformation. Additional evidence was recently provided (13) when exogenous thyroxine fed to radiothyroidectomized mice promptly stopped cell proliferation in the pituitary.

Summary. Pituitary glands from mice radiothyroidectomized for various periods of time were simultaneously studied for their proliferative capacity (thymidine-^{3}H uptake) and for their transplantability in isologous hosts. Correlation of the latter two phenomena became obvious only after the pituitary donors had been radiothyroidectomized since 12 months.

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Received Oct. 3, 1968. P.S.E.B.M., 1969, Vol. 130.