Cell Population Kinetics during the Induction of Thyrotropic Pituitary Tumors

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Summary

Changes in weight and in the incorporation of infused TdR-3H into the DNA of the pituitary gland were determined at intervals during the development of thyrotropic tumors in radiothyroidectomized mice. The relative specific activity of the DNA was found to increase markedly during the final stage of tumor development. In an effort to correlate the information on the sequence of changes during tumor induction, 5 mathematical models of cell population changes were considered. A model that assumes persistence of most of the original cell population at a constant level throughout tumor induction, an exponential increase in the size of a cell population that initially constitutes a small percentage of the total population, and the appearance of a 3rd more rapidly increasing cell population some time after radiothyroidectomy was described. This model was found most consistent with the experimental data presented and with published results of others and may be an aid in the design of future experiments.

Introduction

1. Neoplasia is a state in which cells, normally limited, proliferate without restraint. 2. This state can be brought about by changes either in the host or in the cell. The former type of neoplasm is termed dependent, the latter autonomous. 3. The basic derangement in the host can be due to sustained excessive stimulation or to a deficiency of natural restraining forces. 4. The basic derangement in the cell can be cytogenic, or it can reside outside the self-replicating apparatus of the cell, as in parasitism by virus. The former is an autonomous, the latter, a virus-dependent, neoplasm. 5. Some autonomous tumors respond in varying degrees to their physiologic regulators; others do not. 6. The trend is for progression "from bad to worse" led to autonomous TtT, which were capable of growth in intact animals. The latter secreted large quantities of thyrotropic hormone, which could result in tumorigenesis in the thyroid glands of the host mice. Despite the work of Furth, of Gorbman, and of others stimulated by them, several questions remain about the timing and sequence of changes in pituitary cell populations during TtT induction and growth. It can be helpful in such cases to examine 1 or more mathematical models with the aim of correlating the available information, and as an aid in suggesting further experiments.

Two models of cell population kinetics during the induction of pituitary tumors have been suggested. The 1st was based on the changes in weight and histology of the pituitary glands of the Sprague-Dawley line of rats during the induction of mammary tumors by chronic estrogen administration (3). According to this model, the cells from which the tumors ultimately arose initially comprised but a small percentage of the anterior pituitary cell population. These cells were postulated to increase exponentially at a constant rate during chronic estrogen exposure.

Kwa's application of this model to his data on TtT induction in mice was unsuccessful. He suggested instead that most or all of the hypophyseal cells were involved in tumor formation. The kinetic consequences of this suggestion were not examined in detail.

The introduction of TdR-3H, a specific DNA precursor, has made possible the study of cell kinetics in a variety of tissues. As the duplication of DNA is a necessary prelude to cell division, under most circumstances the rate of DNA synthesis of a cell population as measured by TdR-3H incorporation is an index of the mean rate of cell proliferation.

The aims of the current studies were (a) to obtain correlated information on the sequence of change in weight and proliferative rate during TtT induction and (b) to examine some kinetic model systems in the light of the available data and of information from the literature.
Materials and Methods

**Biol logic and biochemical procedures.** Groups of young, adult male C57BL/6J mice were fed a low-iodine diet and distilled water for 10–14 days, after which they were injected i.p. with 60–70 μg mL⁻¹. This procedure has been found to result in complete thyroidectomy in all mice so treated (2, 8). At intervals after radiothyroidectomy, groups of animals were infused i.p. with a solution of TdR-H (1.9 c/mmol, Schwarz Biochemicals, Orangeburg, N. Y.) in a nutrient medium containing antibiotics (Eagle’s basic medium, Microbiological Association, Inc., Bethesda, Md., with penicillin and streptomycin added) at a concentration of 100 μg/ml and a total dose of 360 μg/mouse in 48 hr. The infusion apparatus consisted of a Braunwill pump outfitted with up to 6 syringes and operated at a flow rate of 0.075 ml/hr. A polyethylene tube (i.d. 0.034 inches; o.d. 0.06 inches) was inserted into the abdominal cavity through a ventral incision and was passed s.c. to the dorsal area between the shoulder blades and thence to the infusion pump. The small infusion tubing was protected by a larger polyethylene tube (i.d. 0.106 inches; o.d. 0.138 inches), the heat-expanded end of which was sewn s.c. in the dorsal incision, as described by Mendelsohn (19).

In a preliminary autoradiographic study of the distribution of incorporated TdR-H, the mice were killed by cervical dislocation 16–19 hr after the end of the infusion. The pituitary glands were immediately removed, fixed in acetic acid-formol:ethyl alcohol fixative, paraffin imbedded, and sectioned at 2–3 μ. Mounted deparaffinized sections were hydrolyzed for 10 min in 1 N HCl at 60°C to remove unincorporated TdR-H and were coated with Kodak NTB-3 liquid emulsion. After exposure and development, the nuclei were stained with Harris’s hematoxylin.

For the biochemical studies, the mice were removed from the infusion apparatus and either were killed immediately for collection of tissue samples as above or were kept alive for 21–69 days before sacrifice. The pituitary glands were removed immediately after death and immersed in chilled 0.5 N PCA. After a few minutes of fixation in PCA, the tissue samples were blotted on filter paper and rapidly weighed. Those glands weighing less than 10.0 mg were weighed to the nearest 0.02 mg, and larger samples to the nearest 0.5 mg on torque balances.

Tissue samples were homogenized in chilled glass homogenizers with small amounts of cold 0.5 N PCA and centrifuged at 1500 rpm in a refrigerated centrifuge. The supernatant fractions were discarded. The precipitates were resuspended, washed, and re-centrifuged 5 times with chilled PCA. The washed precipitates were extracted twice with 0.5 N PCA in a boiling water bath to remove the DNA. The DNA extracts were adjusted to a final concentration of 10 mg equivalent of wet tissue weight per ml for larger glands, or to a minimum volume of 0.5 ml for smaller glands. The DNA concentrations of the PCA extracts were determined by a semimicro adaptation of Burton’s modification (1) of the Dische reaction. Total protein determinations were performed on 0.3 N KOH hydrolysates of the acid-insoluble precipitates, according to the method of Lowry et al. (17).

For determination of radioactivity, aliquots of 0.05–0.20 ml of the DNA extracts were pipetted into plastic bottles, and PCA or internal standard (known quantities of TdR-H) in PCA was added to a final volume of 0.3 ml. Ten ml of a dioxane scintillation mix (21) was then added to each, and the radioactivity was determined as cpm in a liquid scintillation counter. Standards containing unlabeled pituitary extracts to which known amounts of TdR-H were added were prepared for comparison. The radioactivity measurements were normalized according to the measured radioactivity of the TdR-H solution used for infusion of each experimental group. To reduce the variation between groups due to infusion with different batches of TdR-H, the results are expressed as relative specific activity; that is, the cpm/μg of DNA of the extracts of tissues from thyroidectomized mice were divided by the mean cpm/μg of DNA of their respective controls. Radioactivity measurements on tissues taken on the day after infusion were made to a maximum S.D. of 10% for the smallest tissue samples. The radioactivity detection efficiency was about 7%.

**Calculation of the specific activity of the DNA of the rapidly proliferating cells.** As the anterior pituitary is a mosaic of functional cell types of which the normal thyrotropic cells comprise a fraction (13, 20), the observed specific activity of the DNA of a pituitary gland from a thyroidectomized mouse will consist of 2 components: the specific activity of that quantity of DNA from the proliferating Tt and the specific activity of the DNA of the fraction of normal cells remaining. Although the methods used in these experiments do not provide for the determination of the absolute percentage of the thyroidectomized mouse pituitary gland that is composed of the rapidly proliferating cells, the 2 logical extremes of the specific activity of the rapidly proliferating cells can be calculated. For this purpose, the following symbols are defined: \( X_t \), the observed total DNA in the pituitary gland of a thyroidectomized mouse; \( X_n \), the quantity of DNA contributed by normal cells; \( X_p \), the quantity of DNA in the rapidly proliferating fraction of cells in the pituitary of a thyroidectomized mouse; \( Y_t \), the total radioactivity in cpm in the DNA of a thyroidectomized mouse pituitary; \( Y_n \), the total radioactivity in the normal DNA; and \( Y_p \), the total radioactivity in the rapidly proliferating cells in the thyroidectomized mouse pituitary. Then \( Z_t = Y_t/X_t \) is the observed total specific activity of the DNA of the thyroidectomized mouse pituitary; and \( Z_n = Y_n/X_n \) and \( Z_p = Y_p/X_p \). Now, \( X_n \) in the pituitary of a thyroidectomized mouse represents some fraction, say \( f \), of the total DNA in the pituitary of a thyroidectomized mouse; \( X_p = (1 - f)X_t \). Therefore, the total radioactivity in the DNA of a thyroidectomized pituitary is:

\[
Y_t = fX_pZ_n + (1 - f)X_tZ_p
\]

and the specific activity of the DNA of the rapidly proliferating cells is:

\[
Z_p = (Y_t - fX_pZ_n)/(1 - f)X_t
\]

or:

\[
Z_p = (Z_tX_t - fX_pZ_n)/(1 - f)X_t
\]

and

\[
Z_p = (Z_t - fZ_n)/(1 - f)
\]

Where relative specific activity is used as in the current studies, \( Z_n \) equals 1 by definition. The latter equation then is simplified to:

\[
Z_p = (Z_t - 1)/(1 - f)
\]
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**Chart 1.** Weight of the mouse pituitary gland at intervals during TtT induction. *Vertical bars* indicate the S.D. of weight; *horizontal bars*, the S.D. of time. *Digits* indicate the number of glands included in the respective points.

\[ Z_p = (Z_t - f)/(1 - f) \]

where \( Z_p \) and \( Z_t \) are the respective RSA-DNA values.

The lower limit for \( Z_p \) has been calculated by taking \( f \) as equal to 0; that is, by making the assumption that all pituitary cells take part in the tumorous proliferation and that following radiothyroidectomy there is no normal DNA. Under these circumstances, \( Z_p \) is equal to \( Z_t \), and the RSA-DNA of the proliferating fraction of cells in the thyroidectomized pituitary is equal to the observed RSA-DNA of such glands.

The upper limit of \( Z_p \) was calculated by taking \( Z_p \) as equal to the observed RSA-DNA from normal control pituitary glands (i.e., as 1) and taking \( f \) as equal to the weight of the thyroidectomized mouse pituitary gland. This requires the following assumptions: (a) that the cells from which the tumors are derived initially comprise an insignificant fraction of the total cells present in the normal pituitary and that the normal cells persist throughout the tumor-induction period in the same total number as in the normal gland; and (b) that the proliferative rate of the normal cells remains constant throughout the tumor-induction period. If the total number of normal cells decreases during tumor induction, \( f \) tends toward 0, and the real value of \( Z_p \) would fall within the prescribed limits.

**Results**

Fifty days after administration of \(^{131}I\), pituitary gland weights of thyroidectomized animals were approximately 50% greater than their respective controls (Chart 1). The weight increased slightly during the next 70 days, and progressively greater increases were observed during subsequent experimental intervals. By 455 days, the glands of thyroidectomized animals averaged over 50 mg. Pituitary gland weights of control mice did not significantly change during the experimental period.

The minimum (observed) RSA-DNA of the pituitaries of the radiothyroidectomized mice averaged 1.7 by 50 days following \(^{131}I\) administration and fluctuated from 1.6 to 4.6 during the ensuing 336 days (Chart 2). By 455 days after radiothyroidectomy, when the mean pituitary weight was 52 mg, however, the observed RSA-DNA increased to 17.6. The difference between the minimum and the maximum calculated RSA-DNA was greatest at the early time intervals, becoming less marked as the ratio of the control to the thyroidectomized pituitary weights decreased.
The pattern of hypophyseal growth following radiothyroidectomy of C57BL male mice is grossly similar to that described during the 1st 2 months after thyroidectomy of mice or the initiation of estrogen treatment in Sprague-Dawley rats, there is a period of glandular hypertrophy. This is followed by a prolonged tumor-induction phase, during which the weight of the gland increases slowly, and finally by rapid tumorous growth.

Initial period of glandular hypertrophy in the hypophyses of estrogen-treated rats was attributed to cell enlargement and an increase in the volume of vascular spaces (3). Similar hypertrophic changes in the presumptive Tt of mice during the 1st 60 days after thyroidectomy have been described by Halmi and Gude (13). In the latter study, no histologic evidence of rapid proliferation was found during the 1st 60 days. In the following discussion and for the purpose of the calculations, therefore, it is assumed that the hypertrophic changes in the pituitaries of mice are complete by 50 days following 131I administration. Changes in total gland weight thereafter are assumed to be due primarily to proliferation.

Five possible mathematical models designed on the basis of pituitary weights following thyroidectomy have been considered in the current studies (Table 2). Values were derived for the terms of the equations by trial and error. All 5 models yielded curves that are reasonably consistent with the weight changes (Chart 3).

Model I is the same as that proposed for estrogen-induced hypophyseal tumors in rats (3) and follows the equation:

$$W = A_0e^{B_0} + B_0e^{T/T_B}$$

where $W$ is the weight of the pituitary gland of a thyroidectomized mouse, $A_0$ is the weight of the normal cells that remain in the gland during tumorigenesis and is assumed constant, $B_0$ is the weight of the cells present in the pituitary gland 50 days following radiothyroidectomy from which the tumors arise, $e$ is the base of the Naperian system of logarithms, $T$ is the time in days following radiothyroidectomy minus 50, and $T_B$ is a constant reflecting the volume-doubling time of the proliferating cells. If $B_0$ is taken as 2% (0.042 mg) of the gland weight at 50 days, and $T_B$ as 58.3 days (Table 2), the curve obtained approximates the experimental data (Chart 3).

Models II and III were designed from the suggestion of Kwa (16). In Model II, all cells are considered to be involved in the

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**TABLE 1**

**Cumulative Mean Protein and DNA Concentrations of Pituitary Gland Extracts**

<table>
<thead>
<tr>
<th>No. of DNA/mg Tissue</th>
<th>No. of Mean ± S.D. Protein/mg Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroidectomized</td>
<td>45 5.7 ± 1.8</td>
</tr>
<tr>
<td>Control</td>
<td>48 8.0 ± 3.4</td>
</tr>
</tbody>
</table>

Following TdR-H infusion, several small groups of animals were set aside in order to observe the decrease in RSA-DNA due to loss of the labeled DNA by cell death or from dilution of the label by synthesis of unlabeled DNA. The decreases in RSA-DNA apparently exceeded that to be expected from dilution during growth alone and were in general in the same range in treated and in control mice (Chart 2). The level of radioactivity in these samples was, however, below that necessary for accurate quantitation.

Although no attempt was made to obtain quantitative recovery, it is of interest that there were no significant differences at any experimental interval in the concentrations of either DNA or protein between the hypophyseal pituitary preparations from the thyroidectomized mice and those from their respective controls. Furthermore, there was no apparent trend in either group with time after initiation of treatment. The data were thus combined and the over-all means are presented in Table 1.

In the radioautographic studies, a few labeled nuclei occurred at random in the control glands (Fig. 1) and appeared more frequently by 150 days after thyroidectomy (Fig. 2). By 270-330 days following radiothyroidectomy, the labeled nuclei occurred primarily in focal areas (Figs. 3, 4), which increased in size as tumorigenesis proceeded (Fig. 5). As a result of the variation in size, shape, and location of these centers of DNA-synthesizing cells, estimation of the fraction of cells involved in tumor formation or of the rate of DNA synthesis by radioautographic means would be subject to large sampling error.

**Model Systems**

The pattern of hypophyseal growth following radiothyroidectomy of C57BL male mice is grossly similar to that described for the anterior pituitary glands of male rats of the Sprague-Dawley line subjected to chronic estrogen administration (3). During the 1st 2 months after thyroidectomy of mice or the initiation of estrogen treatment in Sprague-Dawley rats, there is a period of glandular hypertrophy. This is followed by a prolonged tumor-induction phase, during which the weight of the gland increases slowly, and finally by rapid tumorous growth.

**TABLE 2**

**Equations and Values for Theoretical Pituitary Weight Curves Plotted in Chart 3**

<table>
<thead>
<tr>
<th>Equation</th>
<th>Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_0, mg$</td>
<td>$A_0 = A_0e^{B_0} + B_0e^{T/T_B}$</td>
</tr>
<tr>
<td>$z, days^{-1}$</td>
<td>$z = T_{BO}^{T/T_B}$</td>
</tr>
<tr>
<td>$B_0, mg$</td>
<td>$B_0$ is the weight of the normal cells that remain in the gland during tumorigenesis and is assumed constant.</td>
</tr>
<tr>
<td>$T_B, days$</td>
<td>$T_B$ is the time in days following radiothyroidectomy minus 50, and $T_B$ is a constant reflecting the volume-doubling time of the proliferating cells.</td>
</tr>
<tr>
<td>$T_{BO}, days$</td>
<td>$T_{BO}$ is the time at which the tumors arise.</td>
</tr>
<tr>
<td>$y, days^{-1}$</td>
<td>$y$ is the base of the Naperian system of logarithms.</td>
</tr>
<tr>
<td>$C_0, mg$</td>
<td>$C_0$ is the weight of the normal cells that remain in the gland during tumorigenesis and is assumed constant.</td>
</tr>
<tr>
<td>$T_C, days$</td>
<td>$T_C$ is the time at which the tumors arise.</td>
</tr>
</tbody>
</table>

$* T$, days after thyroidectomy minus 50 days; $e$, base of the Naperian logarithms.
tumorous proliferation from 50 days onward. To obtain a curve approximating the experimental data, it was necessary to assume that the doubling time of the proliferating cells was continually reduced. Thus, if $T_B$ is initially 45.6 days and continually decreases at the rate indicated by $\gamma$ in Table 1, a fair approximation of the experimental growth is obtained (Chart 3). It should be noted that values could probably also be found for the situation in which $T_B$ is assumed to decrease linearly with time in a model otherwise similar to Model II. For reasons noted in the discussion, however, such calculations were not performed.

In Model III, the proliferative rate of the cells from which the tumors arise is considered to be constant, but new cells are assumed to enter the rapidly proliferating component from the normal cell component throughout tumor induction. Thus the weight of normal cells symbolized by $A$ decreases at a rate that is dependent on time.

Model IV is a generalized form of Model II. For reasons discussed below, it was of interest to take $B_0$ as a small percentage of the total gland weight and determine the necessary rate of increase in $T_B$ for a curve that would approximate the experimental data.

Model V was developed with the assumption that normal cells persist throughout tumor development and that 2 rapidly proliferating populations of cells develop during tumor induction. The 2nd rapidly proliferating cell population, symbolized by $C$, was assumed either to initially comprise a very small percentage of the total gland weight or to arise by transformation from cells in another component during tumor induction. The models presented do not, of course, exhaust the possibilities. For example, a series of additional components, i.e.

$$
1/T_B = 1/(T_{bo}e^{-\nu T})
$$

and mean rate, Model V =

$$
[(B_{o}e^{\gamma T_A})/T_B] + [(C_{o}e^{\gamma T_C})/T_C] / (B_{o}e^{\gamma T_B} + C_{o}e^{\gamma T_C})
$$

The degree of similarity between the pattern of change in the increase rates so calculated for Model V and the pattern of
change in RSA-DNA values is illustrated in Chart 4. As would be expected, the agreement is somewhat better with the estimated maximum than with the minimum RSA-DNA values of the proliferating cell population.

The calculations with Model IV showed a maximum total increase in mean proliferative rate of less than 10% during the entire experimental period and, with Model II, a 40-fold increase during the same time. The value of $1/T_B$ does not change with time in Models I and III. Models I through IV are thus inconsistent with the TdT—H incorporation data, and Model II, with the histologic data.

If Model V is indeed a valid approximation of the cell population kinetics during TIT induction, $B_0$ would be interpreted as the initial Tt population. The cell population symbolized by $C$ would be taken as the altered cells that are capable of continued proliferation when grafted at sites remote from the hypothalamus (2).

The value of $C_0$ in mg in Table 2 is less than the weight of a single cell. Assuming the 1st altered cell weighed $1 \times 10^{-4}$ to $30 \times 10^{-4}$ mg (i.e., was 10–30 µ in average dimension), it would 1st appear 200–250 days after radiothyroidectomy. The model thus suggests the time after I$^{131}$ administration when pituitary tissue from thyrotoxic thyroidectomized mice may 1st give rise to tumors on transplantation to thyroid-deficient hosts. This change would appear to be the earliest known step in TtT progression "from bad to worse" on the road toward malignant autonomy (6).

Acknowledgments

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References

17. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall,
Figs. 1–5. All figures are of radioautographs of sections of pituitary glands of mice that were infused with TdR-\(^{3}H\). All were stained with hematoxylin and are enlarged 60 times. Fig. 1 is from a normal control mouse of the same age as that in Fig. 5; pars distalis at right and left, pars intermedia and nervosa in center. Fig. 2 is from a mouse radiothyroidectomized 150 days earlier; pars intermedia at left border. Fig. 3 is from a mouse radiothyroidectomized 270 days earlier. Fig. 4 is from a mouse radiothyroidectomized 330 days earlier; pars intermedia at left center. Fig. 5 is from a mouse radiothyroidectomized 390 days earlier.
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