Alterations Induced by Thyroidal Hormones in the Response to Temperature by T-1 Kidney Cells[1] (37611)

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Over the years it has been supposed in one form or another that the actions of the two thyroidal hormones, thyroxine (T_4) and triiodothyronine (T_3), are principally calorigenic, involving the release of heat and the elevation of body temperature. The relationship of metabolic processes, especially those having to do with respiration and energy transformation, are classically linked to these elaborations and to disorders afflicting their source, the thyroid gland. Although the existence of a thermoregulatory center within the mammalian brain has been recognized for well over a century (1), the mechanisms governing and generating the body heat of homeotherms still remain unclear. Complicating the picture is the nature of the contribution to thermal regulating and producing processes made by the peripheral cells acting under the influence of the circulating thyroidal hormones.

In the hope that experimentation with an *in vitro* system will shed new light on the nature and role of intracellular thermal mechanisms, studies were undertaken with the T-1 human kidney cell line (2) exposed to different temperatures and concentrations of T_4 and T_3. This cell line was chosen since it has been found very sensitive to and specific for these hormones and related amino acids. For example, treating the T-1 cells with T_4 and T_3 results in modifications of morphology, dimensions, proliferation, and colony-forming ability, as well as of the metabolism of proteins, nucleic acids, and lipids; for eliciting most of these effects, T_3 is 5–10 fold more potent with regard to concentration than T_4 (3, 4). Reducing the fetal bovine serum content of the growth medium unmasks the profound sensitivity of the T-1 cells to the presence of low hormonal concentrations, even those as dilute as 10^{-9} *M* T_4 or 10^{-10} *M* T_3; it was thus found that L-T_4 is more active than D-T_4 (5). The response of these cultured kidney cells to the two thyroxine analogs, diiodothyropropionic acid and triiodothyroacetic acid accords with their effects *in vivo* as well as on other *in vitro* systems (6). In the T-1 cells these thyroidal hormones trigger an increase in the number of nucleoli and in their synthesis of nuclear RNA (3). Studies with tagged thyroxine and the antibiotics puromycin and actinomycin D also implicate the nucleus as a primary locus of action in the T-1 cell line (7), a finding consonant with the recent report (8) of T_3 specific nuclear binding sites being encountered *in vivo*.

Materials and Methods. The T-1 cultured cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum as monolayers onto glass or plastic in a water-saturated atmosphere of CO_2 (5%) and air (95%). Normally maintained at 37°, the temperature of incubation for these experiments was varied between 34.5 and 38.5°. To monitor the constancy of the internal temperature of the incubators while these studies were underway, frequent determinations (at least four times daily) were made using calibrated laboratory thermometers. For determinations of cellular proliferation (9), 3×10^4 isolated cells were

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seeded onto plastic Petri dishes, and the multiplicity (*i.e.*, the average number of cells per colony) was estimated as a function of time utilizing an inverted tissue culture microscope equipped with phase contrast optics. The slope of the straight-line segment of the resulting semi-log plot was employed to estimate the mean doubling time (T_d). Plating efficiency (PE) determinations were made as reported previously (3), by introducing 300 single cells in the exponential phase of growth into 100 mm diameter plastic Petri dishes; after 2 weeks of incubation, the colonies made visible following staining with 1% methylene blue were scored. As noted subsequently, for some experiments the numbers of individual cells introduced were increased from 300 to 500, 3000, or 30,000. L-thyroxine and L-triiodothyronine obtained from Calbiochem were each dissolved in triple distilled water with a drop of 1 N NaOH and sterilized by filtration; osmolarity was maintained by preparing highly concentrated solutions of each hormone, so that only 0.1 ml of the required amino acid was diluted with 10 ml of the growth medium.

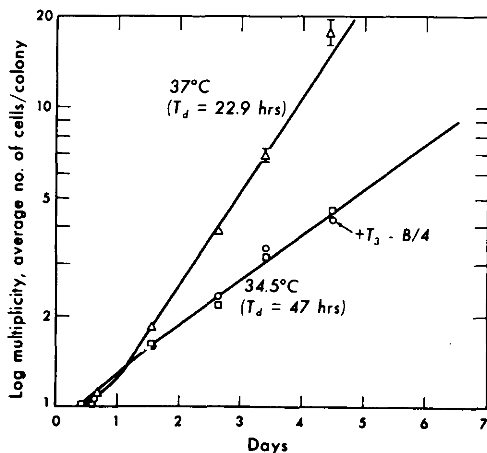

FIG. 1. Depression of cellular proliferation resulting from reduction of incubation temperature from 37° to 34.5°; mean doubling time (T_d) more than doubles. Introduction of 4.45 $\times$ 10^{-7} M T_3 (plotted as circles, designated T_3-B/4), a concentration shown earlier to reduce T_d at 37°, does not stimulate proliferation at 34.5°. Plotted points are the means of duplicate dishes based on scoring 200 colonies/dish with bars indicating SD when large enough. T_d is estimated from the linear segment of the semi-log curves.

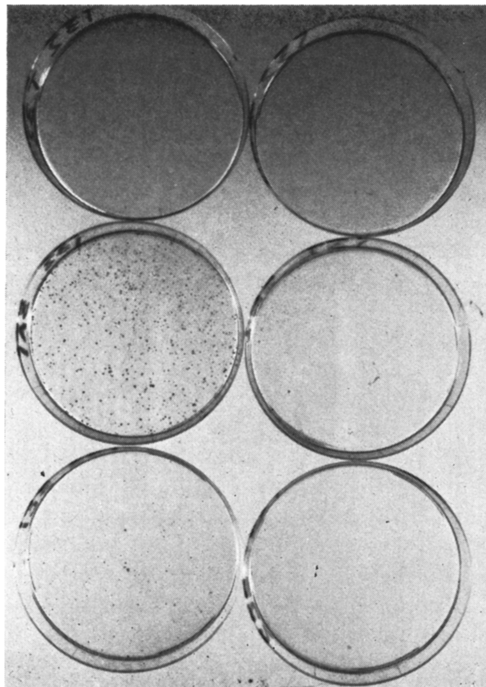

FIG. 2. Effect of T_4 and number of cells seeded on PE of T-1 cells at 35.5°. Top row, control (no hormone); middle row, 4.45 $\times$ 10^{-6} M T_4; and bottom row, 1.78 $\times$ 10^{-7} M T_4. Left column, 3000 cells seeded per dish; right column, 300 cells per dish.

Results. Dropping the temperature at which the T-1 cells were incubated greatly increased the mean doubling time (T_d). Thus, T_d rose from 22.9 to 47.0 hr when the temperature was lowered from 37° to 34.5°. At the lower temperature, 4.45 $\times$ 10^{-7} M T_3, a hormone concentration strongly affecting the rate of proliferation of these cultured cells (3) at 37°, was without effect (Fig. 1). When incubated at the lower temperature, very few of the colonies that develop attained four or more cells, even in the presence of T_4 or T_3.

When grown at lower temperatures, colony-forming ability for the T-1 cells was markedly depressed with as well as without the addition of these thyroidal hormones. Thus, PE for the cultured kidney cells at 34.5° is zero for control and T_4 treated populations alike. A few colonies do develop in T_4 treated dishes grown at 35.5°, but not in the corresponding controls (Fig. 2). Incubation at

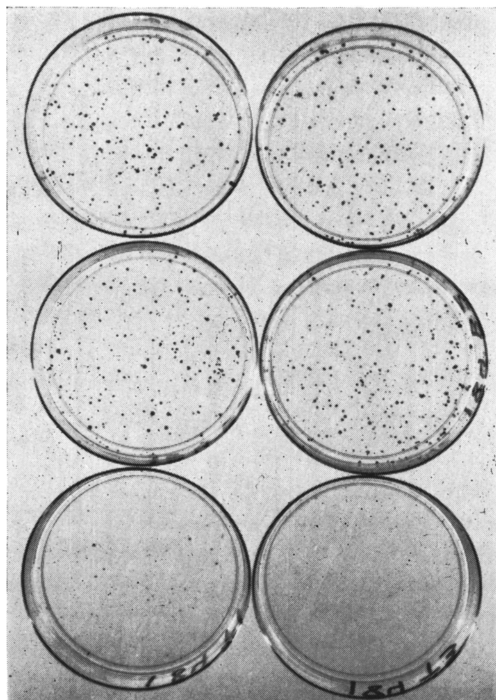

FIG. 3. Effect of high temperature (38.5°) on PE of T-1 cells as modified by T_3. Top row, 1.78×10^{-7} *M* T_3; middle row, 4.45×10^{-7} *M* T_3; and bottom row, control (no hormone). Results of scoring 4 replicate dishes: 1.78×10^{-7} *M* T_3, 79.6 ± 3.2%; 4.45×10^{-7} *M* T_3, 97.4 ± 2.5%; and controls, 11.7 ± 3.9%. Values given are mean PE followed by SD.

36° marked the initiation of significant colony development as well as of appreciable hormonal response. At the highest temperature studied (38.5°), although PE was greatly depressed for the untreated cells, it was profoundly elevated (to normal levels) by the presence of these hormones (Fig. 3). The variation of PE for the T-1 cells over the entire temperature range investigated as well as the alterations of the response induced by 4.45×10^{-6} *M* and 1.78×10^{-7} *M* T_4 are plotted in Fig. 4.

The inhibition in colony formation associated with lower temperatures can be partially overcome by increasing the number of cells plated. Thus, by initiating PE experiments with 500, 3000, or 30,000 cells rather than seeding the standard 300 individual cells, some colonies do develop even at 34.5°, and the effects of the thyroidal hormones become very obvious as illustrated by T_4 in Fig. 5. Similar modifications produced by the introduction of T_4 which result from increasing the number of cells seeded at 35.5° are illustrated in Fig. 2.

Discussion. From these studies of the role played by temperature as well as those described previously concerned with the influence of serum content, it is apparent that perturbing this *in vitro* system displays profound dependence of the T-1 cells on the presence of the thyroidal hormones, suggesting once again the possibility that the latter are ultimately essential for the life of higher animals (5). Lowering the temperature at which the T-1 cells are grown impairs survival and markedly retards proliferation, an unsalutary condition overcome partially at least by T_4 and T_3, responses analogous to those that occur widely *in vivo*. The *in vitro*

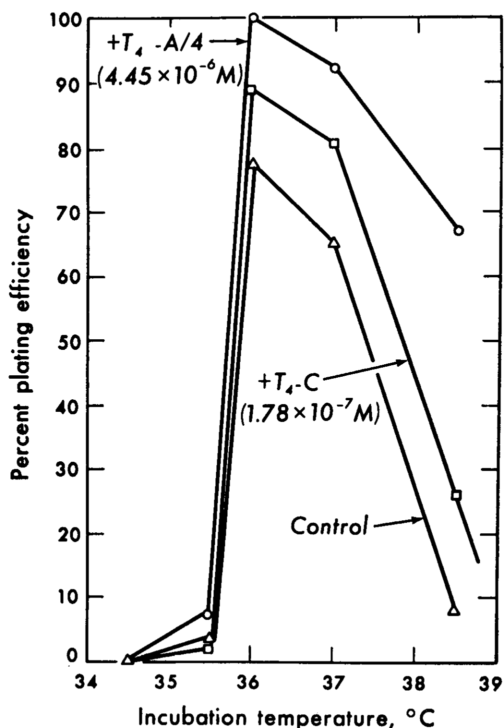

FIG. 4. Variation of PE for T-1 cells over the entire temperature range studied (34.5°–38.5°) as modified by T_4. 4.45×10^{-6} *M* T_4, circles; 1.78×10^{-7} *M* T_4, squares; and control (no hormone), triangles. Points plotted represent the means of scoring 4 replicate Petri dishes seeded initially with 300 dispersed cells; in no instance did SD exceed 5%.

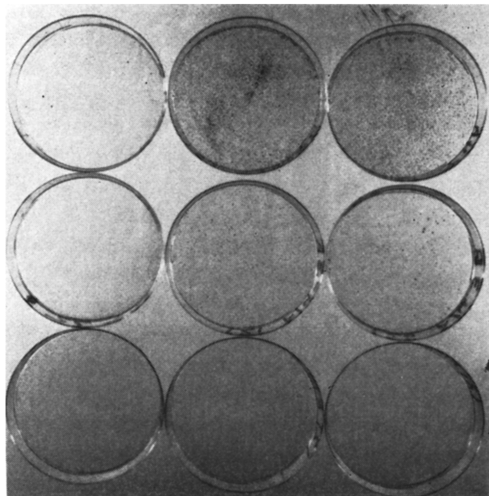

FIG. 5. Effect of number of cells seeded at 34.5° on PE of T-1 cells as modified by T_4 (4.45×10^{-6} *M*). Top row, 30,000 cells seeded; middle row, 3,000 cells seeded; and bottom row, 300 cells seeded. Left column, control (no hormone); middle and right columns duplicate T_4 treated Petri dishes.

response to temperature changes as modulated by T_4 and T_3 resembles those encountered in many organisms, further support for the view that these cultured human kidney cells constitute a valuable system for studying peripheral behavior and processes.

In opposing temperature effects that inhibit colony formation and growth (expressed by determinations of PE and T_d), the thyroidal hormones, among their many actions, enhance the ability of the cultured kidney cells to adhere and aggregate. These hormones thus promote cytological processes important for multicellular development and differentiation with which these secretions are intimately associated. That such cell-cell interactions come into play is apparent also from our experiments in which the number of T-1 cells constituting the inoculum was increased. The results imply that cell number and the thyroidal hormones act in concert to nullify the adverse effects of low temperatures. This novel synergism warrants further exploration, perhaps with scanning electron microscopy, to ascertain whether intercellular processes do develop as the T-1 cells proliferate and form colonies in response to the presence of T_4 and T_3. Since the efficacy of these hormones is augmented by cell number, this may signify that the more cells, the more hormone that can be transformed or "activated,"—a relationship in keeping with long held, but elusive, notions regarding hormonal behavior.

Much more study of the events occurring at the sub-cellular level is needed if we are to account for the cellular response to environmental temperature changes. In view of their functions and reactions to cold and heat, a number of organelles and structures are under suspicion for contributing significantly to the thermal response. As the temperature of cultured cells is elevated, the mitochondrion may be the first organelle affected; under phase contrast microscopy, it ceases to move, swells irregularly, and increases its pallor (10). Another corroborating report is that hamsters exposed to 35° for 60 days, which results in a generalized decrease in body thermogenesis, have diminished mitochondrial metabolism (11). In the T-1 cells, nucleoli may be concerned with temperature changes, since these organelles, under the influence of T_4 and T_3, increase in number and their synthesis of RNA (3); this conjecture is strengthened by the report (12) of a heat-sensitive function concerned with DNA-dependent RNA synthesis located in nucleoli of cultured cells.

These investigations of the cultured kidney cells suggest that some of the response exhibited by homeotherms to environmental temperature as modulated by the circulating thyroidal hormone involves interactions occurring at the level of the peripheral cells. Just how such organisms, especially man, maintain their characteristic body temperatures, despite wide thermal fluctuations of their milieu, eludes us (13). For mammals, there are two distinct modes of heat production, thermomechanical interactions (*e.g.*, shivering) and chemical transformations (14). Among the latter, the oxidative respiratory activities associated with the mitochondrion are the principal source of cellular heat, as mentioned earlier. It has been long known that the homeostatic controls over oxidative mechanisms are linked to the thyroid gland and its elaborations and to temperature (15, 16). In effecting the response

to temperature, thyroid gland activity is modulated by the anterior pituitary's output of thyrotropin (TSH) which, in turn, is regulated by the neurohormonal agent TRH released by the hypothalamus (17, 18). The so-called "set-point" for body temperature is believed situated within the posterior hypothalamus, the setting itself determined by the inborn ratio of the concentrations of Ca^{2+} and Na^{+} (19). It is tempting to speculate that the fine regulation of temperature may reside within the cells of homeotherms (1), since the *in vitro* phenomena presented here occur over a narrow temperature range.

Summary. Varying the incubation temperature (34.5°–38.5°) significantly affects the mean doubling time (T_d) and plating efficiency (PE) of the T-1 human kidney cell line. These alterations can be modified to some extent by the introduction of T_4 and T_3 as well as by the cell number of the inoculum. It is speculated that these *in vitro* responses to temperature raise the possibility of the existence of intracellular thermal controls for mammals.

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