otes suggest that mitochondria and chloroplasts arose, and reside in eukaryote cells, as symbionts with origins related to those of present-day bacteria and blue-green algae, respectively. (iii) We have observed what appears to be a normal HeLa nuclear division stage (Fig. 1E) after 6 days of growth in M3, a medium that was devised specifically for plant protoplast culture. The HeLa cells apparently can tolerate a range in cultural conditions, and a cell line is now under selection for growth in a mixed medium also adapted for protoplast maintenance.

If the fused HeLa-GGLL cells remain viable, a number of new avenues of research are opened. With the use of various immunological tools (for example, fluorescence antibody labeling and immune precipitation) it should be possible to answer questions about the continued synthesis of both tobacco cell and HeLa cell products, as well as the intracellular distribution of those products having specific intracellular locations. If nuclear and cell divisions occur in the fused cells and are accompanied by loss of human chromosomes, as in man-mouse somatic hybrids, it may be possible to extend the number of human genes located by association of particular chromosomes with biochemical products more likely to be uncharacteristic of the widely disparate plant host cell, provided that the necessary regulatory components are present.

A number of differences have been observed between normal and tumorous or transformed cells of both plant and human tissues. These include membrane differences (*8*), differences in rate of uptake and transport of certain solutes (*9*), and changes in the organization of membrane-associated components (*10*). Since fusion is essentially a membrane phenomenon, the altered membrane condition of the tumorous cells used in our experiments may have influenced, and possibly aided, in the fusion process.

Lastly, we call attention to the fact that the *N. glauca* × *N. langsdorffii* tumor cells are totipotent. Complete mature hybrid tobacco plants can be routinely differentiated from single GGLL tumor protoplasts. The consequences of introducing a human tumor cell, which has not been shown to be capable of differentiation, into a plant cell that retains this capability remains to be explored.

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Thyrotropin Releasing Hormone: Development of Inactivation System During Maturation of the Rat

Abstract. *Whereas thyrotropin releasing hormone is rapidly and extensively degraded by plasma of adult rats, no appreciable loss of biological or immunological activity is caused by plasma from rats 4 or 16 days old. The plasma of neonatal rats does not appear to contain an inhibitor of thyrotropin releasing hormone peptidase or a peptidase with altered substrate affinity. The development of an active peptidase in rat plasma suggests a physiological role for inactivation of thyrotropin releasing hormone.*

Hypothalamic releasing hormones act directly on the brain in addition to their well-established endocrine functions of releasing pituitary hormones. For example, thyrotropin releasing hormone (TRH) elicits behavioral changes—including mood-elevating effects in some depressed women (*1*), neurochemical responses in brain tissue (*2*), electrophysiological alterations in neurons (*3*), and neuropharmacological effects in counteracting phenobarbital toxicity (*4*)—in addition to releasing thyrotropin (TSH) and prolactin from the pituitary (*5*). The hypothesis that hypothalamic releasing hormones have neurotropic functions has been strengthened by the finding that TRH is present in extrahypothalamic regions of the brain (*6*).

In order to understand the extent and duration of the neurotropic and endocrine activities of hypothalamic releasing hormones and the manner in which these activities are regulated, the process of inactivation must be considered. Although active TRH degradation systems have been reported in adult plasma (*7, 8*) and brain tissue (*9*), the role of degradation in controlling TRH activity has not been clearly elucidated. However, the rapid degradation of TRH by plasma may account for the brevity of the antidepressant effects of TRH in women and for some of the controversy surrounding these effects (*1, 10*).

In an effort to explore the possible role of degradation in the regulation of TRH activity, we investigated the degradation of TRH by rats during the postnatal period, a time in which thyroid hormone is vital for the normal development of the central nervous system (*11*). Here we present evidence that the plasma of neonatal rats does not actively degrade TRH whereas that of adult rats rapidly inactivates TRH.

The biological and immunological activity of TRH was measured following incubations of TRH with plasma from rats 4 days, 16 days, and about 1 year old. Immunoreactive TRH was measured by a radioimmunoassay for TRH similar to that of Bassiri and Utiger (*12*). (Modifications of the assay are given in the legend to Fig. 1.) The sensitivity of our assay is 20 pg of TRH in a 300-μl assay system. Some likely TRH degradation products such as the free acid form of TRH (pglu-his-proOH), pglu-his, proNH$_2$, pglu (*13*), histidine, and proline do not cross-react with our TRH antibody.

The plasma of adult rats rapidly destroys the immunological activity of TRH (Fig. 1). The half-life of TRH (about 30 minutes) is in general agreement with that reported by others (*14*) if differences in plasma and TRH concentrations are considered. The striking feature of Fig. 1 is the inability of the plasma of 16-day-old rats to degrade TRH. Even after a 3-

hour incubation of TRH with plasma of these rats at 37°C, at least 90 percent of the TRH remains intact. We obtained similar results with plasma from 4-day-old rats.

The biological activity of TRH was determined by measuring the ability of

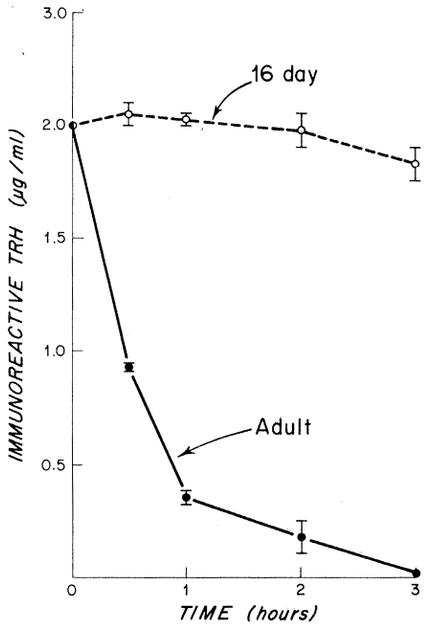

Fig. 1. Immunological activity of TRH after incubation with plasma of neonatal or adult rats. Plasma was obtained from male and female rats 16 days after birth and from adult females. All rats (plasma donors as well as those used in biological tests) were of the Charles River CD strain. The animal quarters were lighted only from 0700 to 1900 hours; Purina Lab Chow and tap water were always available. Neonatal rats were caged with lactating females. Bleeding was by decapitation without anesthesia. Blood was collected into heparin-containing tubes. After separation, the plasmas were either used immediately for incubations or stored at −20°C. Adult rats were bled individually. The blood of neonates of the same age and sex were pooled to obtain adequate volumes. For the inactivation studies, 200 μl of plasma were added to 800 μl of phosphate buffer (0.25 percent BSA, 0.01*M* potassium phosphate, 0.15*M* NaCl, *p*H 7.5) containing 2 μg of TRH (Abbott Laboratories) which had been warmed to the incubation temperature, 37°C. Portions (100 μl) were removed at the time points indicated, diluted to 1 ml with phosphate buffer, and immediately placed in a boiling water bath for 3 minutes to stop the inactivation reaction. The samples were further diluted with phosphate buffer for the TRH radioimmunoassay. Amounts of immunoreactive TRH were determined by a TRH radioimmunoassay procedure similar to that of Bassiri and Utiger (*12*) except that the time of incubation of [^{125}I]TRH, unlabeled TRH (standard or experimental sample), and diluted antiserum against TRH was 90 minutes. The data shown are for plasma from 16-day-old males and adult females. No significant differences were observed between male and female plasma at 16 days or between fresh, unfrozen plasma and plasma stored frozen at −20°C.

TRH to stimulate the release of TSH in rats. This test of biological activity was performed under conditions in which TRH caused a dose-dependent increase in the plasma TSH concentrations of rats (*15*).

Figure 2 shows the difference between the plasma of neonatal rats and that of adult rats with respect to the capacity to destroy the biological activity of TRH. The mean plasma TSH concentrations of rats injected with TRH previously incubated with the plasma of neonates were five to six times greater than those of controls injected with plasma alone. By contrast, rats treated with TRH previously incubated with the plasma of adults showed circulating TSH levels virtually indistinguishable from control levels. The responses to TRH previously incubated with plasma of neonatal rats are not grossly different from the responses observed in our earlier study of untreated TRH (*15*), but because we have encountered considerable variation within groups with respect to TSH response to TRH, we cannot at present conclude that TRH retains 100 percent of its biological activity after incubation with the plasma of neonatal rats. However, if the plasma of neonates has any capacity to degrade TRH, this capacity is very small by comparison with that of the plasma of adults. The evidence presented in Fig. 2, together with the immunological data of Fig. 1, clearly demonstrates the marked difference between the capacity of plasma from neonatal and adult rats to degrade TRH.

The nature of the TRH degradation systems in plasma of adults and in brain homogenates and the metabolic products of degradation have not been clearly elucidated, but inactivation of TRH has been reported to result in the deamidation of TRH or the release of one or more amino acids, or both (*7–9*). This suggests that inactivation is mediated by a peptidase. Thus, some possible explanations for the observation reported here are (i) a neonatal TRH peptidase with a different affinity for TRH than that of the adult peptidase, (ii) an inhibitory factor in the plasma of neonates which prevents degradation of TRH, or (iii) an inactive proenzyme form of TRH peptidase in the plasma of neonates. We attempted to evaluate these possibilities experimentally by measuring immunoactive TRH after several types of incubations with plasma. First, plasma from neonatal rats had no effect on the immunoreactivity of TRH when the TRH concentration was varied by a factor of 100. Second, dialyzed plasma of neonatal rats did not significantly degrade TRH during a 3-hour incubation at 37°C, whereas dialyzed plasma of adult rats was fully capable of degrading TRH. Furthermore, addition of plasma of neonatal rats to adult plasma did not inhibit the adult peptidase activity. Thus, it does not appear that the plasma of neonatal rats contains a TRH peptidase with an altered affinity for TRH or an inhibitor of TRH peptidase. Third, the similarity of the rate of TRH degradation by the adult and the combined plasma also suggests that a proenzyme form of TRH peptidase is not present in the plasma of neonates, but this possibility has not been examined closely. Alternately, a new protein species, unrelated to any present in the plasma of neonates, may develop during the maturation of the rat.

The lack of an active TRH degradation system in the plasma of neonatal rats raises several interesting questions concerning the development of TRH peptidase and other plasma peptidases and proteases and the possible physiological importance of the TRH degradation system. Although the induction of many en-

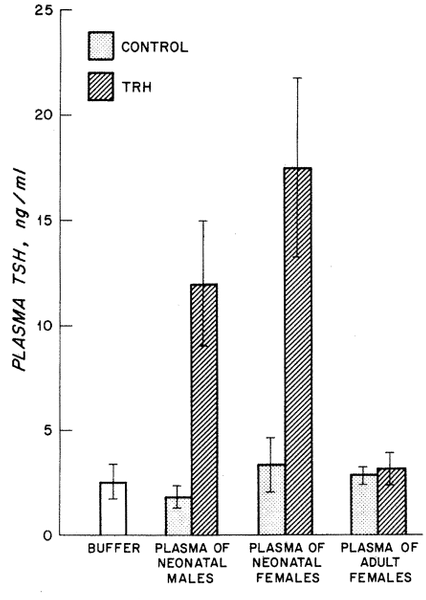

Fig. 2. Biological activity of TRH after incubation with plasma of neonatal or adult rats. Plasma (200 μl) from adult or neonatal rats was incubated either alone or with TRH (2 μg) in the phosphate buffer described for Fig. 1 (final volume, 1 ml) for 3 hours at 37°C. The contents of five incubation tubes for each type of plasma were pooled and injected intraperitoneally into groups of five adult male rats (800 μl per rat). An additional group of rats was injected with similar volumes of buffer. All rats were bled 30 minutes after injection. The plasmas thus obtained were assayed for TSH as described (*15*). A significant response ($P < .02$) to TRH was found after incubation with the plasma of neonates of either sex. After incubation with the plasma of adult females, TRH caused no significant response ($P \geqslant .05$). Standard errors are shown.

zyme activities in fetal and neonatal tissues has been studied (*16*), little is known about the development of plasma peptidases or proteases. However, plasma renin activity in sheep (*17*) and plasma vasopressinase activity in monkeys (*18*) are reported to be higher in fetal and newborn animals than in maternal or nonpregnant animals. In addition, the proteases involved in clotting activity are also present in the plasma of newborn mammals. In view of the possible neurotropic functions of TRH, it will be of interest to determine the time of appearance of plasma TRH peptidase activity and to compare it with the development of the central nervous system of the rat, most of which occurs after birth (*19*).

In regard to a possible physiological function of TRH degradation in plasma, TRH travels from the hypothalamus to the pituitary via the hypophyseal portal vessel (*20*), and hypophyseal portal blood inactivates TRH (*8*). Plasma inactivation of TRH could be one means of controlling the activity of TRH at the level of the pituitary or at other as yet unknown tissue sites. The lack of an active TRH degradation system in neonatal rats and the later development of an active degradation system suggests some physiological function for degradation of TRH by plasma. The specificity of the TRH peptidase is not known, but a high degree of specificity would lend further weight to a physiological role for the plasma inactivation of TRH. The role of plasma peptidases in controlling the activity of polypeptide hormones warrants further study.

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Mg^{2+},Ca^{2+}-Dependent Adenosine Triphosphatase as Receptor for Divalent Cations in Bacterial Sensing

Abstract. *The Mg^{2+},Ca^{2+}-dependent adenosine triphosphatase responsible for interconversion of the energized membrane state to adenosine triphosphate is the receptor for divalent metal ions in bacterial chemotaxis. The receptors controlling bacterial behavior show a common pattern of dual functions.*

Bacteria can respond to chemical stimuli by means of a prototypic sensory system. This system comprises a number of distinct chemoreceptor proteins, a mechanism to analyze and transmit chemical information, and a motor response requiring energy transduction (*1, 2*). Although it is contained in a single cell, the bacterial system is analogous in many ways to those of higher organisms.

In cells of higher species electrochemical gradients play a key role in the propagation of action potentials; the receptor-chemoeffector complex which is formed ultimately signals a depolarization involving massive changes in cation gradients (*3*). In lower species such as paramecium, ciliary activity is controlled by Ca^{2+} gradients (*4*).

Cation gradients have also been implicated in energy transduction processes in microorganisms and higher species. A central role for proton and cation gradients is proposed in the chemiosmotic hypothesis of Mitchell (*5*). Kashket and Wilson (*6*) have correlated the transport of sugars with proton gradients in artificial membranes and in intact bacteria. Racker and Stoeckenius (*6*) have shown that a light-induced proton gradient in *Halobacterium halobium* can generate adenosine triphosphate (ATP) in a reconstructed membrane system (*7*). Alternative approaches of Boyer (*8*), Ernster (*9*), and others also invoke an energized membrane state, but differ in the way it is generated. A central role in the conversion of the energized membrane state to ATP molecules in bacteria is played by the membrane-bound Mg^{2+},Ca^{2+}-dependent adenosine triphosphatase (Mg^{2+},Ca^{2+}-ATPase) protein, which is extensive and highly conserved throughout all living systems (*10*). This protein is involved in the synthesis of ATP in the final steps of oxidative phosphorylation (*11*), energy-dependent transhydrogenation (*12*), and the coupling of the hydrolysis of ATP to the transport of certain amino acids and sugars (*13*). The role of the ATPase is of particular interest since Larsen *et al.* (*14*) have established that the energized state is required for motility and that ATP is required for chemotaxis.

Two key questions can be asked in relation to bacterial chemotaxis: The first is whether the general chemotactic signal is generated by a pure Nernst-type electrochemical gradient across the membrane, or whether it operates through a membrane-bound protein. The second is whether a cation gradient is a general means of signal transmission from recep-