and deaths of newborn children is also higher for mothers who smoke, the rates being approximately dose-related (*11*, *14*). There are undoubtedly several mechanisms for these effects since tobacco smoke contains many chemicals. Because HbCO concentrations of 5 to 10 percent are common in persons who smoke one to two packs of cigarettes per day, it is conceivable that CO-induced hypoxia may be an important factor. For instance, in a pregnant woman smoking one pack of cigarettes a day, with an HbCO concentration of about 4.8 percent, fetal O_2 tension would decrease 2 to 3 torr. In a woman smoking two packs a day, with an HbCO concentration of about 9 percent, fetal O_2 tension would decrease 4 to 6 torr. In a woman exposed to ambient CO concentrations of 30 ppm for prolonged periods, as in industrial exposure or severe air pollution, the increases in maternal and fetal HbCO concentrations and decreases in fetal O_2 tensions would be equivalent to those expected if the women smoked a pack of cigarettes per day. Thus, fetuses of pregnant women in these environments may be exposed to CO concentrations that are not innocuous.

These results raise numerous questions regarding the biologic effects of relatively low CO concentrations on the developing embryo and fetus. For instance, what are the physiologic effects of these decreases in blood O_2 tension on availability of O_2 to the fetal brain, heart, and other vital tissues? Are cells of the developing embryo or fetus more or less sensitive to the effects of CO than those of adults? To what extent does CO interference with fetal oxygenation also result in problems such as mental retardation, cerebral palsy, and perhaps subclinical neurologic, intellectual, or behavioral deficits? Is there a threshold level above which adverse effects are noted? If so, what are the maximum allowable CO exposures for pregnant women and their fetuses?

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19 November 1975; revised 23 July 1976

Thyroxine-Induced Activation of Hypothalamo-Hypophysial Axis in Neotenic Salamander Larvae

Abstract. *Thyroxine injected into the hypothalamus of neotenic* Ambystoma tigrinum *induces metamorphosis by activating hypothalamo-hypophysial stimulatory control of thyroid activity, thereby removing the hypothalamic block to metamorphosis.*

We have examined the role of thyroxine (T_4) on hypothalamic function in tiger salamander larvae with respect to hypothalamic maturation and control over metamorphosis. A number of reports have dealt with interactions among thyroid hormones, thyrotropin [thyroid-stimulating hormone (TSH)], prolactin (PRL), gonadotropins, corticotropin, melatonin, gonadal steroids, and corticosteroids in neotenic populations of tiger salamanders, *Ambystoma tigrinum*, in Colorado (*1–3*). These animals normally become sexually mature and breed without first undergoing metamorphosis to adult terrestrial body form and are termed neotenes. This neotenic condition cannot be explained by reduced sensitivity of peripheral tissues to thyroid hormones or of the thyroid gland to TSH since both neotenes and immature larvae from populations that normally undergo metamorphosis before sexual maturation do not differ in sensitivities to these hormones (*2*, *4*). Neotenes occasionally undergo spontaneous metamorphosis, especially when brought into the laboratory in the spring and fall.

We examined the influence on metamorphosis of a small amount of T_4 placed in the region of the hypothalamus. Neotenes were anesthetized lightly with urethane, and a small hole was drilled in the roof of the mouth posterior to the optic chiasma but anterior to the pituitary. We injected T_4 into the region of the hypothalamus in a single dose of 2.0, 0.2, or 0.02 μg in 5 μl of saline buffer (*5*). The hole in the skull was occluded with dental cement. Placement of the injected dose was aided by the use of a stereotaxic device (*6*). A high incidence of metamorphosis was observed in all but the group receiving the lowest dose (Table 1). Animals receiving saline buffer intrahypothalamically (i.h.) did not metamorphose. This experiment was repeated and almost identical results were obtained. Neotenes or immature larvae receiving a single intraperitoneal (i.p.) dose of T_4 do not metamorphose. Injection of the largest dose of T_4 (2.0 μg) into the olfactory region of the brain induced metamorphosis in only three of eight neotenes.

In a second series of experiments, we measured the uptake of radioiodide by the thyroid of neotenes following a single i.h. injection of 2.0 μg of T_4, compared to effects of daily injections of 0.2 μg of T_4 administered i.p. for 10 days (Table 2). Daily injections of 0.2 μg of T_4 had been shown previously to induce metamor-

Table 1. Effectiveness of single i.h. injections of T_4 in inducing metamorphosis in immature and neotenic larvae of *Ambystoma tigrinum*. Larvae were maintained at 15°C on a photoperiod of 12 hours light and 12 hours darkness. The injection vehicle was 5 μl of saline buffer, *p*H 10.4 (*2*, *4*). Mean body weight values are ± standard errors.

Group	*N*	Initial mean body weight (g)	T_4 dose (μg)	Proportion induced
		Neotenes		
1	8	64.1 ± 4.13	2.0	8/8
2	8	58.2 ± 1.81	0.2	7/8
3	8	58.7 ± 2.95	0.02	3/8
4	8	59.0 ± 2.16	0	0/8
		Immature larvae		
5	9	6.0 ± 0.61	2.0	9/9
6	9	6.0 ± 0.61	0.2	0/9
7	9	6.8 ± 0.62	0	0/9

Table 2. Uptake of ^{131}I by the thyroid of metamorphosing neotenes induced with a single i.h. injection or daily i.p. injections of T_4. Group 1 differs significantly ($P < .01$) from all others. Larvae were maintained at 15°C on a photoperiod of 12 hours light and 12 hours darkness. Body weights are means ± standard errors. Uptake of ^{131}I is expressed as percentage of the injected dose (mean ± standard error).

Group	Treatment	N	Mean body weight (g)	Total dose of T_4 (μg)	Mean ^{131}I uptake (%)
1	T_4, 2.0 μg i.h.	8	66.9 ± 4.38	2.0	8.5 ± 2.33
2	T_4, 0.2 μg/day i.p. for 10 days	8	66.8 ± 3.24	2.0	1.0 ± 0.20
3	Saline buffer	8	65.6 ± 3.07	0	0.5 ± 0.25

phosis in neotenes (*2, 4*). Radioiodide (1.0 μCi of ^{131}I, carrier-free) was administered i.p. to metamorphosing larvae when each group had achieved a mean resorption of approximately 50 percent of the right proximal gill rachis. The animals were killed 24 hours later and thyroidal radioiodide uptake was measured (*3*). Radioiodide uptake by animals receiving a single i.h. injection of T_4 was significantly greater than uptake by metamorphosing animals induced with i.p. injections. Although neotenes receiving daily i.p. injections of T_4 were at the same stage of metamorphosis as those injected i.h., radioiodide uptake was not significantly different between i.p. injected metamorphosing neotenes and saline-injected nonmetamorphosing controls. These data suggest that T_4 injected directly into the hypothalamus is capable of activating the hypothalamo-hypophysial-thryoid axis.

Etkin (*7*) attempted to explain induction of metamorphosis in larval anurans as a result of "positive feedback" of T_4 on the hypothalamo-hypophysial-thyroid axis. This hypothesis was later modified to include demonstrated antimetamorphic actions of exogenous mammalian PRL on metamorphosis (*8*). Etkin explained the slow induction of anuran metamorphic events as a consequence of "turning on" of a hypothalamic stimulatory center controlling TSH release from the adenohypophysis and a hypothalamic inhibitory center for reducing release of PRL. According to Etkin's model, low circulating levels of T_4 in the premetamorphic tadpole cause a maturation of these neurosecretory centers, resulting in greater T_4 secretion. The increase in circulating T_4 accelerates the rate of hypothalamic maturation until T_4 reaches the threshold for inducing metamorphosis (*9*). Etkin supported his model with data on sensitivities to thyroid hormones and changes in neurosecretory activity in the hypothalamus and median eminence. However, his experiments all involved immersion of tadpoles in solutions of T_4, so that a specific direct action on the hypothalamus could not be confirmed.

As predicted by Etkin's hypothesis, a decrease in pituitary PRL accompanies T_4-induced or spontaneous metamorphosis of tiger salamander larvae (*10, 11*). Platt (*12*) has suggested that PRL is responsible for maintaining the neotenic condition in this species by blocking the action of thyroid hormones at the level of the hypothalamus. Therefore, it is probable that a stimulatory factor such as a rise in circulating thyroid hormone levels is responsible for overcoming the proposed hypothalamic PRL block to metamorphosis.

In a separate experiment, we removed the pituitary gland from neotenes and grafted a pituitary gland into the lower jaw near the thyroid glands. These neotenes showed no metamorphic tendencies 3 weeks later following injection of 2.0 μg of T_4 directly into the region of the transplant. The failure to observe metamorphosis under these conditions could have been due to increased PRL release once this gland has been freed of direct hypothalamic inhibition. The transplanted glands were secreting melanophore-stimulating hormone (MSH) for up to 6 weeks, as evidenced by a marked darkening of the skin (animals whose pituitary glands have been removed appear blanched). Since increased release of MSH and PRL is observed from pituitaries deprived of contact with the hypothalamus (*13*), it might be assumed that PRL was being released from these transplanted pituitaries. However, treatment of pituitary-grafted neotenes with ergocornine, a drug that acts directly on the pituitary gland to block release of PRL and MSH (*14*), did not influence the response to T_4. As shown for other amphibian species (*7*), there is little stainable neurosecretory material in the hypothalamus of immature tiger salamander larvae. A marked increase in neurosecretory stainability occurs after treatment with thyroid hormones. Neotenic larvae already possess large quantities of hypothalamic neurosecretory material, and treatment with T_4 does not produce any obvious change in stainability (*15*). As mentioned above, target tissues for T_4 in neotenes and immature larvae are equally sensitive to T_4. However, a much greater i.h. dose is required to initiate metamorphosis in immature larvae (Table 1), and the onset of metamorphosis is delayed as compared with that in neotenes (*15*). It appears that in neotenes the role of T_4 is to activate an inhibited hypothalamic center, whereas in immature larvae it must first bring about a general maturation of the hypothalamus.

We propose that the PRL block to metamorphosis in this species can be overcome by a moderate increase in circulating thyroid hormones or a marked decrease in PRL secretion, which would allow currently circulating thyroid hormones to produce a maturation (immature larvae) or an activation (neotenes) of the hypothalamo-hypophysial-thyroid axis (*16*).

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6. The location of the hypothalamus was facilitated by use of an atlas of the tiger salamander brain [C. J. Herrick, *The Brain of the Tiger Salamander* (Univ. of Chicago Press, Chicago, 1948)]. Previous use of a stereotaxic device to trace the pathway of India ink–coated microsyringe needles made it possible to localize the injection site.
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ly collected neotenes is correlated with the lowest tail height. Tail height is readily increased by treatment with ovine PRL or reduced by treatment with such ergot alkaloids as ergocornine and ergocryptine. These drugs enhance the sensitivity of neotenes to thyroid hormones, but this treatment in itself will not induce metamorphosis in the absence of exogenous thyroid hormones.
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17. We would like to thank Dr. Richard E. Jones for his thoughtful criticisms of the manuscript and Sandoz Pharmaceuticals for the gift of ergocornine.

26 February 1976; revised 14 June 1976

Discharge of Aminoacyl–Viral RNA by a Factor from Interferon-Treated Cells

Abstract. *Extracts of cells treated with purified interferon discharge the amino acid previously esterified to viral RNA but fail to affect aminoacylated transfer RNA. A similar activity is also present in crude interferon preparations.*

The RNA from several different viruses can react in place of transfer RNA (tRNA) in the esterification of specific amino acids by eukaryotic aminoacyl-tRNA synthetases (*1*). The biological role in viral replication of such aminoacylation is not yet known, but the suggestion has been made that it may be involved in the regulation of protein synthesis (*2*). Interferon, a protein produced by cells exposed to viruses and certain other materials, causes cells to become resistant to viral infection and also alters specific functions of the treated cells, including protein synthesis (*3*). Incubation of exogenous messenger RNA (mRNA) with extracts of interferon-treated cells results in the translation of less protein than is observed with extracts of untreated cells; this low level of translation can be increased by the addition of certain tRNA preparations (*4, 5*). We report here another activity shown by extracts of cells exposed to interferon: specific deacylation of aminoacylated viral RNA.

The RNA extracted from tobacco mosaic virus (TMV) was esterified with histidine by partially purified rabbit liver histidinyl-tRNA synthetase. The unpurified human, rabbit, and mouse interferons used had all been induced in cell culture by virus; the residual virus was inactivated by dialysis at *p*H 2, and the neutralized fluids were then stored at −70°C. Mock preparations of unstimulated cell cultures were similarly prepared from supernatant fluids that, like the interferon preparations, contained 2 percent fetal bovine serum in Eagle's minimal essential medium.

In order to show that a factor interfering with aminoacylation of viral RNA is produced by cells after exposure to interferon, mouse L_{929} cells grown in 60-mm petri plates were treated with Sepharose beads bearing covalently linked mouse interferon (30,000 beads per dish) (*6*); control cells were treated with unmodified Sepharose beads. After incubation at 37°C for 6 hours, the beads were removed and the cells were washed and homogenized; supernatant fluids from the homogenates were then added either to an acylating system (Fig. 1a) or to a solution of histidinyl–TMV-RNA (Fig. 1b). As seen in Fig. 1, the extracts of cells that had been treated with interferon diminished the esterification of the TMV-RNA and increased the breakdown of preformed histidinyl–TMV-RNA.

The deacylating factor may be related to the development of resistance to viruses. Cells treated with Cantell's highly purified human interferon (*7*) (PIF) (with a specific antiviral activity of 3.7×10^6 units per milligram of protein) became progressively more resistant to virus challenge; the activity in extract supernatants increased in parallel with antiviral resistance until about 7 hours after interferon treatment, when deacylating activity began to decline and a plateau in antiviral resistance was reached (Fig. 2). The dissociation of the curves after 7 hours may be explained by mechanisms for disposing of the factor, such as binding to insoluble components or secretion from the cell.

Crude interferon preparations also contain a deacylating activity. The activity is clearly not attributable to interferon itself: no deacylating activity could be detected in as much as 170,000 units of PIF, but an active factor was present in crude preparations of human, rabbit, and mouse interferons (even after they were diluted > 1 : 250). The deacylating activ-

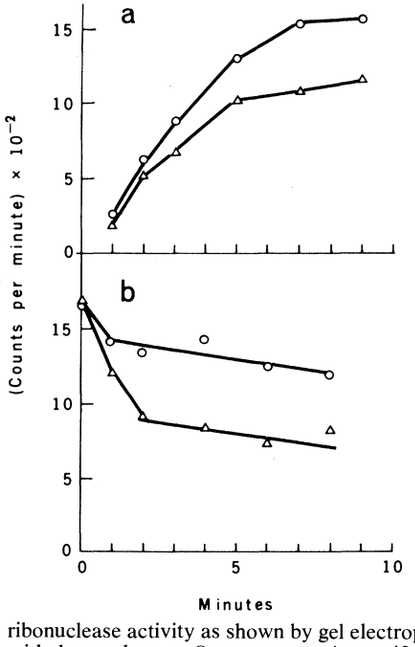

Fig. 1. The in vitro activity of L_{929} cell homogenates treated with Sepharose-bound mouse interferon (△) or with control, untreated Sepharose beads (○) on (a) the inhibition of histidine acylation to TMV-RNA and (b) the accelerated discharging of histidine TMV-RNA. The amount of interferon bound to the beads was about 3×10^4 units, as measured in L_{929} cells with GDVII virus as challenge (*12*). Cells were exposed to the beads for 6 hours at 37°C, then washed, homogenized in 1 ml of 0.02*M* tris-HCl buffer (*p*H 7.5), and centrifuged for 20 minutes at 2000*g*. Supernatant fluids were assayed. Rabbit liver histidinyl-tRNA synthetase was prepared from the 105,000*g* supernatant fluid of clarified liver homogenates. The fraction eluted from diethylaminoethyl (DEAE)-cellulose with 0.25*M* NH_4Cl served as a crude synthetase preparation from which the histidine synthetase was purified by column chromatography on DEAE-cellulose followed by affinity chromatography on a tRNA (*Escherichia coli*)–Sepharose column, and subsequently by TMV-RNA-Sepharose column chromatography, according to methods described by Remy *et al.* (*13*). Although not homogeneous, this preparation was devoid of ribonuclease activity as shown by gel electrophoresis of TMV-RNA before and after treatment with the synthetase. One enzyme unit esterifies 1 pmole of tRNA in 10 minutes. The TMV-RNA was prepared from a CsCl-banded virus preparation, which was then further concentrated, suspended in 1 percent sodium dodecyl sulfate, and extracted with an equal volume of phenol. The TMV-RNA was esterified with histidine in the following reaction mixture (0.1 ml): 0.02*M* tris-HCl (*p*H 7.5), 5 m*M* $MgCl_2$, 2 m*M* ATP, 10 μM [^{3}H]histidine, 4 to 10 pmole of TMV-RNA per 0.1 ml, 10 μl of extract from treated L_{929} cells, and 20 units of purified histidine synthetase. Aminoacylation was measured as trichloroacetic acid–precipitable tritium at the times indicated. Discharging was measured in a system containing 1 pmole of histidinyl–TMV-RNA in 0.02*M* tris (*p*H 7.5), 5 m*M* $MgCl_2$, 2 m*M* ATP, and 10 μl of cell extract. Samples were precipitated with trichloroacetic acid at the times indicated, filtered on glass fiber disks, washed, and counted in a scintillation counter.