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Hypothalamo–Hypophysial Relations in Amphibian Larvae

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The results of studies on the differentiation of the preoptic nucleus and of experiments regarding the effects of propylthiouracil and extirpation of the preoptic area demonstrate that in *Xenopus laevis* tadpoles a thyrotropin-releasing factor (TRF) is formed in peptidergic cells in the dorsal part of the preoptic nucleus. The differentiation of this nucleus depends on thyroid hormones, but at the same time these hormones inhibit the activity of the TRF cells.

Young larvae of *X. laevis* acquire the ability to adapt themselves to a white background simultaneously with the appearance of aminergic neurosecretory centers in the caudal hypothalamus. During prometamorphosis these centers comprise a paraventricular organ (PVO), a nucleus infundibularis, fiber tracts to the telencephalon and to the pars intermedia of the adenohypophysis, and a nucleus in the caudalmost part of the dorsal tuber cinereum. The PVO consists of sensory nerve cells and cells secreting products into the cerebrospinal fluid; the infundibular nucleus contains numerous liquor-contacting neurons. The aminergic neurosecretory cells contain catecholamines such as dopamine. Depletion of these cells by reserpine leads to a dispersion of melanin granules in the skin melanophores. It is believed that among the functions of the aminergic neurosecretory centers is the production of a melanotropin-inhibiting factor. In the ventral tuber cinereum of *X. laevis*, neurosecretory cells are situated that are neither peptidergic nor aminergic. They are more active in adult animals than in tadpoles.

Ever since the pioneering work of Adler (1914), Smith (1916), and Allen (1916) evidence has accumulated that in amphibian larvae the adenohypophysis regulates background adaptation, growth and metamorphosis, the last via the thyroid glands. To that end it produces a melanotropic (MSH), a growth-promoting and a thyrotropic hormone (TSH). It seems likely that the adenohypophysis of amphibian larvae also produces an adrenocorticotropic hormone, but this needs experimental verification (cf. van Oordt, 1971).

From the beginning of its differentiation, the adenohypophysis lies in close contact with the infundibulum. This seems to imply a functional relation between the hypothalamo–neurohypophysial complex and the adenohypophysis. Among the early work demonstrating such a relationship are the transplantation experiments of Etkin (1938). He removed the adenohypophysis of *Rana pipiens* larvae from the original site and allowed it to continue its development in the tail region. This led to a darkening of the skin and impairment of metamorphosis. In addition, many larvae reached a gigantic size. This work was repeated by Etkin and Lehrer in 1960; they concluded that for adequate functioning the adenohypophysis of amphibian larvae depends on a melanotropin-inhibiting factor (MIF), a larval growth-hormone inhibiting factor and thyrotropin-releasing factor (TRF).

Heterotopic transplantation of the thyroid has also been carried out by Pasteels (1957) in larval *Pleurodeles waltlii*. As a result, the TSH cells regress, the thyroid becomes inactive and metamorphosis is blocked. Etkin and Sussman (1961) have demonstrated that this effect may be attributed entirely to a disconnection of the vascular link between the median eminence

and the pars distalis of the adenohypophysis. Indeed, these authors observed that after sectioning the portal vessels in larval *Ambystoma mexicanum*, metamorphosis does not take place unless the vessels are allowed to regenerate. This led to the hypothesis that the portal vessels are needed for passing on information from the hypothalamus and the median eminence.

From more than a dozen papers (cf. Etkin 1968, 1969; Goos 1969a) it appears that lesioning of the hypothalamo–hypophysial tract and removal of the infundibulum, the caudal hypothalamus or the entire diencephalon have almost invariably led to a retardation or a complete blockage of metamorphic processes. So, it is generally believed that in amphibian larvae TRF is formed in the preoptic nucleus, and is transported via the preoptico–hypophysial tract towards the outer zone of the median eminence. This interpretation is supported by the results of histological studies of the hypothalamus, the pituitary and the thyroid during larval development. Several authors (cf. Etkin 1968, 1969; Goos 1969a) have noted a correlation between the development of the median eminence and the progress of metamorphosis. The outer zone of the median eminence and the portal vessels do not develop before late prometamorphosis, i.e., the stage of maximal activity of TSH cells and the thyroid. During premetamorphosis and early prometamorphosis the median eminence consists of an inner zone only; a network of capillaries is situated between the median eminence and the developing pars distalis of the adenohypophysis.

Etkin (1963, 1964, 1965) has found that after thyroidectomy the median eminence of *R. pipiens* tadpoles retains its larval character. When thyroidectomized animals were allowed to swim in thyroxine solutions, the outer zone of the median eminence developed normally, and metamorphosis was completed. However, treatment of young larvae during premetamorphosis with thyroxine led to an increase in thickness of the median eminence only when the animals were over 40 mm long (Etkin, 1966a).

On the basis of these results Etkin (1966a, 1966b, 1968, 1969) developed the theory that the hypothalamus is not essential for the activity of the TSH cells, the thyroid and metamorphic processes during premetamorphosis and early prometamorphosis. These early stages may depend on the pituitary–thyroid axis only. However, during prometamorphosis the thyroid hormones are supposed to induce the hypothalamus to develop TRF-secreting neurons. Because the thyroid hormones lead to the onset of and the continuous increase in TRF production, Etkin refers to the influence of the thyroid hormones upon the hypothalamus as a "positive feedback." According to Etkin the TRF in its turn stimulates TSH secretion and by doing so counteracts the negative feedback influence of the thyroid hormones upon the TSH cells. As a result, the activity of the hypothalamus–hypophysis–thyroid axis rapidly increases and becomes very high at the beginning of metamorphic climax. From that moment the hypothalamic TRF-system is believed to change its sensitivity to thyroid hormones, so that the positive feedback is replaced by a negative feedback. The complete change in sensitivity of the TRF neurons for thyroid hormones might explain the rapid decrease in activity of the TSH cells and the thyroid during climax.

Goos (1968, 1969a,b; Goos *et al.*, 1968a, b) has tested Etkin's hypothesis on tadpoles of *Xenopus laevis*. By using the pseudoisocyanine (PIC) method of Schiebler and Schiessler (1958) peptidergic neurosecretion could be demonstrated in cells situated in the dorsal preoptic area of the hypothalamus from early premetamorphosis. In fact, these neurosecretory cells developed at the very moment when the first TSH cells in the adenohypophysis and the first thyroid follicles appeared. During prometamorphosis the number of PIC-positive cells in the dorsal preoptic area increased, and at the end of prometamorphosis numerous large neurosecretory cells formed the dorsal part of the preoptic nucleus. By that time also the ventral, parvocellular part of the preoptic nucleus

had developed. However, when prometamorphic larvae were treated with propylthiouracil (PTU), two things happened: Firstly, there was an extrusion of all secretory granules from the existing neurosecretory cells in the dorsal preoptic nucleus and from the TSH cells in the pituitary, combined with a hyperactivity of the thyroids. Secondly, a complete halt occurred in the development of the entire hypothalamo–hypophysial system, including the neurosecretory cells in the preoptic nucleus. This was part of an overall impairment of larval development and metamorphosis. When, after such a PTU treatment, the animals were transferred to normal tapwater, the neurosecretory cells and the TSH cells regranulated, the thyroids resumed normal activity, and larval development including that of the hypothalamus was restored. The effects of PTU could also be counteracted by thyroxine.

The close correlation among the development and the activity of the PIC-positive cells in the dorsal preoptic nucleus, the TSH cells and the thyroid seems to indicate that in *X. laevis* TRF is formed in cells belonging to the dorsal, magnocellular part of the preoptic nucleus, and that these cells enhance the thyrotropic potency of the pituitary from early premetamorphosis. The effects of the goitrogen and thyroxine point to a dual function of the thyroid hormones with regard to the TRF center. On the one hand, the thyroid hormones seem to stimulate the differentiation of the TRF cells, and in doing so to enlarge the activity of the entire TRF center; on the other hand, the thyroid hormones exert a negative feedback by lowering the secretion of TRF by the individual PIC-positive cells in the dorsal preoptic nucleus. The stimulatory influence of the thyroid hormones seems to be part of the general morphogenetic function of these hormones during larval growth and development (Fox, 1967) rather than a positive feedback.

Goos demonstrated the value of this hypothesis in a series of experiments on the effects of extirpation of the dorsal preoptic area in combination with PTU treatment. It appeared that removal of the forebrain did not hamper the activity of the TSH cells and the thyroids unless the dorsal part of the preoptic nucleus had been removed at the same time. When the last had been left intact, PTU had the same dramatic effects upon the TSH cells and the thyroids as in unoperated animals. However, even after extirpation of the dorsal part of the preoptic nucleus some goitrogenic effect of PTU remained, particularly if some PIC-positive cells had remained or regenerated after the operation. This first of all confirms the supposition that TRF is formed in PIC-positive cells of the dorsal, magnocellular part of the preoptic nucleus. It also shows that the thyroid hormones exert a direct negative feedback influence on the TSH cells as well as an indirect one via the TRF center. This means that in *X. laevis* tadpoles TRF cells in the dorsal preoptic nucleus stimulate the TSH cells and these in their turn the thyroid follicles, whereas the latter directly inhibit the activity of these two cell types. The functioning of the hypothalamus–pituitary–thyroid axis does not differ principally from that in adult *X. laevis* (Rosenkilde, 1969). Yet, there is one major difference between the larvae and the adults: the activity of the system leads to homeostasis in the adult but not in the tadpole. The reason for this difference lies in the morphogenetic activity of the thyroid hormones before the end of metamorphosis and the ensuing differentiation of many cell types, tissues and organs, among others the TRF cells in the hypothalamus. As a logical consequence of this situation the activity of TRF center-TSH cells-thyroid system will gradually increase, until the differentiation of the TRF cells is completed and the thyroid hormones lose their morphogenetic activity, and the negative feedback remains.

Recent experiments by Neuenschwander and Weber (1970) have shown that the activity of the TRF center in larval *X. laevis* is temperature–dependent, and stops at low temperatures. Thus, ambient factors such as temperature may influence metamorphosis via the TRF center in the hypothalamus. However, it would be wrong to

suppose that the activity of the TRF center-TSH cells-thyroid system is sufficient to explain the endocrine regulation of amphibian metamorphosis. Etkin and Gona (1967) have demonstrated that during metamorphosis prolactin counteracts the thyroid hormones and retards metamorphosis by inhibiting the secretion of TSH. In addition, prolactin seems to inhibit the morphogenetic effect of thyroid hormones both *in vivo* and *in vitro* (Berman *et al.*, 1964; Derby, 1968; Etkin *et al.*, 1969). This means that during metamorphosis prolactin affects the differentiation of the TRF cells, the median eminence and the TSH cells, and thereby lowers the TSH output and thyroid activity. At the same time, prolactin seems to act as the major growth-promoting hormone in amphibian larvae (cf. Bern and Nicoll, 1968).

Etkin *et al.* (1969) have confirmed the observation of Etkin and Lehrer (1960) that production and secretion of prolactin increases after heterotopic transplantation of the adenohypophysis. It, therefore, seems justified to suppose that the production of prolactin is normally inhibited by a hypothalamic factor. However, during premetamorphosis the acidophilic prolactin cells in the adenohypophysis are very active, and they regress in the course of prometamorphosis, only to redevelop during postmetamorphosis (cf. van Oordt, 1968, 1971). This seems to indicate that the prolactin-inhibiting factor (PIF) mainly exerts its influence during prometamorphosis and climax.

The center of PIF production has not yet been determined. It might be part of the peptidergic preoptic nucleus, but it might just as well be part of the aminergic nucleus recently observed by Goos and van Halewijn (1968) in the caudal hypothalamus of prometamorphic *X. laevis* tadpoles. The rostral tip of this paired nucleus is situated against the narrow opening between the third ventricle and the infundibular lumen. From that point the nucleus runs ventrocaudally along the cavity of the infundibulum and turns in a more lateral direction along the narrow side of the lateral recess of the infundibular lumen.

Peute (1969) has made a detailed electron microscopical study of the rostral part of this nucleus, and pointed out that it is homologous with the organon vasculosum hypothalami or paraventricular organ (PVO) of other vertebrates. The PVO of *X. laevis* tadpoles is characterized by an ependymal lining containing a few cilia and two types of nerve cells. Both cell types have processes forming a dense network in the ventricle. The type-I cells contain dense-cored vesicles with a diameter of 800–1100 Å. These vesicles have a relatively wide electron-lucent zone around the dense core. In the PVO of both larval and adult *X. laevis* Peute (1971) observed type-I cells that make synaptic contacts with dendrites of other nerve cells. These so-called somato–dendritic synapses are characterized by the presence of clusters of small, electron-lucent synaptic vesicles, 500–600 Å in diameter, localized in the narrow cytoplasmic zone between the nucleus and the cell membrane. Many of these vesicles are capable of reacting with zinc iodide–osmium, as described by Akert and Sandry (1968), indicating that their contents might contain neurotransmitter substances (Martin *et al.*, 1969). It thus seems that the type-I cells possess the morphological basis for transmitting information to other cells. Moreover, many protrusions of the type-I cells bear cilia which have the $8+1$ pattern and might therefore be adapted to a chemoreceptive function (Leeson, 1962). These morphological data seem to indicate that in *X. laevis* the type-I cells of the PVO are sensory nerve cells passing on information from the ventricle to deeper lying neurons.

The protrusions of the type-II cells seldom bear cilia, and the cells are innervated by aminergic nerves (Peute, 1969). They have dense-cored vesicles with a diameter of approximately 1350 Å. The dense core of these vesicles is surrounded by a narrow electron-lucent zone. It is suggested that these type-II cells have a secretory function, releasing products into the cerebrospinal fluid (cf. Goos and Peute, 1970).

The laterocaudal part of the aminergic

nucleus described by Goos and van Halewijn (1968) adjoins the narrow lateral side of the infundibular recess. Pending a detailed description, the term "nucleus infundibularis" is proposed. Between the PVO and this infundibular nucleus Terlou and R. E. Ploemacher (unpublished) observed a narrow zone, 50 to 150 μ wide, depending on the developmental stage of the larvae. This zone contains very few aminergic neurons (Peute, unpublished). In a preliminary note Peute (1968) described the ultrastructural aspects of the cells belonging to the nucleus infundibularis. It appears that the cells are neuronal and contain moderate amounts of dense-cored vesicles, 700–1000 Å in diameter. Most of the cells have a club-shaped protrusion into the infundibular lumen, and may thus be regarded as liquor-contacting neurons. Occasionally, a thin axonal process was observed, running in the opposite direction (Peute, unpublished).

Using Falck's method (Falck and Owman, 1965) for the demonstration of catecholamines and tryptamines, Goos (1969c) did not detect fluorescent fibers from the infundibular nucleus to other parts of the hypothalamus in prometamorphic *X. laevis* tadpoles. However, Terlou and Ploemacher (unpublished) succeeded in finding such a tract running towards the neurohypophysis and the pars intermedia of the adenohypophysis in dark-background-adapted animals, kept on a white-background for 20 min prior to autopsy. Even better results were obtained by treating the tadpoles with iproniazide which inhibits monoaminoxidase. Thus, in cross sections of the caudal hypothalamus a paired tract of fluorescent fibers was noticed, originating on the lateral side of the nucleus, first curving ventro-caudo-laterally and then ventro-caudo-medially. In the ventromedial hypothalamus the fibers turn caudally and combine into a single, flat tract in the bottom of the infundibulum and in the inner zone of the median eminence. The tract does not end in the neurohypophysis, but fans into the pars intermedia, where the fluorescent fibers form a network surrounding the MSH-producing cells. This infundibulo–hypophysial tract could also be vizualized by applying the tryptamine–tetrazolium method of Glenner *et al.* (1957) for the demonstration of monaminoxidase. Along the tract a strong enzyme reaction has been observed (M. Terlou and H. W. Stroband, unpublished).

The PVO, infundibular nucleus and infundibulo–hypophysial tract were not the only fluorescent structures found by Terlou and Ploemacher in the brains of iproniazide-treated *X. laevis* larvae. Sections stained with Falck's method (Falck and Owman, 1965) also revealed two paired fiber tracts from the PVO to the telencephalon. One of these belongs to the basal forebrain bundle. It ends in a fiber-rich zone in the region of the ganglion basale or pars subpallialis (Gaupp, 1899). The other runs more medially and dorsally together with other fibers of the medial forebrain bundle. Moreover, a greenish fluorescence was observed in a paired nucleus situated in the dorsal part of the tuber cinereum, immediately in front of the proximal end of the thin dorsal wall of the infundibular process. In median sections this nucleus consisted of scattered neurons with relatively weak fluorescence. However, in more lateral sections it contained a larger number of strongly fluorescent perikarya. This nucleus may be related to the nucleus reticularis mesencephali, recently described by Braak (1970) in *R. esculenta*. Indeed, it continued into the mesencephalon; immediately behind this nucleus, serotonin-containing neurons were observed.

The functional significance of many of the aminergic structures is completely unknown. However, the existence of an infundibulo–hypophysial tract, and the fact that this tract fills with neurosecretory material when animals are transferred from a dark to a light background, seem to point to a relation between the aminergic neurosecretory centers and the pars intermedia of the adenohypophysis. This may mean that aminergic rather than peptidergic neurosecretory fibers control the pars intermedia. Jørgensen and Larsen (1963) were the first to doubt the significance of

the peptidergic neurosecretory system in this respect. They noted that denervation of the pars intermedia in *X. laevis, B. bufo* and *A. mexicanum* was followed by a remarkably slow recovery. This made them believe that "ordinary" nerves and not neurosecretory fibers actually controlled the activity of the pars intermedia. Electron-microscopical studies have shown that apart from occasional peptidergic neurosecretory fibers many aminergic fibers penetrate the pars intermedia and make synaptic contact with the melanotropic cells. This applies to *B. arenarum* (Iturriza, 1964), *X. laevis* (Cohen, 1967; Pehlemann, 1967), *R. catesbeiana* (Saland, 1968), and *R. pipiens* (Saland, 1968; Nakai and Gorbman, 1969). Doerr-Schott and Follenius (1969, 1970) found three types of nerve fibers in electron micrographs of the pars intermedia of *R. esculenta*. One type selectively accumulated tritiated DL-noradrenaline and is thus believed to be aminergic. With Falck's method for the demonstration of certain biogenic monoamines, such fibers have also been visualized in the neurohypophysis and the pars intermedia of *R. temporaria* (Enemar and Falck, 1965) and *B. arenarum* (Enemar *et al.*, 1967). Moreover, the pharmacological and surgical experiments of Burgers *et al.* (1958) with *X. laevis*, of Iturriza (1966, 1967, 1969) with *B. arenarum*, and of Dierst-Davis *et al.* (1966) and Bercu and Brinkley (1967) with *R. pipiens* demonstrate that hypothalamic monoamines are involved in inhibiting the MSH activity of the pars intermedia.

Yet, in none of these experiments was the actual site of MIF production ascertained. Goos (1969c) has attempted to do so by studying the effect of reserpine on the skin melanophores of dark-background-adapted prometamorphic *X. laevis* tadpoles with and without infundibular nucleus. When the nucleus had been removed, reserpine did not affect the melanophores, but in intact larvae it caused an initial aggregation of the melanin granules in the melanophores which some hours later was followed by a redispersion. This coincided with the disappearance of bioamines from the infundibular nucleus. He concluded that the initial aggregation was the result of an enhanced secretion of MIF, and that the ensuing dispersion of the melanin granules in the melanophores followed upon the depletion of the infundibular nucleus.

H. J. Th. Goos and G. E. van Ree (unpublished) have tried to identify the hypothalamic bioamines in *X. laevis* tadpoles. To that end they first determined the excitation and emission spectra of the fluorescent products derived from biogenic monoamines with the method of Falck (Falck and Owman, 1965), present in the PVO, the infundibular nucleus, the median eminence and the pars intermedia of prometamorphic *X. laevis* tadpoles, treated with iproniazide. These spectra were determined with the microspectrophotofluorometer method of Jonsson (1967a,b). It appeared that the fluorescent material had an excitation maximum of 405–410 mμ and an emission maximum of 480 mμ. These maxima were identical with those of fluorescent products of catecholamines, enclosed in dried protein and treated with formaldehyde gas according to Falck. Fluorophores of indoles such as 5-hydroxytryptamine (5-HT) had an excitation maximum of 395 mμ and an emission maximum of 525 mμ. It is thus indicated that the aminergic neurosecretory material in *X. laevis* tadpoles contains catecholamines rather than indoles.

Goos and van Ree (unpublished) also studied the presence of several catecholamines and 5-HT in a homogenate of 1450 pooled midbrains of prometamorphic *X. laevis* larvae. The homogenate was fractionated according to the column adsorption chromatographic technique of Atack and Magnusson (1970). In the fraction the presence of DOPA, noradrenaline and adrenaline was determined with the trihydroxyindole method as modified by Bertler *et al.* (1958), that of dopamine by using the fluorimetric method of Carlsson and Waldeck (1958), and that of 5-HT with the fluorimetric method of Andén and Magnusson (1967). In all cases a Zeiss spectrophotofluorometer was used. Following these procedures it appeared that the

excitation and emission maxima of the fractions that might contain DOPA, noradrenaline plus adrenaline and 5-HT, respectively, differed considerably from those of the respective standard solutions. On the other hand, the excitation and emission spectra of the fractions that should contain dopamine were 337 and 383 mμ respectively, very different from those of tissue blanks, and identical with those of a standard solution. This means that the presence of dopamine was demonstrated, but not of the other catecholamines and 5-HT.

The presence of dopamine in the hypothalamus of *X. laevis* larvae does not imply that this substance should be considered as the natural MIF, produced in the infundibular nucleus and transported to the pars intermedia via the infundibulo–hypophysial tract. Indeed, according to Jørgensen (1968), Spies has found that dopamine cannot directly inhibit MSH release. Other catecholamines as well as acetylcholine, serotonin, histamine and pars nervosa hormones also fail to bring about this effect (Ralph and Sampath, 1966; Jørgensen, 1968). There is circumstantial evidence against the original hypothesis that substances such as the pars nervosa hormones, produced in the peptidergic preoptic nucleus exercise a MSH-inhibiting activity (cf. Jørgensen, 1968). However, it would be wrong to suppose that none of the neurohumors is involved in the inhibition of MSH secretion. Apart from the strong arguments in favor of an aminergic regulation of the melanotropic cells, Doerr-Schott and Follenius (1969, 1970) and Oshima and Gorbman (1969) have demonstrated the presence of a cholinergic innervation of the pars intermedia cells. Likewise, Dierst-Davis *et al.* (1966) have obtained pharmacological evidence that part of the pathway mediating the hypothalamic control of the pars intermedia function is cholinergic. It thus seems possible that it is not one single MIF, but at least two different substances—one aminergic, another cholinergic—that together inhibit the production and extrusion of MSH.

It may well be that the substances regulating the activity of the pars intermedia are not exclusively transported via nerve fibers. Dierickx *et al.* (1970) have called attention to a capillary network in the PVO of *R. temporaria*. It is believed that these capillaries give rise to the hypothalamic branch of the encephalo-posthypophysial portal vein (Cruz, 1959; Rodríguez and Piezzi, 1967) that drains into the secondary capillary plexus in the neurointermediate lobe of the pituitary. The authors suggest that by this way monoamines from the PVO might influence the pars intermedia.

Moreover, Rodríquez (1969) has described ependymal cells in the median eminence of *B. bufo* and *B. arenarum* that link the capillaries in the median eminence with the infundibular lumen. Many of the vascular endings of the ependymal processes have dense-cored vesicles with a diameter of 700–1400 Å. Possibly, these granules represent transport material derived from the cerebrospinal fluid. If so, they might contain substances secreted into the infundibular lumen by cells such as the type-II cells of the PVO and by the liquor-contacting neurons of the infundibular nucleus. Such a situation might explain why the skin melanophores of very young larvae of *X. laevis* can adapt themselves to the background even before a nervous link between the infundibulum and the adenohypophysis is established (Nyholm, 1969).

The secretion of MSH is the first endocrine function of the adenohypophysis of amphibian larvae (Pehlemann, 1962, 1965; Thurmond, 1967; Doerr-Schott, 1968). In studying the relation between hypothalamus and adenohypophysis in very young *X. laevis* tadpoles, M. Terlou and H. W. M. van Straaten (unpublished) observed that the abilities to adapt to a white background and to inhibit MSH secretion were acquired simultaneously with the appearance of the first aminergic cells in the hypothalamus. This early aminergic nucleus extended from immediately behind the optic chiasma to the lateral recess of the infundibulum. Its cells have club-shaped intraventricular processes, but axons

have not been observed. Some hours after this first aminergic nucleus a second one appears. This nucleus runs along the narrow side of the recessus lateralis and extends into the infundibular lobe. Cells of this nucleus, bordering the ventricle, have clubshaped protrusions into the cerebrospinal fluid, but no axons filled with aminergic material. On the other hand, cells not bordering the ventricle were seen to have axons running in a lateroventral direction. Moreover, in the infundibular lobe some multipolar aminergic neurons were observed. It is not impossible that these neurons contribute to the aminergic nervous connections between the neurohypophysis and the adenohypophysis, which—according to Nyholm (1969)—develop at the beginning of premetamorphosis.

The regulation of the MSH activity of the pars intermedia is not the only neurosecretory function of the caudal hypothalamus. Dierickx (1966) has made it clear that in *R. temporaria* the gonadotropin-releasing factor (GRF) is formed in aldehyde fuchsin-negative neurons, situated in the ventral part of the tuber cinereum. It has even been suggested (Goos, 1969c; Doerr-Schott and Follenius, 1970) that cells belonging to the aminergic infundibular nucleus might be the source of GRF. The main reason for such a suggestion was that these aminergic cells were the only neurosecretory elements seen in that region. However, Peute (unpublished) has recently observed a new center of apparently neurosecretory activity in electron micrographs of the caudal hypothalamus of *X. laevis* tadpoles, autopsied at the beginning of metamorphic climax. The Falck-negative and Gomori-negative cells composing this center were located in the ventral part of the tuber cinereum, ventrocaudally to the infundibular nucleus. The nucleus–cytoplasm ratio of its cells was less than in the aminergic elements; the rough endoplasmatic reticulum and the Golgi apparatus were far better developed. The cells were bipolar, one nerve ending protruding into the infundibular lumen, the other running in the opposite direction. The cells contained granulated vesicles, about 1350 Å in diameter. In adult *X. laevis* and *R. esculenta* these cells were also observed, but with a larger number of granulated vesicles. In adults the cells contained a variable number of lysosome-like organelles as well as large colloid-like droplets, apparently built up of fused granulated vesicles. It seems that during metamorphic climax the cells in the ventral part of the tuber cinereum were at the beginning of development and that in the adult animals they showed full activity. This situation resembles the development of the gonadotropic activity of the adenohypophysis (cf. van Oordt, 1968, 1971). Consequently, it is not impossible that the neurosecretory cells in the ventral part of the tuber cinereum are the source of the GRF.

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DISCUSSION

BAGNARA: Is it possible that the control of MSH release might operate through one of the fluorescent adrenergic nuclei by regulating production or release of an MIF? The MIF could then reach the pars intermedia by either some portal system or even by passage through the ventricle. This suggestion would fit both the requirement of an adrenergic mechanism and the presence of a hypothalamic inhibiting factor.

VAN OORDT: First: I would like to say that Rodríquez has found that in *Bufo arenarum*, ependymal cells in the median eminence can transport substances from the cerebrospinal fluid to the portal system. Moreover, Dierickx recently described a capillary system in the paraventricular organ of *Rana temporaria*. He also found portal vessels connecting this capillary system with the pars nervosa and the pars intermedia. Thus, I would think that there can be more than one system connecting the hypothalamus with the pars intermedia. In addition, we should not rule out the possibility that several hypothalamic factors might act together to accomplish the inhibition of MSH release.

RODRÍGUEZ: I would support Dr. van Oordt's view concerning the probable role of the cerebrospinal fluid (CSF) as a vehicle for the transport of substances which affect MSH release. In amphibians, capillaries connecting the median eminence with the neurointermediate lobe, do exist. These vessels run along the neural lobe stalk and are partly surrounded by ependymal processes. These ependymal cells contain a highly developed tubular system, whose contents may be stained by a modification of the zinc iodide-osmium tetroxide impregnation method. Furthermore, these ependymal cells incorporate exogenous peroxidase when this is injected into the CSF.

PESETSKI: The ability of ependymal cells to take up exogenous substances and transport them is a characteristic not restricted to the median eminence. In my laboratory, for example, we have studied similar processes in ependymal cells of the amphibian medulla oblongata. Do you have evidence that this transport has anything to do with hypothalamic–hypophysial function?

RODRÍGUEZ: I agree that transporting ependymal cells are not only seen in the median eminence but in many other regions of the ventricular wall also. However, the fact that those in the median eminence are interposed between the CSF and the portal capillaries, would suggest that they are involved in hypophysial function. Furthermore, there are a few publications reporting changes in the ependymal lining of the median eminence following castration.

VAN OORDT: Terlou observed a correlation between the appearance of aminergic neurons in the caudal hypothalamus and the adaptation of young *Xenopus* larvae to a white background. If we consider the possibility of a causal relationship between these two phenomena, we will have to accept the possibility of transport of an MIF via the cerebrospinal fluid, for in these young larvae, the direct innervation of the pars intermedia has not yet developed. Also, the early aminergic cells do not possess axons running towards the neurohypophysis.