Immunohistochemical Localization of Gonadotropin-releasing Hormone (GnRH) in the Fetal and Early Postnatal Mouse Brain [1]

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ABSTRACT The objectives were to (a) determine the age in development when GnRH is first detectable in the brain and (b) observe the distribution of GnRH throughout the fetal and early postnatal period. GnRH was localized immunohistochemically in fetal (15, 16, 17 and 19 days of gestation) and early postnatal (1- and 7-day-old) mice with the peroxidase-antiperoxidase (PAP) method of Sternberger.

In the organum vasculosum of the lamina terminalis (OVLT) and in the median eminence of the fetus, GnRH was first detected at 17 days of gestation. In the OVLT, GnRH was found ventral to the preoptic recess of the third ventricle near the ventral surface of the brain. In addition, GnRH was located adjacent to the superficial portal capillaries near the surface of the median eminence. At 19 days of gestation, the distribution of GnRH was similar to that observed at 17 days and there was a marked increase in amount.

In the newborn mouse, GnRH was undetectable in the OVLT and its content in the median eminence was decreased as compared to that observed in the fetus. By the seventh postnatal day, a considerable accumulation of GnRH had occurred in the OVLT and median eminence. In the OVLT, it was associated with capillaries ventral to the preoptic recess, and its distribution in the median eminence was similar to that in the adult mouse.

In both the OVLT and median eminence of the fetal and early postnatal mouse GnRH appeared to be stored in axons and axon endings, but was not detectable in nerve cell bodies or ependymal cells. These observations suggest that the potential for neuroendocrine control of gonadotropin secretion exists in the fetal mouse as early as 17 days of gestation.

Considerable evidence indicates that several endocrine systems, including the pituitary-gonadal axis, are active in the fetus and neonate (Jost, '53, '54, '62), but little is known about when, and to what degree, the brain is able to regulate these systems. With respect to the hypophysis, it has been suggested that in the fetal rat (Glydon, '57) and rabbit (Cambell, '66), neurovascular control by the brain is unlikely because capillary loops of the primary portal vascular plexus do not penetrate the median eminence until late in the first postnatal week. However, several authors have reported the presence late in gestation of superficial capillaries on the surface of the median eminence, and of portal vessels which connect these capillaries with sinusoidal capillaries of the developing pituitary gland (Enemar, '61; Halász et al., '72; Monroe et al., '72; Smith, '70). They postulated that capillary loops are not essential to neurovascular function, and concluded that crucial morphological criteria for establishing the existence of a neurovascular link between the hypothalamus and pituitary pars distalis exist late in gestation.

In support of these morphological observations, some experimental evidence demonstrates that the adrenal (Jost et al., '70; Zarrow et al., '68) and thyroid (Conk-

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lin et al., '73) glands are under neuroendocrine control during fetal life. In addition, the hypothalamus probably plays a role in sexual development, particularly in terms of gonadotropin secretion. Monoamines, which may regulate LH and FSH secretion (McCann, '74), have been detected in the fetal median eminence (Bjorklund et al., '68; Smith and Simpson, '70). Moreover, the fetal hypophysis appears to be responsive to exogenous gonadotropin-releasing hormone (GnRH) (Foster et al., '72; Schafer and McShan, '74). However, evidence for the existence of GnRH in the developing brain is scarce and often conflicting (Barry and Dubois, '74; Cambell and Gallardo, '66; Corbin and Daniels, '67; Araki et al., '75). In view of the important role played by GnRH in the control of reproductive physiology, evaluation of the development of this control is crucial to an understanding of the neuroendocrine regulation of gonadotropin secretion. Thus, the investigation reported here had as its major objectives to (a) determine the age in development at which GnRH is first detectable in the brain and (b) observe the development of the adult pattern of distribution of GnRH, as previously reported (Gross, '76), throughout the fetal and early postnatal period.

MATERIALS AND METHODS

Mice used in this study were of the Swiss-Webster strain. They were maintained in a breeding colony in air-conditioned quarters, with a controlled lighting schedule and feeding *ad libitum*. The mice were mated overnight and the females checked for the presence of a vaginal plug the following morning. If a plug was present, the female was removed to a separate cage and considered to be in day 1 of pregnancy. The average gestation period in this colony was 19 days. Following are the numbers of fetal mice studied during gestation: nine at 15 days, five at 16 days, 12 at 17 days and nine at 19 days. Also examined were 14 newborn mice and nine after seven days of postnatal life. The fetal mice were removed from the uterus at 10 A.M., decapitated, and the cranium, with the brain intact, was immersed in Bouin's fluid for 48 to 72 hours. The postnatal mice were killed by decapitation at 10 A.M. Subsequent removal of the superficial cranium and brain stem provided rapid access to the base of the brain, which was flooded immediately with Bouin's fluid. The brain was excised, with care being taken to leave hypothalamo-hypophysial relations intact, although this was difficult in the case of the neonatal mice. Fixation was completed by immersion in Bouin's fluid for 24 to 48 hours. The tissue from all mice was embedded in Paraplast and sectioned serially at 4 μm in either a transverse or sagittal plane. Every twentieth section was stained with cresyl violet to aid in the selection of sections for immunohistochemical study and identification of hypothalamic nerve cell nuclei. The cephalocaudal distribution of GnRH will be described throughout the organum vasculosum of the lamina terminalis (OVLT) and the median eminence.

Immunohistochemistry

Following removal of paraffin and hydration, the sections were prepared immunohistochemically by the peroxidase-anti-peroxidase (PAP) technique of Sternberger et al. ('70), with 3,3'-diaminobenzidine (DAB) being used as the label for GnRH. All antisera were applied for 30 minutes at room temperature. Nonspecific background was reduced by treating sections with normal sheep serum before application of anti-GnRH,[3] anti-IgG, and PAP (Petrali et al., '74). The majority of the specimens were labeled with antiserum No. 173, prepared by W. C. Dermody of Parke, Davis Research Laboratories.[4] This was an antiserum to unconjugated, synthetic GnRH which was used at a dilution of 1/600. In addition, several specimens from 17- and 19-day-old fetal and newborn mice were labeled with either antiserum No. 154 from Doctor Dermody, which was generated against synthetic GnRH conjugated to limpet hemocyanin and used at a dilution of 1/300, or antiserum No. 12, obtained from Doctor R. P. Kelch of the University of Michigan which was generated against synthetic GnRH conjugated to bovine serum albumin and employed at a dilution of 1/300. These were the maximal dilutions at which optimal labeling of GnRH was ob-

[3] Antisera will be indicated by the prefix "anti-" added to the name of the antigen.

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tained regularly. The preparation and specificity of antisera Nos. 173 and 154 for demonstration of GnRH have been discussed previously (Baker et al., '75; Dermody et al., '73; Gross, '76).

In order to verify the specificity of the immunohistochemical method for localization of GnRH, several control procedures were performed. First, GnRH antisera were preabsorbed with synthetic GnRH (Parke, Davis and Company). In the case of antiserum No. 173, 20 μl of a 0.1% solution of GnRH was added to 20 μl of a 1/300 dilution of antiserum. This produced a preabsorbed antiserum at a final dilution of 1/600, the same as that used without preabsorption. When antiserum No. 154 was preabsorbed with GnRH, the final dilution was either 1/300 or 1/800, with 0.1% or 1% solutions of GnRH being used. The absorbed antisera were preincubated overnight. Second, to show that GnRH antisera were not contaminated with antibodies to other hypothalamic peptides they were preabsorbed with 1% arginine-vasopressin, oxytocin, or thyrotropin-releasing hormone (TRH). Third, to insure that labeling with antiserum No. 154 was not due to antibodies to hemocyanin, this antiserum was preabsorbed with a 0.1% solution of hemocyanin. Finally, background staining was reduced by preabsorbing antiserum No. 154 with acetone-dried mouse liver powder for one hour at room temperature and then centrifuging at 18,000 rpm for 15 minutes. Also normal rabbit serum was substituted for GnRH antiserum.

RESULTS

Fifteen and 16 days of gestation

The OVLT was not well differentiated at 15 days of gestation. Although identifiable, the median eminence could not be differentiated into internal and external laminae. In all nine mice, GnRH was not detectable in any area of the brain at this stage of development (fig. 9).

At 16 days there was little change in the morphology of the OVLT or median eminence. GnRH was still undetectable in both the OVLT and median eminence in all mice studied at this age.

Seventeen days of gestation

OVLT

At 17 days the morphology of the OVLT was still rather indistinct. Only a few blood vessels had penetrated the lamina terminalis in this region. A small amount of GnRH was present in the most cephalic portion of the OVLT of all 17-day-old fetuses (fig. 1). Here it was located near the ventral surface of the brain, lateral and dorsal to the blood vessels invading the lamina terminalis. Caudally, an increased amount of GnRH was detected ventral to the preoptic recess (fig. 5).

Median eminence

At 17 days, the superficial capillary plexus was prominent in the area between the ventral surface of the median eminence and the well-defined pars tuberalis. GnRH was present in the median eminence of all 12 17-day-old fetuses. In the cephalic region of the median eminence, GnRH occurred in two narrow transverse bands close to the ventral surface of the brain (fig. 10). These bands extended quite far laterally, past the lateral limits of the pars tuberalis, median eminence and developing arcuate nucleus. No GnRH was present under the infundibular recess in the medial part of the median eminence. More caudally, in the broad region of the median eminence, a slight aggregation of GnRH occurred dorsal to the lateral side of the beginning of the tuberoinfundibular sulcus, close to the ventral surface of the brain (fig. 11). The GnRH in this region was adjacent to the superficial capillaries of the developing portal vascular system. No reaction product was present in an adjacent section stained with an antiserum to GnRH preabsorbed with an excess of synthetic GnRH (fig. 12), thus demonstrating the specificity of the immunohistochemical labeling. In addition, no immunohistochemical labeling was observed when normal rabbit serum was substituted for any GnRH antiserum. Absorption of antiserum No. 173 or No. 154 with arginine-vasopressin, oxytocin, or thyrotropin-releasing hormone, and absorption of antiserum No. 154 with hemocyanin, was without effect. When employed on consecutive sections of specimens from 17- and 19-day-old fetal, and newborn mice, identical localization of GnRH was obtained with antisera Nos. 173, 154 and 12. Caudal to this region, GnRH was located along the lateral sides of the deepened tuberoinfundibular sulci (fig. 13a). As in the more cephalic regions, the

foci [5] of GnRH were close to the ventral surface of the brain and often were adjacent to capillaries located between the median eminence and the pars tuberalis (fig. 13b). Again, foci were present far laterally, while no GnRH was seen medial to the tuberoinfundibular sulci. In the postinfundibular median eminence, GnRH was scattered in two narrow bands near the ventral surface of the brain (fig. 14). In the most caudal part of the median eminence, very little GnRH was present. When viewed in sagittal sections, the distribution described above for transverse sections was confirmed (fig. 15a), with most of the GnRH occurring near the ventral surface of the lateral region of the postinfundibular median eminence. The superficial capillaries of the supratuberal plexus were evident along the surface of the median eminence and, when viewed at high magnification, the close relationship of GnRH to these capillaries was striking (fig. 15b). No GnRH was detectable within any neuronal cell bodies, nor in the cytoplasm of any ependymal cells.

Nineteen days of gestation

OVLT

At 19 days of gestation, the blood vessels along the surface of the OVLT were conspicuous, although only a few capillaries were present within the nervous tissue of the developing OVLT. GnRH was localized in the cephalic part of the OVLT (fig. 2), with bodies labeled for GnRH [5] being present dorsal and dorsolateral to the ependyma of the most cephalic end of the preoptic recess. More caudally, several foci were present ventral and ventrolateral to it (fig. 6). In both regions, GnRH was near the superficial capillaries and the ependyma of the OVLT. A small increase in the amount of GnRH in the OVLT had occurred subsequent to day 17.

Median eminence

By the end of gestation, early differentiation of the internal and external laminae was evident, and the arcuate nucleus was becoming more well-defined. The superficial capillary network of the primary plexus was increasingly well-developed. A marked augmentation in the amount of GnRH present within the median eminence, compared to the 17-day fetus, was apparent. Cephalically, GnRH was again located in two lateral bands at the ventral surface of the median eminence, with several of these foci lying adjacent to the capillaries of the supratuberal plexus (fig. 16). As the tuberoinfundibular sulci formed, the bodies labeled for GnRH were distributed over the apices of these shallow sulci, and were found in two longitudinal bands along their lateral sides (fig. 17). As in the cephalic zone, no GnRH was localized under the infundibular recess. As these sulci deepened, GnRH accumulated in longitudinal bands located dorsal to the apices and lateral borders of the sulci (fig. 18). Near the region of separation of the infundibulum from the brain, the greatest accumulation of GnRH occurred near the ventral surface of the median eminence (fig. 19a). Again, the midline region, which in the adult contained most of the reaction deposit, was generally free of GnRH. The proximity of these putative granular deposits of GnRH to the ventral surface of the brain was apparent at high magnification (fig. 19b). In the caudal part of the median eminence, a few foci of GnRH were scattered along the ventral surface of the most lateral aspect of the median eminence. In sagittal section, the accumulation of GnRH in the postinfundibular median eminence was evident, as was the small amount near the ventral surface of the cephalic region (fig. 20). In no animals was GnRH detected in neuronal cell bodies or ependymal cells and their processes.

The newborn mouse

OVLT

In the newborn mouse, capillaries of the OVLT were somewhat more prominent than those of the 19-day fetus, and the density of nerve fibers adjacent to these capillaries had increased slightly. GnRH was not consistently detectable in the OVLT of the newborn mouse (figs. 3, 7). Traces of positive reaction appeared in only two of 14 animals, and the amount of DAB deposited was so slight as to render its significance questionable.

Median eminence

In the newborn mouse, the internal and

[5] The terms "foci" and "bodies labeled for GnRH" are used synonymously to denote individual sites where oxidized DAB was deposited.

external laminae were distinguishable, although not well defined. The arcuate nucleus and pars tuberalis had attained a relationship to the median eminence similar to that seen in the adult. The superficial, but not deep, capillaries of the primary portal plexus were present. At all cephalocaudal levels of the median eminence, the amount of detectable GnRH was considerably less than that found late in gestation, and in five of the 14 mice studied, no GnRH was observed in the median eminance. In the cephalic part of the median eminence, the distribution of GnRH appeared to be similar to that in fetal mice, but the amount was markedly less (fig. 21). No GnRH was found under the infundibular recess, and only a few diffuse foci were scattered along the lateral part of the median eminence. More caudally, GnRH was almost invisible, being restricted to a small cluster of light foci capping the shallow tuberoinfundibular sulci (fig. 22). Slightly more GnRH was found dorsal to the deepened sulci (fig. 23a), but still the amount was considerably less than that seen in the fetus. When viewed at high magnification, the immunoreactive deposits were much lighter and more diffuse than those observed at the end of gestation (fig. 23b). A marked reduction in GnRH content was also evident in the postinfundibular median eminence (fig. 24), where it appeared only as a few diffuse deposits of DAB scattered laterally near the ventral surface. GnRH was not found in the caudal median eminence from 11 of the 14 animals studied.

The 7-day postnatal mouse

OVLT

At seven days, the OVLT was well-developed. This was particularly evident in its component blood vessels which were prominent. In the cephalic part of the OVLT, GnRH was found in the nervous tissue along the lateral sides of the central core of blood vessels (fig. 4). At this level, ependymal elements were not extensive and the foci of GnRH were often associated closely with capillaries of the OVLT. More caudally, GnRH accumulated ventral to the preoptic recess of the third ventricle, where the bodies labeled for GnRH appeared to encircle the cluster of capillaries found in this region (fig. 8). The close relationship of GnRH to these blood vessels was apparent in high-magnification views of the OVLT (fig. 8, inset).

Median eminence

By the seventh postnatal day, the essential morphological components of the adult median eminence were present. The internal and external laminae were clearly defined and the arcuate nucleus and pars tuberalis were well-developed. In addition to the presence of the superficial portal plexus, some capillary loops had penetrated the external lamina. The GnRH-containing neural processes in the cephalic portion of the median eminence were conspicuous as two bands in the lateral portions of the median eminence (fig. 25a). In addition, for the first time, GnRH was detected in the external lamina, under the infundibular recess. The DAB deposits occurred near the ventral surface of the external lamina, adjacent to the pars tuberalis and the superficial portal capillaries (figs. 25b). Proximal to the shallow tuberoinfundibular sulci (fig. 26), GnRH was concentrated in two longitudinal bundles dorsal to these sulci. Several foci of GnRH were observed adjacent to the ependyma of the infundibular recess, and a few were occasionally present across the external lamina. More caudally, GnRH accumulated over the apices of the deepened tuberoinfundibular sulci (fig. 27). Several foci were present along the lateral sides of the sulci, and some occurred along the medial sides. Although GnRH did not extend across the median eminence in the external lamina, labeled neural processes were sometimes evident in the internal lamina, ventral to the ependymal cells lining the floor of the third ventricle. In addition, GnRH appeared in foci scattered from the apex of the tuberoinfundibular sulci medially to the region adjacent to the ependyma of the lateral recesses of the third ventricle. The greatest concentration of GnRH was found in the postinfundibular median eminence (fig. 28), where the labeled neural processes formed narrow bands near the ventral surface and extended outward past the lateral boundary of the arcuate nucleus. GnRH was also conspicuous in the midline, under the infundibular recess. Here some bodies labeled for GnRH occurred proximal to the ependymal cells lining the

third ventricle. The foci, when viewed at high magnification, were beginning to acquire the vesicular appearance characteristic of those in the adult (fig. 28, inset). Only a few foci of GnRH were found in the lateral aspect of the caudal region of the median eminence. The distribution of GnRH at this age was similar to that in the adult; a major difference was the lesser amount demonstrable seven days postnatally. Although not observed at any earlier stage of development, by seven days postnatally a few GnRH-containing neural processes had begun to show a beaded appearance similar to that apparent in the adult (fig. 25b).

DISCUSSION

The organum vasculosum of the lamina terminalis

This study has revealed, for the first time, the presence of GnRH in the OVLT of the fetal mouse. GnRH appeared at 17 days of gestation and was present in a higher concentration by 19 days. As in the adult (Gross, '76), it was located proximal to the blood vessels of the OVLT. GnRH appeared in the OVLT at the same time in development as did GnRH in the median eminence, but the functional significance of GnRH in the fetal OVLT is not presently understood. Since no portal vessels are known to connect the OVLT with the pituitary gland, GnRH in the OVLT may not be involved directly in pituitary secretion of gonadotropin; rather it may function as a neurotransmitter, affecting the surrounding nervous tissue. In fact, behavioral effects, presumably mediated by a central nervous system site of action, are well documented for GnRH, as well as for some other neurohormones, including TRH and somatostatin (Marx, '75). This probability is of particular interest in view of the presence of GnRH in the OVLT during a time when the neural control of sexual development may be especially active (Barraclough, '66).

GnRH was virtually undetectable in the OVLT of the newborn mouse, demonstrating that a significant decrease in the content of GnRH took place sometime between the last day of gestation and the morning of the first postnatal day. Further, at birth a marked decrease was noted also in the GnRH content in the median eminence. Thus the unknown factor or factors responsible for the decrease may be similar for both the OVLT and the median eminence.

As compared with the condition immediately after birth, a significant increase in the store of GnRH in the OVLT had occurred by the seventh postnatal day. Concurrently the vascular pattern of the OVLT had become more complex, with the appearance of a well-developed central core of blood vessels. These changes indicate that the morphological relationship between GnRH, presumably stored in axons, and the vascular system had attained a stage of development similar to that in the adult. The increased concentration of GnRH in the postnatal OVLT may play a mediating role in sexual differentiation of the hypothalamic neuroendocrine regulatory system which is known to occur during the early postnatal stages of development (Barraclough, '66). High doses of exogenous GnRH can induce mating behavior in the rat (Moss and McCann, '73; Pfaff, '73), and McCann ('74) has suggested that GnRH may be released into the preoptic area, where it elicits this behavioral response. While the physiological role of GnRH in the OVLT remains obscure, on morphological grounds the OVLT appears to be functionally competent by seven days after birth.

The median eminence

The fetal mouse

In this investigation, GnRH was detected in the median eminence of the 17-day-old mouse fetus. The position of the bodies labeled for GnRH is significant in light of the nature of the developing portal vascular system. Glydon ('57) and Cambell ('66) have suggested that fetal hypophysiotropic releasing factors would not be able to reach the pituitary pars distalis because no capillary loops are present in the fetal median eminence. However, the GnRH found in both 17- and 19-day-old fetuses was located close to the ventral surface of the median eminence. In this position, GnRH foci were almost directly adjacent to some of the superficial blood vessels of the developing supratuberal plexus. This plexus, in the rat fetus, contains portal vessels connecting the superficial capillaries on the surface of the median eminence with the sinusoidal capillaries in the developing

anterior pituitary gland (Glydon, '57; Florsheim and Rudko, '68; Jost et al., '70). Possibly, GnRH located near the surface of the median eminence may pass through the fenestrations of these superficial capillaries (Smith, '70) and reach the pituitary pars distalis. If this is true, fetal GnRH may stimulate gonadotropin release, since exogenous GnRH can induce release of LH and FSH from the hypophysis of fetal sheep (Foster et al., '72) and rats (Schafer and McShan, '74).

While the subcellular location of GnRH in the fetus remains unclear, considerable evidence suggests that, in the adult rat, GnRH is stored in intra-axonal granules (Pelletier et al., '74; Goldsmith and Ganong, '75; Taber and Karavolas, '75; Clementi et al., '70). The appearance of GnRH in the median eminence of the 17- and 19-day-old fetus can be correlated with the ontogenesis of such intra-axonal granules in the median eminence. In the mouse, nerve endings containing small dense-core granules first make contact with the perivascular space surrounding capillaries of the supratuberal plexus at 16 (Eurenius and Jarskar, '71) or 18 (Beauvillain, '73) days of gestation. Endothelial fenestrations also appear in the superficial capillaries at this time (Beauvillain, '73).

Several other developmental processes related to neuroendocrine control of gonadotropin secretion also appear to mature at 17 to 19 days of gestation. The hypothalamic nuclei most commonly implicated in gonadotropin control (arcuate, ventromedial, suprachiasmatic) are well differentiated by this age (Coggeshall, '64; Hyppa, '69), and the appearance of GnRH in the brain is coincident with the appearance of monoamines, which are detectable in axons of the median eminence in the mouse at 17 days of gestation (Bjorklund et al., '68) and in the rat at two days prior to parturition (Simpson and Smith, '70).

The demonstration, in the present investigation, of the presence of GnRH in the OVLT and median eminence of the 17-day-old mouse fetus represents the earliest age in development at which GnRH has been found in the brain. GnRH was not detected with bioassay in the fetal rabbit median eminence by Cambell and Gallardo ('66), or with radioimmunoassay in the fetal rat brain by Araki et al. ('75). Our immunohistochemical identification of GnRH during late pregnancy supports, in general, the findings of Corbin and Daniels ('67), who used bioassay to detect GnRH in the 20-day-old rat fetus, of Barry and Dubois ('74), who localized GnRH-containing nerve fibers with immunofluorescence near the end of gestation in the guinea pig, and of Eskay et al. ('74), who found GnRH in the 18-day fetal rat brain with radioimmunoassay. The discrepancies in the results of different investigators are probably due to great variability in the sensitivity of the various assays used. It is well established that the unlabeled-antibody immunohistochemical technique is extremely sensitive in detecting tissue antigens (Petrali et al., '74; Sternberger and Petrali, '75). This high sensitivity may explain the early detection of GnRH in the present study in concentrations which would be missed by some assays. Also in agreement with our findings, Barry and Dubois ('74) reported that the amount of immunoreactive GnRH in axons of the fetal median eminence increased toward the end of gestation in the guinea pig. Whether the increase in GnRH content at term implies increased functional activity at the pituitary level remains to be determined.

The cellular source of GnRH in the fetal mouse was not clarified by our study since GnRH was not localized in any neuronal cell bodies. This result stands in contrast to the report of Barry and Dubois ('74), who found GnRH in several neuronal cell bodies at the end of gestation in the guinea pig. A similar discrepancy has been noted in adult animals, where these authors, but few others, have been able to demonstrate GnRH within neuronal cell bodies of the hypothalamus.

As in the adult mouse (Gross, '76), no specific labeling of GnRH occurred in ependymal cell bodies or their tanycyte processes. It has been suggested, on the basis of morphological evidence in the fetal guinea pig, that tanycytes may be important in transporting GnRH to the developing portal blood vessels (Silverman and Desnoyers, '75). Our observations do not support this hypothesis in the mouse.

The distributions of GnRH in brains of the fetal and adult mouse differed in several respects. The most marked difference

was quantitative, with only a small fraction of the adult concentration of GnRH being present in the fetus, even at 19 days. Second, GnRH occurred much farther laterally in the fetal median eminence than in the adult. Third, in contrast to the adult, GnRH was absent from the broad expanse of the median eminence under the infundibular recess. This is a significant observation, because in the adult, most of the GnRH-containing nerve fibers terminate near capillaries of the primary portal plexus in this region (Gross, '76).

It thus seems clear, on the basis of our observations and those reported in the literature, that the potential for neuroendocrine control of gonadotropin secretion exists in the fetal mouse as early as 17 days of gestation. The extent to which this system functions during pregnancy remains to be determined.

The newborn mouse

The most striking feature about GnRH in the median eminence of the neonatal mouse was a marked decrease in content as compared to that of the 19-day-old fetus, even though the structure of the median eminence was more developed than at earlier stages, as observed in this and previous studies (Beauvillain, '73; Eurenius and Jarskar, '71; Kobayashi et al., '68; Monroe et al., '72). One may assume that the decreased content of GnRH in the newborn mouse resulted from an extraneous influence, rather than being simply a component of developmental change. Barry and Dubois ('74) reported a similar phenomenon in the guinea pig. They found that GnRH was present in neuronal cell bodies at the end of gestation but disappeared within a few hours after birth. They also reported marked variability in the amount of GnRH in nerve fibers of the median eminence at this time. These observations stand in contrast to studies in the rat (Araki et al., '75) and rabbit (Cambell and Gallardo, '66), in which GnRH was undetectable in the fetal brain, but was present in the newborn animal. Satisfactory interpretation of these data is complicated by the report of Corbin and Daniels ('67), who were able to measure GnRH in the rat fetus, but found an increase in hypothalamic content in the newborn rat.

One explanation for a decreased hypothalamic content of GnRH at birth is the dramatic change in endocrine environment to which the fetus is exposed. A marked rise occurs in the concentration of estrogen in maternal serum during the last few days of gestation in the rat (Waynforth and Robertson, '72; Waynforth et al., '72; Yoshinaga et al., '69; Mori et al., '74) and may be causally related to the early postpartum LH surge and ovulation that occur in the rat (Waynforth and Robertson, '72). On the other hand, an elevated estrogen level may inhibit gonadotropin release in the adult by acting on the hypothalamus (Ramirez et al., '64; Chowers and McCann, '65; Hobson and Hansel, '74; Libertun et al., '74), possibly by inhibiting GnRH release (Blake et al., '74). It is conceivable that the high maternal estrogen level during the last few days of gestation inhibits GnRH release by the fetal hypothalamus, resulting in an accumulation of GnRH in the median eminence. This hypothesis requires that materno-placental steroids have access to the fetal circulation, an assumption for which there is considerable experimental evidence (Deanesly, '66). After birth this inhibition would no longer be present, and the GnRH which was present in the fetal brain might be released, resulting in a reduced content of GnRH in the newborn. Evidence for the release of GnRH by the newborn hypothalamus comes from the data of Döhler and Wuttke ('74), who demonstrated high levels of LH and FSH in the serum of rats during the first two postnatal days, which may have resulted from stimulation of the newborn hypophysis by GnRH. The inhibitory action of gonadal steroids on GnRH release is strongly supported by the observation that when these steroids are removed by castration, the release of GnRH into portal blood increases markedly (Ben-Jonathan et al., '73). Additional evidence for an effect of gonadal steroids on hypothalamic content of GnRH comes from the observations that the amount of GnRH in the stalk-median eminence region fluctuates during the estrous cycle in the rat (Ramirez and Sawyer, '65; Chowers and McCann, '65; Negro-Vilar et al., '70; Asai and Wakabayashi, '75). In addition, the elevated progesterone levels of pregnancy in the mouse (Forbes and Hooker, '57; Murr et al., '74) might be involved in this mechanism, although

the feedback effects of progestins on GnRH are not well understood (McCann, '74). Another possible explanation for the decreased content of GnRH in the brain of the newborn mouse is that the stress of birth may have induced release of GnRH.

Seven days postnatally

The appearance of GnRH in the broad expanse of the median eminence at seven days occurred at approximately the same time that capillary loops begin to penetrate this region (Beauvillain, '73; Cambell, '66; Glydon, '57). In addition, several GnRH foci had assumed the vesicular structure characteristic of the adult median eminence. The presence of GnRH near the capillaries of the primary plexus, as well as the marked increase in the total amount at seven days, are morphological indicators of a maturing hypothalamo-hypophysial neuroendocrine system.

The increased concentration of GnRH found in the mouse median eminence by seven days agrees with the radioimmunoassay data of Araki et al. ('75) and Eskay et al. ('74) in the rat, and the immunofluorescence data of Barry and Dubois ('74) in the guinea pig. Possibly the feedback action of gonadal steroids becomes active at this time and results in an increased hypothalamic content of GnRH. Only small amounts of steroids are required to activate the hypothalamo-hypophysial feedback system in the immature rat (Ramirez and McCann, '63). Regardless of these hypotheses, it is clear that by the seventh postnatal day, the distribution of GnRH within the mouse brain has attained the significant characteristics of its distribution in the adult.

LITERATURE CITED

Araki, S., C. D. Toran-Allerand, M. Ferin and R. L. VandeWiele 1975 Immunoreactive gonadotropin-releasing hormone (GnRH) during maturation in the rat: ontogeny of regional hypothalamic differences. Endocrinology, *97:* 693–697.

Asai, T., and K. Wakabayashi 1975 Changes of hypothalamic LH-RF content during the rat estrous cycle. Endocrinol. Jap., *22:* 319–326.

Baker, B. L., W. C. Dermody and J. R. Reel 1975 Distribution of gonadotropin-releasing hormone in the rat brain as observed with immunocytochemistry. Endocrinology, *97:* 125–135.

Barraclough, C. A. 1966 Modifications in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. Rec. Prog. Horm. Res., *22:* 503–539.

Barry, J., and M. P. Dubois 1974 Étude en immunofluorescence de la differentiation prénatale des cellules hypothalamiques élaboratrices de LH-RH et de la maturation de la voie neurosécrétrice préoptico-infundibulaire chez le cobaye. Brain Res., *67:* 103–113.

Beauvillain, J. C. 1973 Structure fine de l'éminence médiane de souris au cours de son ontogénèse. Z. Zellforsch., *139:* 201–215.

Ben-Jonathan, N., R. S. Mical and J. C. Porter 1973 Superfusion of hemipituitaries with portal blood. I. LRF secretion in castrated and diestrus rats. Endocrinology, *93:* 497–503.

Bjorklund, A., A. Enemar and B. Falck 1968 Monoamines in the hypothalamo-hypophysial system of the mouse with special reference to the ontogenetic aspects. Z. Zellforsch., *89:* 590–607.

Blake, C. A., R. L. Norman and C. H. Sawyer 1974 Localization of the inhibitory actions of estrogen and nicotine on release of luteinizing hormone in rats. Neuroendocrinology, *16:* 22–35.

Cambell, H. J. 1966 The development of the primary portal plexus in the median eminence of the rabbit. J. Anat., *100:* 381–387.

Cambell, H. J., and E. Gallardo 1966 Gonadotrophin-releasing activity of the median eminence at different ages. J. Physiol., *186:* 689–697.

Chowers, I., and S. M. McCann 1965 Content of luteinizing hormone-releasing factor and luteinizing hormone during the estrous cycle and after changes in gonadal steroid titers. Endocrinology, *76:* 700–708.

Clementi, F., B. Caccarelli, E. Cerati, M. L. Demonte, M. Felici, M. Motta and A. Pecile 1970 Subcellular localization of neurotransmitters and releasing factors in the rat median eminence. J. Endocrin., *48:* 205–213.

Coggeshall, R. E. 1964 A study of diencephalic development in the albino rat. J. Comp. Neur., *122:* 241–269.

Conklin, P. M., W. J. Schindler and S. F. Hull 1973 Hypothalamic thyrotropin releasing factor and pituitary responsiveness during development in the rat. Neuroendocrinology, *11:* 197–211.

Corbin, A., and E. L. Daniels 1967 Changes in concentration of female rat pituitary FSH and stalk-median eminence follicle stimulating hormone releasing factor with age. Neuroendocrinology, *2:* 304–314.

Deansely, R. 1966 The endocrinology of pregnancy. In: Marshall's Physiology of Reproduction. A. S. Parkes, ed. Little, Brown and Co., Boston, pp. 891–1063.

Dermody, W. C., E. A. Becvar, B. L. Windsor and J. R. Reel 1973 A radioimmunoassay for luteinizing hormone releasing factor (LRF). Fed. Proc., *32:* 239.

Döhler, K. D., and W. Wuttke 1974 Serum LH, FSH, prolactin and progesterone from birth to puberty in female and male rats. Endocrinology, *94:* 1003–1008.

Enemar, A. 1961 The structure and development of the hypophysial portal system in the laboratory mouse, with particular regard to the primary plexus. Ark. Zool., II. Ser. *13:* 203–252.

Eskay, R. L., C. Oliver, A. Grollman and J. C. Porter 1974 Immunoreactive LRH and TRH

in the fetal, neonatal, and adult rat brain. Proc. Endocrine Soc., Abstract 55.

Eurenius, L., and R. Jarskar 1971 Electron microscopic studies on the development of the external zone of the mouse median eminence. Z. Zellforsch., *122:* 488–502.

Florsheim, W. H., and P. Rudko 1968 The development of portal system function in the rat. Neuroendocrinology, *3:* 89–98.

Forbes, T. R., and C. W. Hooker 1957 Plasma levels of progestin during pregnancy in the mouse. Endocrinology, *61:* 281–286.

Foster, D. L., T. A. Cruz, G. L. Jackson, B. Cook and A. V. Nalbandov 1972 Regulation of luteinizing hormone in the fetal and neonatal lamb. III. Release of LH by the pituitary *in vivo* in response to crude ovine hypothalamic extract or purified porcine gonadotropin releasing factor. Endocrinology, *90:* 673–683.

Glydon, R. J. 1957 The development of the blood supply of the pituitary in the albino rat, with special reference to the portal vessels. J. Anat., *91:* 237–244.

Goldsmith, P. C., and W. F. Ganong 1975 Ultrastructural localization of luteinizing hormone-releasing hormone in the median eminence of the rat. Brain Res., *97:* 181–193.

Gross, D. S. 1976 Distribution of gonadotropin-releasing hormone in the mouse brain as revealed by immunohistochemistry. Endocrinology, *98:* 1408–1417.

Halász, B., B. Kosaras and I. Lengvári 1972 Ontogenesis of the neurovascular link between the hypothalamus and the anterior pituitary in the rat. In: Brain-Endocrine Interaction. Median Eminence: Structure and Function. K. M. Knigge, D. E. Scott and A. Weindl, eds. S. Karger, Basel, pp. 27–34.

Hobson, W., and W. Hansel 1974 Increased *in vitro* pituitary response to LH-RH after *in vivo* estrogen treatment. Proc. Soc. Exp. Biol. Med., *146:* 470–474.

Hyppa, M. 1969 A histochemical study of the primary catecholamines in the hypothalamic neurons of the rat in relation to the ontogenetic and sexual differentiation. Z. Zellforsch., *98:* 550–560.

Jost, A. 1953 Problems of fetal endocrinology: the gonadal and hypophyseal hormones. Rec. Prog. Horm. Res., *8:* 379–413.

——— 1954 Hormones in the development of the foetus. Cold Spring Harb. Symp. Quant. Biol., *19:* 167–181.

——— 1962 Endocrine factors in foetal development. Triangle, *5:* 189–193.

Jost, A., J. P. Dupouy and A. Geloso-Meyer 1970 Hypothalamo-hypophysial relationships in the fetus. In: The Hypothalamus. L. Martini, M. Motta and F. Fraschini, eds. Academic Press, New York, pp. 605–615.

Kobayashi, H., T. Kobayashi, K. Yamamoto, M. Kaibaro and K. Ajika 1968 Electron microscopic observations on the hypothalamo-hypophysial system in rats. IV. Ultrafine structure of the developing median eminence. Endocr. Jap., *15:* 337–363.

Libertun, C., K. J. Cooper, C. P. Fawcett and S. M. McCann 1974 Effects of ovariectomy and steroid treatment on hypophyseal sensitivity to purified LH-releasing factor (LRF), Endocrinology, *94:* 518–525.

Marx, J. L. 1975 Learning and behavior (II): the hypothalamic peptides. Science, *190:* 544–545.

McCann, S. M. 1974 Regulation of the secretion of follicle-stimulating hormone and luteinizing hormone. In: Handbook of Physiology. Vol. 4. Part 2. R. O. Greep and E. B. Astwood, eds. American Physiological Society, Washington, pp. 489–517.

Monroe, B. G., B. L. Newman and S. Schapiro 1972 Ultrastructure of the median eminence of neonatal and adult rats. In: Brain-Endocrine Interaction. Median Eminence: Structure and Function. K. M. Knigge, D. E. Scott and A. Weindl, eds. S. Karger, Basel, pp. 7–26.

Mori, J., H. Nagasawa, R. Yanai and J. Masaki 1974 Changes in serum levels of follicle stimulating hormone and luteinizing hormone shortly before and after parturition in rats. Acta Endocrin., *75:* 491–496.

Moss, R. L., and S. M. McCann 1973 Induction of mating behavior in rats by luteinizing hormone-releasing factor. Science, *181:* 177–179.

Murr, S. M., G. H. Stabenfeldt, G. E. Bradford and I. I. Geschwind 1974 Plasma progesterone during pregnancy in the mouse. Endocrinology, *94:* 1209–1211.

Negro-Vilar, A., M. Sar and J. Meites 1970 Changes in hypothalamic FSH-RF and pituitary FSH during the estrous cycle of rats. Endocrinology, *87:* 1091–1093.

Pelletier, G., F. Labrie, R. Puviani, A. Arimura and A. V. Schally 1974 Immunohistochemical localization of luteinizing hormone-releasing hormone in the rat median eminence. Endocrinology, *95:* 314–317.

Petrali, J. P., D. M. Hinton, G. C. Moriarty and L. A. Sternberger 1974 The unlabeled antibody enzyme method of immunocytochemistry. Quantitative comparison of sensitivities with and without peroxidase-antiperoxidase complex. J. Histochem. Cytochem., *22:* 782–801.

Pfaff, D. W. 1973 Luteinizing hormone-releasing factor potentiates lordosis behavior in hypophysectomized ovariectomized female rats. Science, *182:* 1148–1149.

Ramirez, V. D., R. Abrams and S. M. McCann 1964 Effects of estradiol implants in the hypothalamo-hypophysial region of the rat on secretion of LH. Endocrinology, *75:* 243–248.

Ramirez, V. D., and S. M. McCann 1963 Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology, *72:* 452–464.

Ramirez, V. D., and C. H. Sawyer 1965 Fluctuations in hypothalamic LH-RF (luteinizing hormone-releasing factor) during the rat estrous cycle. Endocrinology, *76:* 282–289.

Schafer, S. J., and W. H. McShan 1974 Gonadotropic hormone release from fetal and adult rat pituitary glands after *in vitro* exposure to synthetic LH-FSH-RH. Neuroendocrinology, *16:* 332–341.

Silverman, A. J., and P. Desnoyers 1975 Postnatal development of the median eminence of the guinea pig. Anat. Rec., *183:* 459–476.

Simpson, R. W., and G. C. Smith 1970 The perinatal development of amine fluorescence in the median eminence of the female rat. J. Anat., *106*: 200.

Smith, G. C. 1970 Ultrastructural studies on the median eminence of neonatal rats. J. Anat., *106*: 200.

Smith, G. C., and R. W. Simpson 1970 Monoamine fluorescence in the median eminence of fetal, neonatal and adult rats. Z. Zellforsch., *104*: 541–556.

Sternberger, L. A., P. H. Hardy, Jr., J. J. Cuculis and H. G. Meyer 1970 The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-horseradish peroxidase) and its use in identification of spirochetes. J. Histochem. Cytochem., *18*: 315–333.

Sternberger, L. A., and J. P. Petrali 1975 Quantitative immunocytochemistry of pituitary receptors for luteinizing hormone-releasing hormone. Cell Tiss. Res., *162*: 141–176.

Taber, C. A., and H. J. Karavolas 1975 Subcellular localization of LH releasing activity in the rat hypothalamus. Endocrinology, *96*: 446–452.

Waynforth, H. B., and D. M. Robertson 1972 Oestradiol content of ovarian venous blood and ovarian tissue in hypophysectomized rats during late pregnancy. J. Endocrin., *54*: 79–85.

Waynforth, H. B., G. S. Pope and Z. D. Hosking 1972 Secretion rates of oestrogens into the ovarian venous blood of pregnant rats. J. Reprod. Fertil., *28*: 191–196.

Yoshinaga, K., R. A. Hawkins and J. F. Stocker 1969 Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. Endocrinology, *85*: 103–112.

Zarrow, M. X., J. E. Philpott and V. H. Denenberg 1968 Postnatal changes in the pituitary-adrenal axis of the rat. Proc. Soc. Exp. Biol. Med., *128*: 269–272.

Abbreviations

C, Capillary
E, Ependyma
EL, External lamina
I, Infundibulum
IL, Internal lamina
ME, Median eminence
NA, Nucleus arcuatus
PC, Portal capillary
PD, Pars distalis
PI, Pars intermedia
PN, Pars nervosa
PT, Pars tuberalis
S, Tuberoinfundibular sulcus
V, Third ventricle

All preparations illustrated were labeled with the PAP immunohistochemical procedure (Sternberger et al., '70) utilizing antiserum No. 173 at a dilution of 1/600.

PLATE 1

EXPLANATION OF FIGURES

Figures 1–4 are transverse sections of the organum vasculosum of the lamina terminalis (OVLT) cephalic to the preoptic recess of the third ventricle. Figures 5–8 are transverse sections of the OVLT just caudal to the cephalic end of the preoptic recess. × 275.

1 Cephalic OVLT, 17 days of gestation. GnRH (black bodies and arrows) is located near the ventral surface of the brain, dorsal and lateral to the blood vessels invading the lamina terminalis.

2 Cephalic OVLT, 19 days of gestation. Several foci of GnRH (arrows) are present dorsal to the ependyma of the cephalic end of the preoptic recess.

3 Cephalic OVLT, newborn. GnRH is not detectable in the cephalic region of the OVLT.

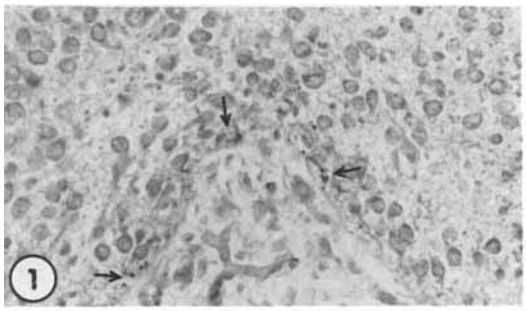
4 Cephalic OVLT, seven days postnatally. Bodies labeled for GnRH (arrows) are concentrated lateral to the central core of blood vessels.

5 Caudal OVLT, 17 days of gestation. Foci of GnRH (arrows) are found ventral and ventrolateral to the preoptic recess of the third ventricle.

6 Caudal OVLT, 19 days of gestation. An accumulation of GnRH (arrows) occurs ventral to the preoptic recess. A slight increase in the content of GnRH has occurred since the seventeenth day of gestation.

7 Caudal OVLT, newborn. GnRH is not detectable in the caudal part of the OVLT of the newborn mouse.

8 Caudal OVLT, seven days postnatally. GnRH is concentrated ventral to the preoptic recess. Note the marked increase in GnRH content of the OVLT that has occurred by this stage in development. The inset shows the accumulation of GnRH surrounding the capillaries in the caudal part of the OVLT. × 600.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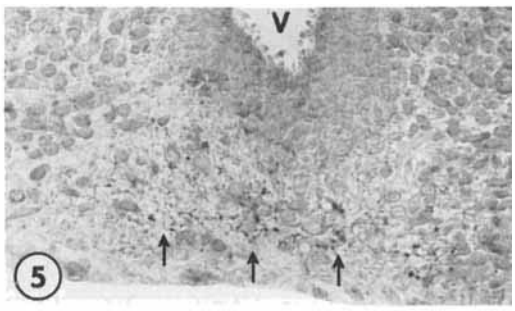
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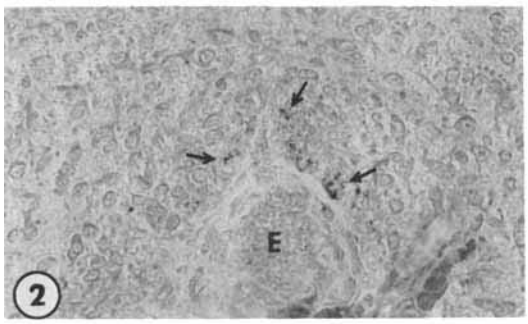
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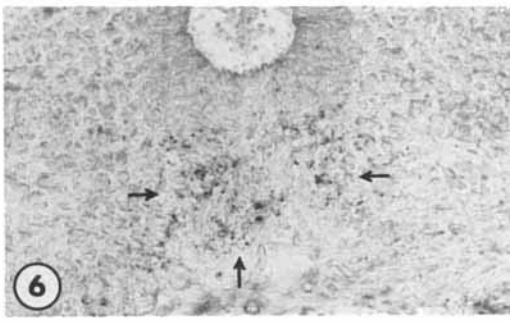
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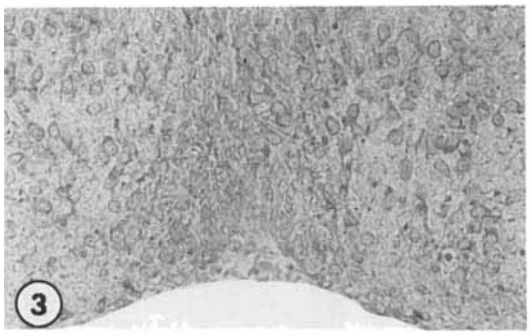
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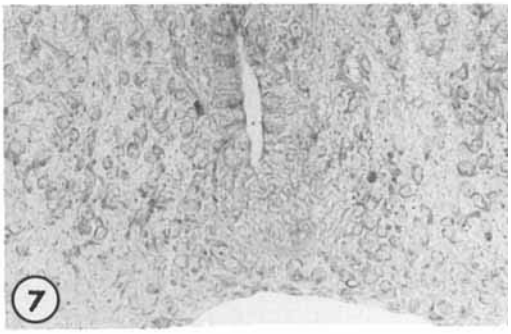
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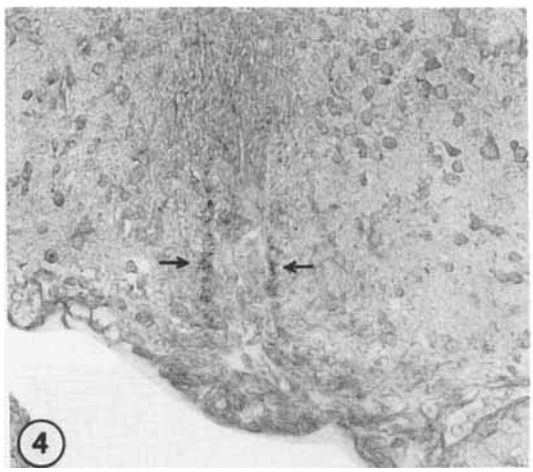
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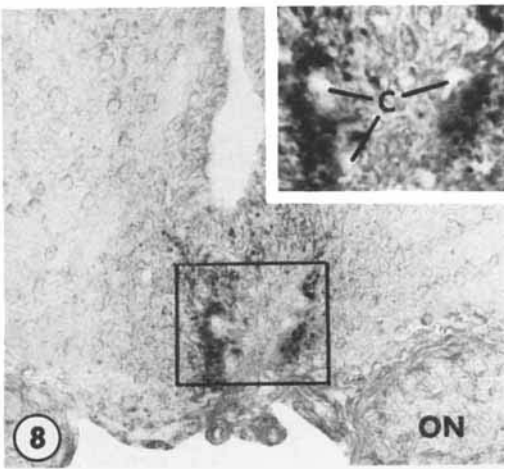
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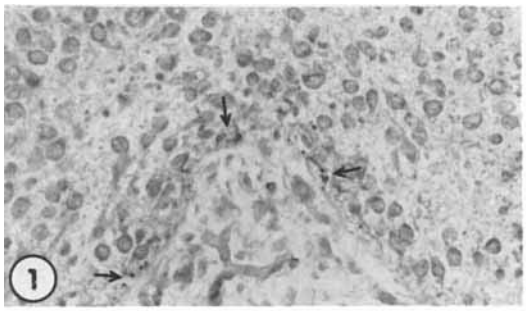
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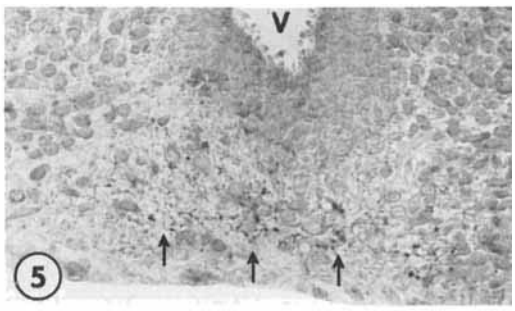
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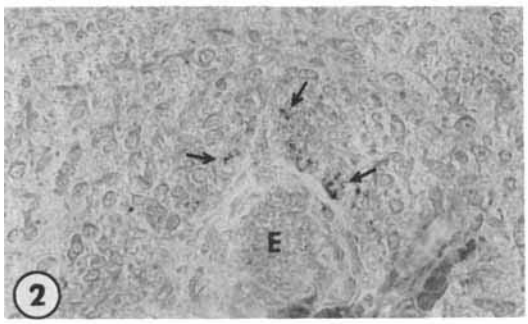
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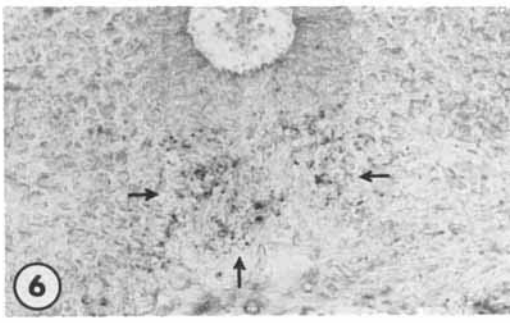
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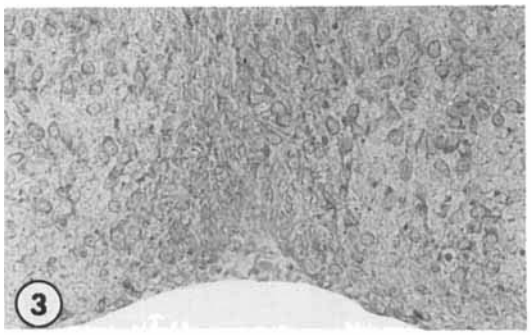
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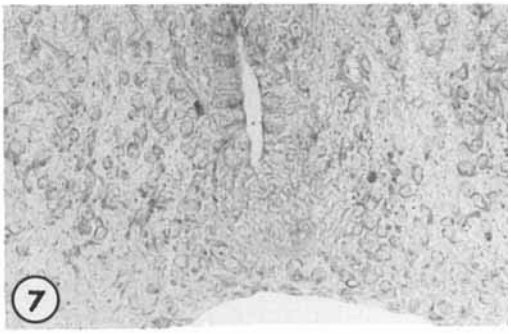
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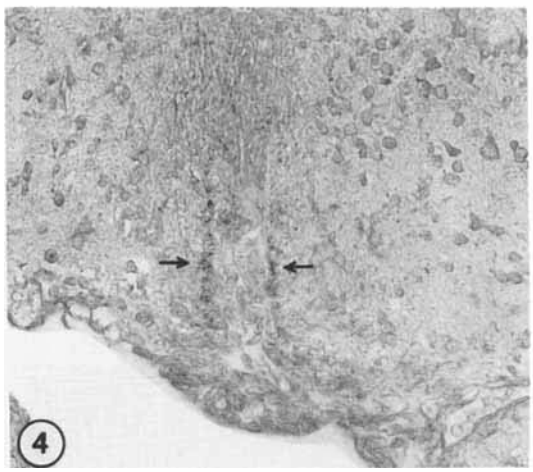
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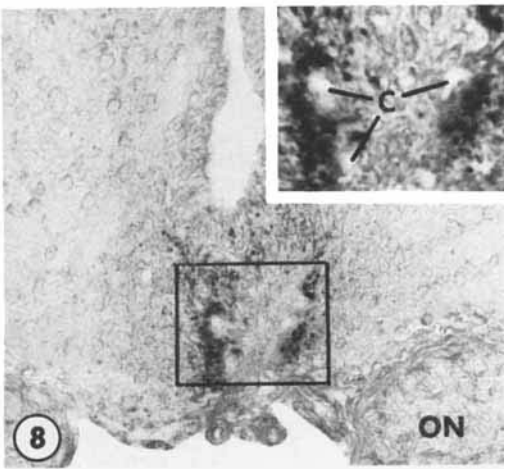
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PLATE 2

EXPLANATION OF FIGURES

9 Median eminence, 15 days of gestation. The median eminence has differentiated and the pars nervosa and pars distalis are well-formed. No GnRH is present at this stage in development. × 140.

Figures 10–14 are transverse sections of the median eminence at 17 days of gestation. × 140.

10 A few foci of GnRH (arrows) are present in the cephalic part of the median eminence and several DAB deposits are located past its lateral boundaries.

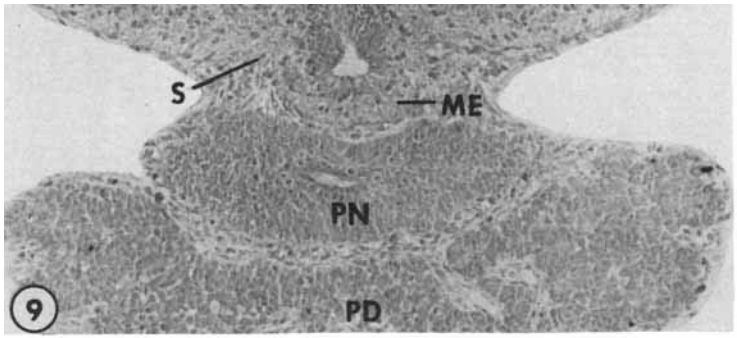
11 The foci of GnRH (arrows) are located near the ventral surface, dorsal and lateral to the shallow tuberoinfundibular sulci.

12 Control section adjacent to that shown in figure 11, labeled with antiserum No. 173 which had been preabsorbed with an excess of synthetic GnRH. Note the absence of bodies labeled for GnRH over the tuberoinfundibular sulci (arrows).

13a In the region of the deep tuberoinfundibular sulci, GnRH (arrows) is found close to the ventral surface of the brain, in bands extending past the lateral limits of the median eminence.

13b The region indicated in figure 13a is shown at high magnification. Note the close relationship of the foci of GnRH (arrows) to the ventral surface, where superficial portal vessels are often located between the median eminence and the pars tuberalis. × 600.

14 Scattered foci of GnRH (arrows) are present in two lateral bands in the region near the separation of the infundibulum from the brain.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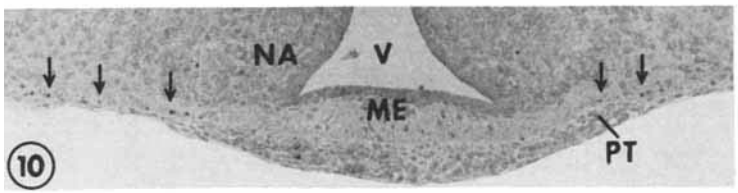
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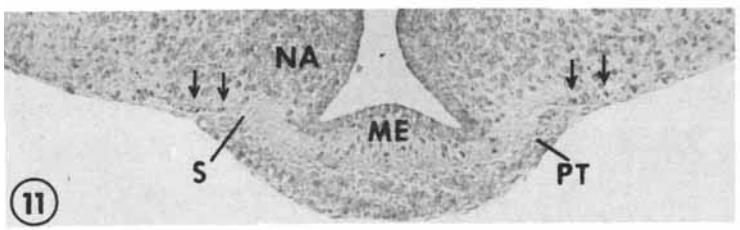
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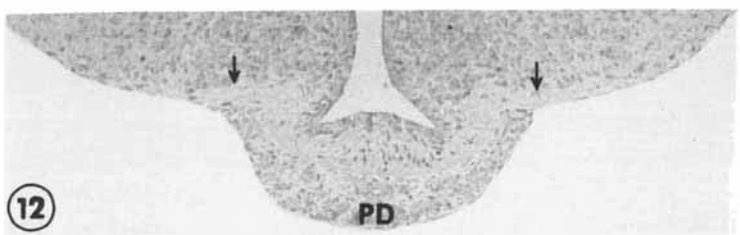
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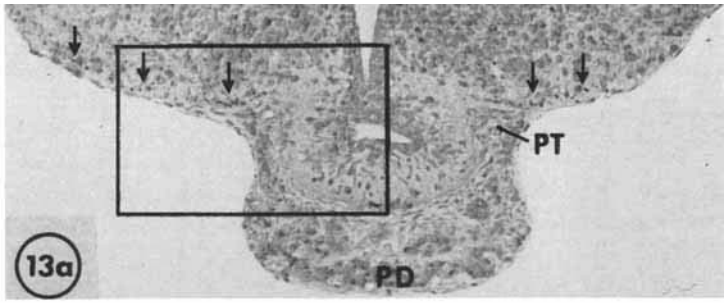
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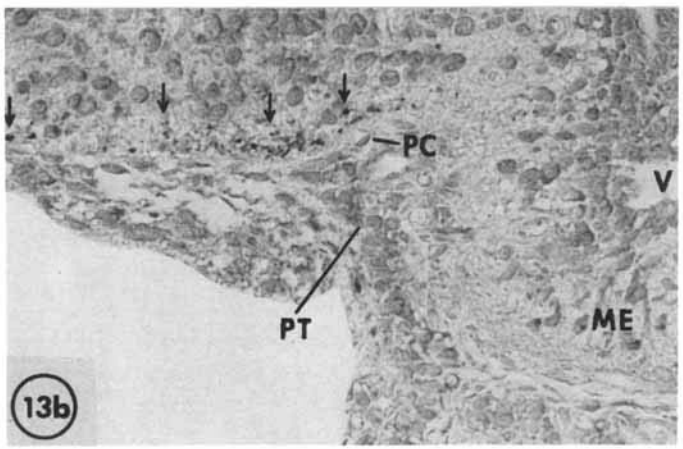
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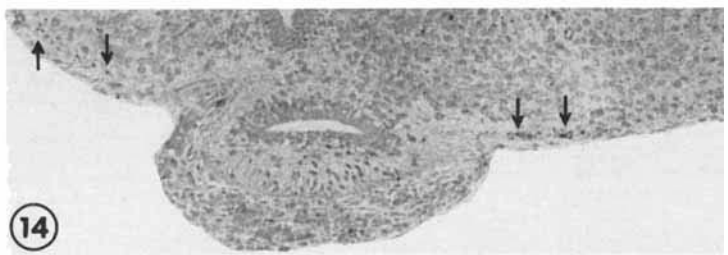
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PLATE 3

EXPLANATION OF FIGURES

15a Sagittal section of the median eminence at 17 days of gestation. GnRH (arrows) is located near the ventral surface in the lateral region of the median eminence. GnRH foci are adjacent to the superficial capillaries of the primary plexus and to the pars tuberalis. Note the well-developed sinusoids in the pars distalis. × 120.

15b High magnification of the region indicated by the center arrow in figure 15a. GnRH-containing deposits are close to the superficial portal vessels of the supratuberal plexus. × 1,000.

Figures 16–19 are transverse sections of the median eminence at 19 days of gestation. × 140.

16 GnRH (arrows) is localized in two small bands in the lateral region of the cephalic part of the median eminence.

17 An accumulation of GnRH occurs in two bundles over the beginning tuberoinfundibular sulci.

18 As the tuberoinfundibular sulci invaginate deeply, GnRH (arrows) is found dorsal to their apices and along their lateral sides.

19a GnRH (arrows) is concentrated in two lateral bands in the postinfundibular median eminence.

19b High magnification of the region outlined in figure 19a, showing the band of GnRH near the ventral surface. Note the increase in content of GnRH which has occurred throughout the median eminence subsequent to 17 days of gestation. × 600.

20 Sagittal section of the median eminence at 19 days of gestation. Several foci of GnRH (arrows) are visible in the cephalic region, while a marked accumulation occurs in the postinfundibular median eminence. × 120.

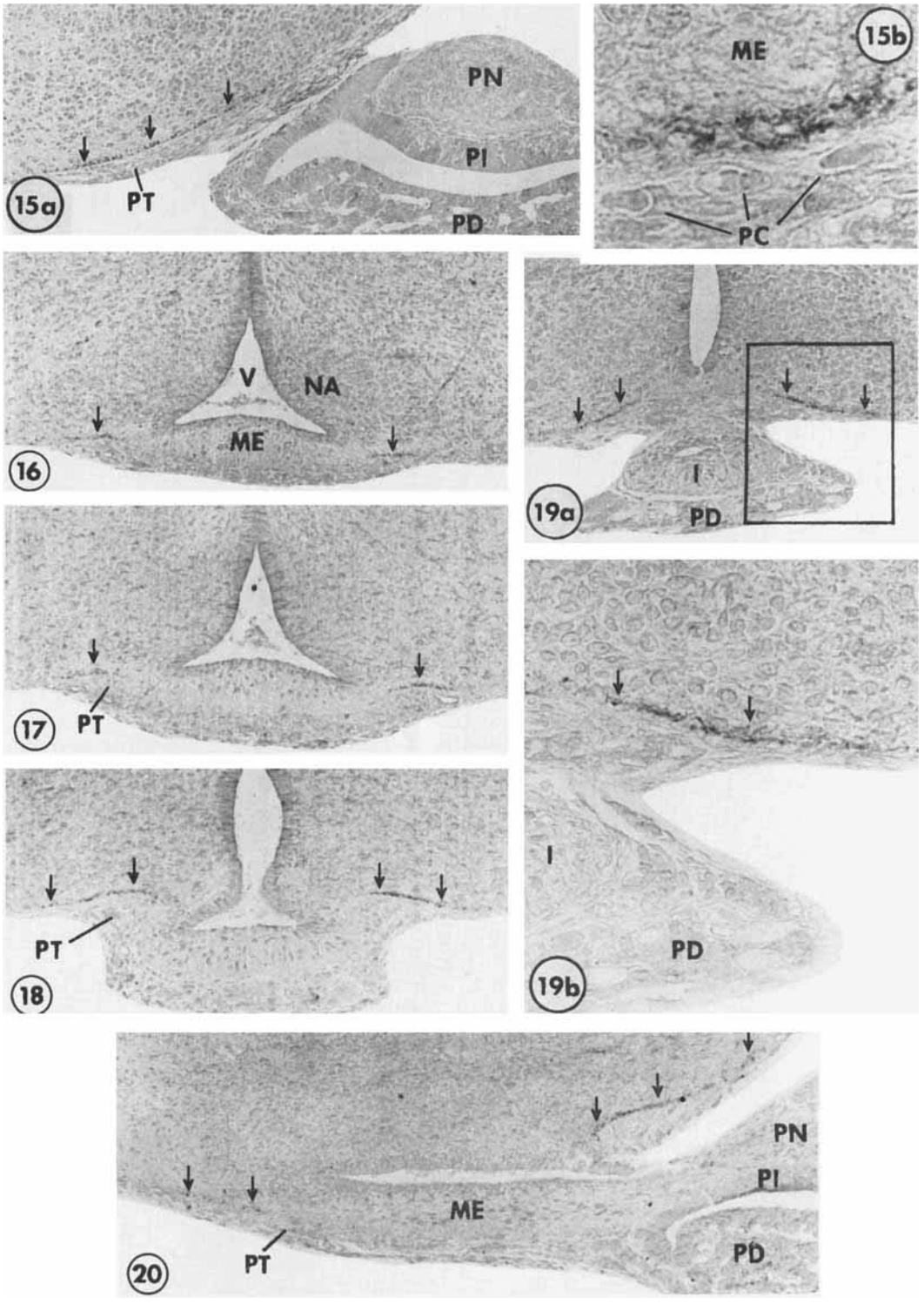
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PLATE 4

EXPLANATION OF FIGURES

Figures 21–24 are transverse sections of the median eminence from a newborn mouse. × 140.

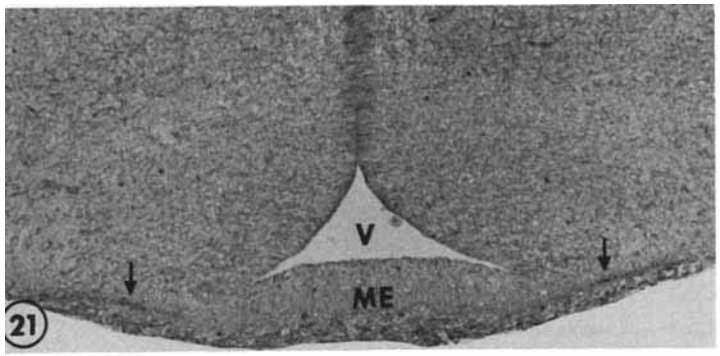
21 Cephalic region of the median eminence. A few light foci of GnRH (arrows) occur laterally.

22 Broad region of the median eminence. GnRH is barely detectable over one tuberoinfundibular sulcus (arrow).

23a GnRH (arrows) is concentrated dorsal to the deepened tuberoinfundibular sulcus.

23b High magnification of the region indicated in figure 23a. Foci of GnRH (arrows) are close to the ventral surface of the median eminence and appear quite diffuse. × 600.

24 Two light bands of GnRH (arrows) are seen in the lateral region of the median eminence near the separation of the infundibulum from the base of the brain. Note the marked decrease in the content of GnRH which has occurred since the end of gestation.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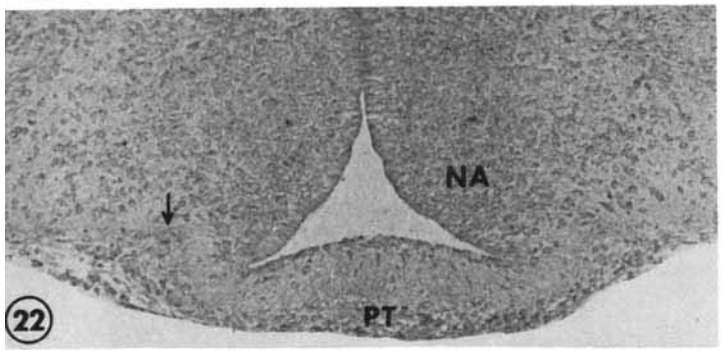
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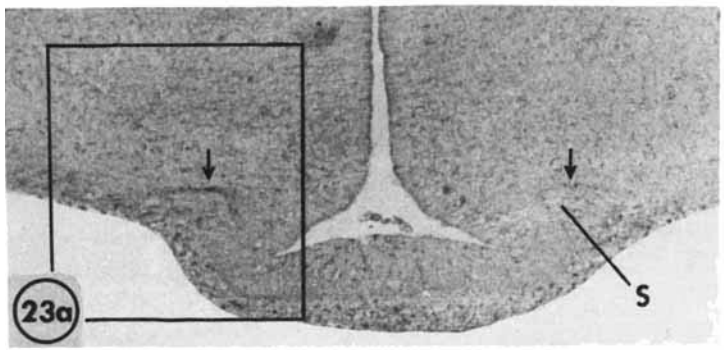
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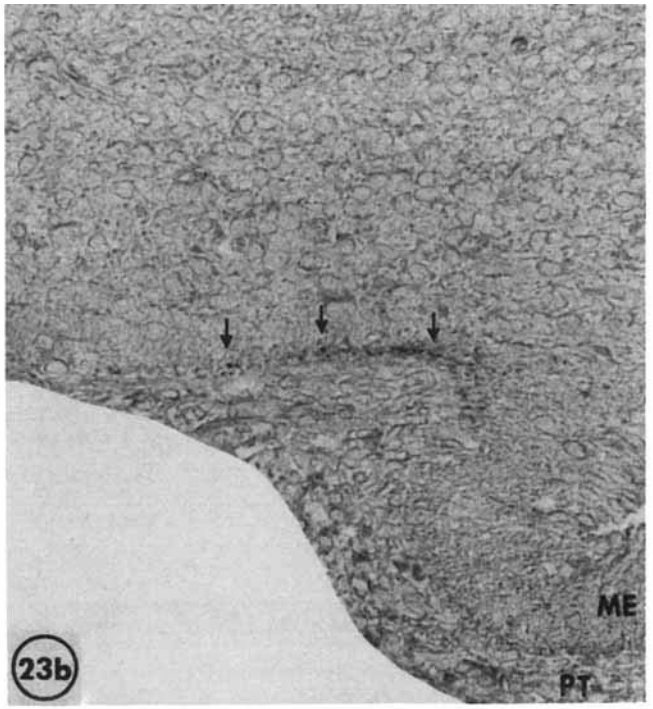
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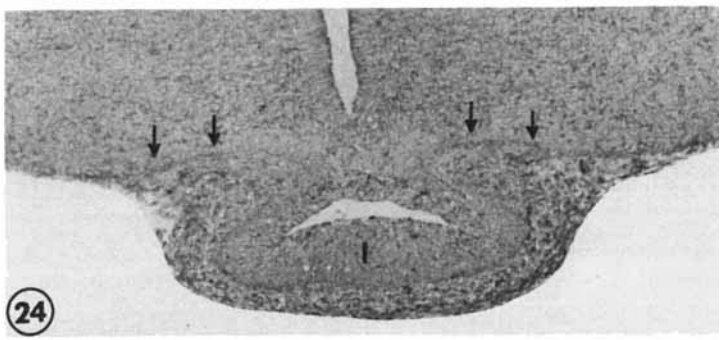
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PLATE 5

EXPLANATION OF FIGURES

Figures 25–28 are transverse sections of the median eminence seven days postnatally. × 140.

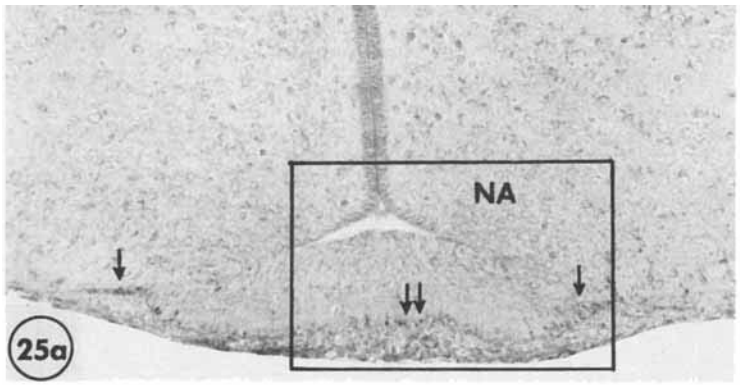
25a GnRH (arrows) is present laterally in the cephalic region of the median eminence. Bodies labeled for GnRH are also present in the medial region, under the infundibular recess (double arrows) for the first time.

25b The beaded appearance of GnRH in the lateral region of the median eminence, as well as its presence near the ventral surface of the broad expanse of the median eminence, are apparent in this high-power view of the area indicated in figure 25a. Note the differentiation of the internal and external laminae. × 600.

26 GnRH (arrows) is concentrated over the apices of the tuberoinfundibular sulci. Several foci extend medially toward the ependyma of the infundibular recess (double arrows), and one beaded process is visible in the external lamina.

27 In the region of the deep tuberoinfundibular sulci, GnRH (arrows) is located over the apices of the sulci and along their lateral sides.

28 In the postinfundibular median eminence, GnRH (arrows) is concentrated in two dense narrow bands, which are conspicuous quite far laterally. Also, foci appear in the midline under the infundibular recess for the first time. The inset shows the indicated area at high magnification. The foci of GnRH (arrows) are beginning to acquire the vesicular appearance characteristic of the adult.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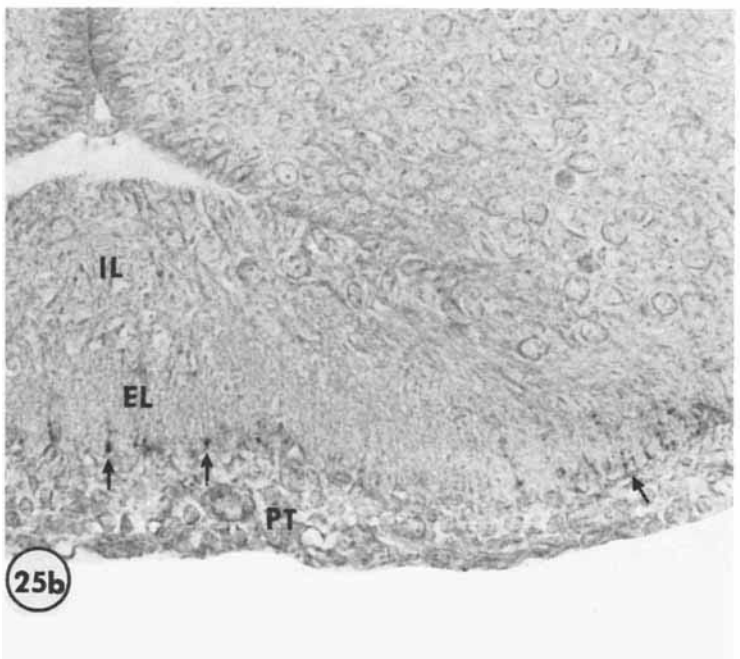
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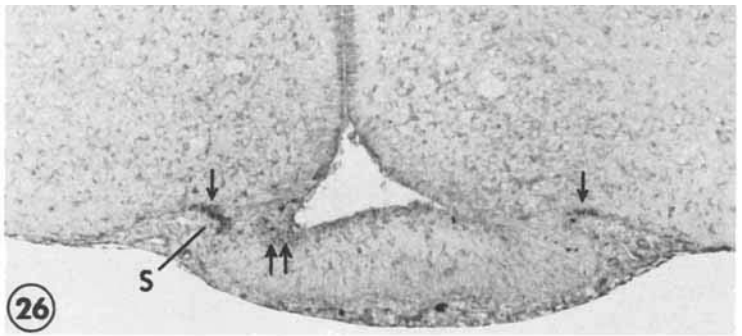
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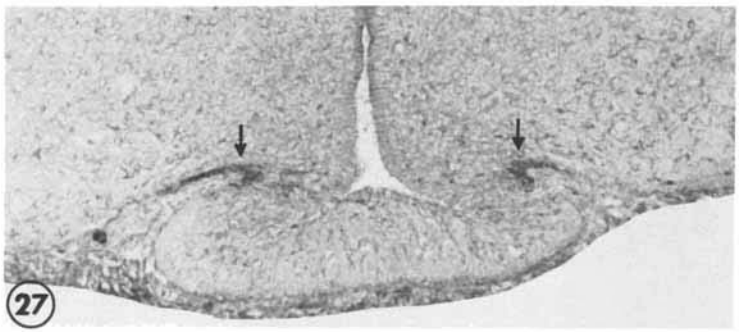
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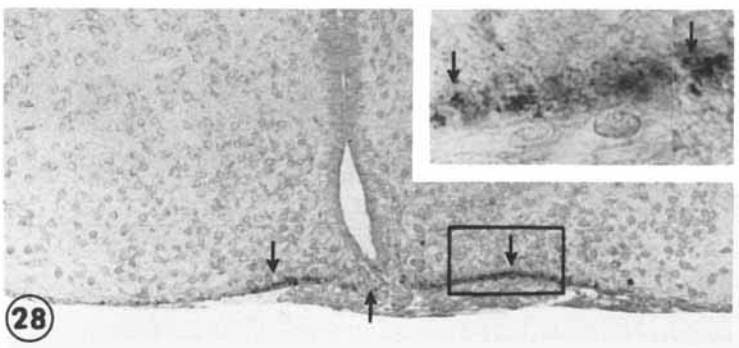
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