The Embryological Development and Cytodifferentiation of the Pars Distalis of the Golden Hamster (Mesocricetus Auratus) With Special Reference to the Localization and Secretion of Prolactin.

Sue Ann Thompson
Louisiana State University and Agricultural & Mechanical College

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THE GOLDEN HAMSTER (MESOCRICETUS AURATUS)
WITH SPECIAL REFERENCE TO THE LOCALIZATION
AND SECRETION OF PROLACTIN.

The Louisiana State University and Agricultural
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Physiology

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THE EMBRYOLOGICAL DEVELOPMENT AND CYTODIFFERENTIATION OF THE
PARS DISTALIS OF THE GOLDEN HAMSTER (MESOCRICETUS AURATUS)
WITH SPECIAL REFERENCE TO THE LOCALIZATION
AND SECRETION OF PROLACTIN

A Dissertation
Submitted to the Graduate Faculty of the
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in
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by
Sue Ann Thompson
B.S., University of Alabama, 1959
M.S., Louisiana State University Medical Center, 1971
August, 1975
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ABSTRACT

An interest in the possible functional roles of prolactin in fetal and neonatal animals has provoked these studies on the cytodifferentiation and localization of the prolactin cell and the determination of fetal and neonatal immunosassayable prolactin serum levels in the golden hamster.

The embryological development and cytodifferentiation of the pars distalis was described to obtain basic information not already available in the literature. The normal chronological events in the development of the hamster pars distalis closely follows the pituitary organogenesis of other laboratory rodents. Rathke's pouch is formed and touches the infundibulum at 8½ days gestation and is closed off from the stomodeum three days later. Penetration into the pars distalis of vascular elements of the developing hypophysial portal system occurs at 12½ days gestation which is the first day small secretory granules are seen in any cells. Further cytodifferentiation during the following prenatal and first few postnatal days of life reveal granulated cells which cannot be identified using morphological criteria or granule size as is done in the adult.

Using the immunoperoxidase technique, a few prolactin cells were localized in the pars distalis at 13½-15½ days gestation, and a dramatic increase in number of positive cells was demonstrated immediately after birth.
Concomitantly, prolactin was measured in fetal and neonatal serum using RIA. Since levels at 13½ days gestation were higher than expected from information based on the small number of immunoreactive cells, and since the fetal levels so accurately reflected maternal levels, studies were undertaken to determine whether fetal prolactin levels could have a maternal source. Radioactive prolactin, injected into maternal circulation, was shown to cross the placenta and enter fetal circulation.

Thus, prolactin cells have been demonstrated and the extracellular hormone has been measured in fetal and neonatal hamsters. It is probable that most of the prenatal hormone is furnished by the mother, whereas after birth, the dramatic increase in the number of peroxidase-positive cells indicate that the serum levels are now a product of the neonatal pituitary cells.

The unquestionable presence of prolactin in the hamster fetus and neonate strongly suggests that this hormone plays an important, though yet undetermined, role in the developing animal.
INTRODUCTION

The pituitary gland has been the object of intensive study and widespread interest for many years. In particular, the pars distalis is of special note because of the six important hormones synthesized and secreted there. Thyrotropin (TSH) acts on the thyroid gland to stimulate the secretion of thyroxine and triiodothyronine. Adrenocorticotropic (ACTH) serves to maintain the adrenal cortex. Somatotropin (STH) is the hormone associated with growth and maturation of the organism as well as acting synergistically with many other hormones to complement or enhance their function. Follicle stimulating hormone (FSH) promotes gamete maturation. Luteinizing hormone (LH) stimulates production of androgen by the interstitial cells of the testes and stimulates the cells of the ovary which secrete progesterone and those which secrete estrogen. Prolactin (luteotropin, lactotropin, mammotropin hormone, LTH) is a multifunctional hormone playing a widely varied physiological role in vertebrates. In mammals, prolactin is mainly concerned with stimulating formation of milk, reproduction and parental care, and general growth and metabolism.

Originally, histological examination of the anterior pituitary using hematoxylin and eosin (H & E) or special trichrome stains, demonstrated the three classic cell types: acidophils, basophils, and chromophobes. Dawson and Friedgood (1938) used Heidenhain's azan modification of Mallory's trichrome connective tissue stain to
differentiate two classes of acidophils. Since that time, with the advent of more sophisticated techniques and more reliable and reproducible staining procedures, an attempt has been made, with some success, to assign tinctorial affinities and physiological responses to a specific cell type. Subsequently, the concept developed that one particular morphological cell type is responsible for the elaboration and secretion of a single hormone (Romeis, 1940; Herlant, 1964). Although there is still some confusion in the terminology and discrepancies in reference to correlating greek letter nomenclature with color affinity, it is now somewhat obsolete to refer to a pituitary cell by any name other than that which relates to its function.

Electron microscopy has been a valuable tool in clarifying some of the earlier confusion in the naming of pituitary cells by revealing morphologically distinct cell types which could consistently be related to a particular physiological function under certain experimental conditions.

Pituitary hormones are produced in the endoplasmic reticulum and packaged in the Golgi. A membrane bound granule of stored hormone is formed which has a characteristic size, shape and density in the adult animal. Costoff and McShan (1969) isolated these granules from the rat anterior pituitary, separated them by differential centrifugation, then stained and measured the granules in the different gradients of the pellet. The fractions were also assayed for specific hormone activity. Their statistical values for the granule size obtained for each cell type have enabled many
Investigators to use this morphological characteristic to further identify and describe other fine structural landmarks related to each cell.

The successful use of immunocytochemical methods, first demonstrated by Coons (1958) with fluorescein-labeled antibodies, was a step in the development of a technique of great importance in the further elucidation of the pituitary hormone picture. Later, the immunoferritin method (Singer and Schick, 1961) which can be used at the electron microscope level, and the immunoperoxidase technique (Nakane and Pierce, 1966, 1967) which can be used with both the electron microscope and light microscope were developed to better localize and identify tissue antigens. The peroxidase-labeled antibody technique, in conjunction with various experimental manipulations, has made it possible to localize and correlate, with even more certainty, the precise hormone being produced by a given cell type.

Although there is a wealth of information now accumulating concerning the cells of the adult mammalian adenohypophysis, little has been done, with a few exceptions, to bring up to date the embryological development and cytodifferentiation of this organ.

In the late 1930's and 1940's, much of the original histological descriptions of pituitary gland development were made thoroughly and accurately, but were hampered by the limitations of the techniques available to investigators at the time. This was also true of studies on the possible role played by fetal hormone
secretions. Electron microscopy, immunocytochemistry, and radioimmunoassay now offer tools for broader, more concise measurements of physiological and morphological parameters involved in the induction and cytodifferentiation of the mammalian pituitary and the determination of hormone levels.

These three techniques have been extensively used in this study to investigate the embryological and cytological differentiation of the hamster pars distalis, especially with reference to the prolactin hormone producing cell, its maturation and onset of secretion. Because each of these investigations constitutes a different aspect of the overall problem, the three studies are presented in separate chapters, as follows:

Chapter 1. The embryological development and cytodifferentiation of the golden hamster, Mesocricetus auratus. A light and electron microscope study. (To be submitted to the American Journal of Anatomy)

Chapter 2. The immunohistochemical localization of the prolactin cell in embryonic and neonatal hamsters. (To be submitted to Cell and Tissue Research)

Chapter 3. The determination of prolactin serum levels of fetal and neonatal hamsters and its relationship to maternal levels. (Has been accepted by The Society for Experimental Biology and Medicine)
Chapter 1

THE EMBRYOLOGICAL DEVELOPMENT AND CYTODIFFERENTIATION

OF THE GOLDEN HAMSTER, MESOCRICETUS AURATUS

A LIGHT AND ELECTRON MICROSCOPE STUDY

The fine structure of the developing pars distalis has been described for only a few mammals; humans (Andersen, 1971), the rabbit (Schecter, 1970, 1971), the mouse (Sano and Sasaki, 1969), and the rat (Fink and Smith, 1971; Daikoku et al., 1973; Dupouy and Magre, 1973).

The golden hamster, Mesocricetus auratus, a well established laboratory mammal, has been sadly neglected in the area of embryology, especially with respect to the cytodifferentiation of the pituitary gland. This is a noticeable oversight especially in light of its extensive use in the area of reproductive physiology. It has the shortest reproductive cycle of any mammal known, completing the estrous cycle every four days and having a gestation time of only 16 days.

The anatomy and cytology of the adult hamster anterior pituitary was first described by Hanke and Charipper (1948), while more recent light microscopy studies of the adult gland include reports by Thompson (1960), Girod and Cure (1965), Reiter and Hoffman (1968), and Avery and Stahl (1969). The fine structure of the various morphological cell types in the adult hamster
adenohypophysis have been described by Girod et al. (1964), and more thoroughly identified and characterized by Dekker (1967, 1968).

The first nine days of embryological development in the hamster has been described by Graves (1945), while McLennen (1974) recently focused on the process of implantation with emphasis on the ultrastructural aspects of this stage of development.

Only one study has been made concerning the further developmental sequences of the pituitary; the stages in morphogenesis are briefly described by Boyer (1968) in his chapter on the embryology of the hamster. Using alcian blue stain and dichloroiodosocyanin histochemical reaction, Klessen (1971) has followed the appearance and development of function of the thyrotropin secreting cells.

Although Krol (1949) has described the development and cytodifferentiation of the hamster pituitary in abstract form, there is, to date, no thorough treatment of the developing hamster pituitary and no study of cytodifferentiation of the pars distalis at the ultrastructural level. The present study was undertaken to fill this gap and to provide information to use as a basis for comparison with the developing pituitary of other animals. Hopefully, greater insight into the inductive processes involved in pituitary organogenesis and cytodifferentiation will be gained.
MATERIALS AND METHODS

Animals

Adult, cyclic female hamsters were mated at approximately 2100 hr. on the evening of estrous. A male was placed in the cage, and when the female was determined to be receptive and when copulation was occurring, the animals were left undisturbed for a period of 1-2 hr, after which time the male was removed. This was determined to be "zero" time of pregnancy. All animals were sacrificed, and tissue samples were collected between 0900-1100 hr. The various gestation and newborn ages are expressed as the day \( \frac{1}{2} \). Thus, animals born in the evening at the beginning of the 16th day after mating were designated as \( \frac{1}{2} \) day old when sacrificed at 0900 hr the next morning.

Light Microscopy

Pregnant female hamsters were sacrificed by decapitation. Fetuses were immediately removed from the uterus through a single longitudinal incision and the entire head removed and placed in Bouin's fixative for 24 to 48 hr. The entire fetus was fixed at 7½-10½ days gestation.

All tissue was dehydrated in a graded series of alcohol, cleared in benzene, and embedded in Paraplast. Serial sections were cut at 7 \( \mu \) and stained with Delafleld's hematoxylin and eosin. Observations were made with a Leitz Ortholux II microscope and photographs were taken with a Leica camera attachment. Studies were made each day, from 7½ days gestation through 3½ days after birth,
using both sagittal and frontal sections from several animals of at least two litters.

**Electron Microscopy**

Animals were sacrificed and fetuses removed as described above. Neonatal hamsters were also sacrificed by decapitation. Tissue was fixed for 3 hr in 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.3), containing 2% sucrose and 1 mM CaCl$_2$. Tissue was washed overnight in buffer, rapidly dehydrated in ethanol, and embedded in Spurr epoxy resin (1969). The entire head from each fetus at 9½ and 10½ days of development was fixed and embedded, while the pituitaries of older animals (11½ days gestation to 3½ days newborn) were removed in a small block of surrounding tissue to insure adequate fixation and infiltration.

Sections were cut on a Sorvall MT-2B ultramicrotome using glass or diamond knives. The pituitary was located in the block by taking 4 μm sections with a glass knife and monitoring the unstained sections mounted on glass slides. When an appropriate area of the pituitary was located, a 1 μm section was stained with methylene blue-azure II and kept as a reference to denote the area from which thin sections were taken. Silver sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and viewed with an Hitachi 11A electron microscope.

**Observations**

The developing hamster pituitary is described beginning at 8½ days gestation until 3½ days after birth, with particular emphasis
on the cytodifferentiation of the pars distalis.

The hamster embryo is not fully implanted until the 6th day of gestation, but from that time until birth, development proceeds at an extremely rapid rate. At 7½ days gestation, there is no evidence of the anterior pituitary anlage, as the embryo is in the last presomite stage of development (Fig. 1). The primitive streak, with only a slight headfold, is apparent (Fig. 1C). In contrast, only 24 hrs later, Rathke's pouch has formed and is already in contact with the infundibulum (Fig. 2). There is a very close association between the cells from the neural and ectodermal components of the developing pituitary. The pouch is wide open to the stomodeum and many cells in mitosis are evident, especially on the neural side.

Rathke's pouch begins to form a pocket by 9½ days of gestation. It retains its vertical orientation, perpendicular to the stomodeum, and is still wide open at the base (Fig. 3). Cells of the pocket have begun to proliferate although the marginal layer, near the stomodeum, is still only a few cells high (Fig. 4). Cells, arranged in palisade fashion, appear totally undifferentiated with finely granular nuclei and little heterochromatin (Fig. 5). A small amount of rough endoplasmic reticulum (RER), many free ribosomes, a few mitochondria and Golgi are evident. The manner in which the points of cytoplasm are thrown up toward each other and touch across wide intercellular spaces, give the cell a scalloped appearance. Cells at the apex of the pouch have a very close association with the cells of the neural tube and some even appear to be crossing over (Fig. 6A).
Cells at the lumenal border of the pocket have no well developed tight junctions (Fig. 4).

By 10½ days of fetal life, the anlage of the pars tuberalis is beginning to form as an anterior projection delineated from the future pars distalis by Atwell's recess (Fig. 7A). External components of the blood vascular system are apparent; however, no internal vascularization is seen (Fig. 7). Many mitotic figures are evident in the developing adenohypophysis (Fig. 8). There is a proliferation of cells in the anterior wall of Rathke's pocket, near the base, forming a globular mound which protrudes into the lumen giving it a slightly inverted S shape (Fig. 8). This mass of cells is the primary component of the future pars distalis. The developing gland is still vertical and open to the stomodeum by a narrow canal only a few μm in diameter (Fig. 9, insert). The cells are still widely spaced with few intercellular contacts (Fig. 10). An amorphous ground substance, containing a few mesenchymal cells, fills the space which separates the brain and pituitary (Fig. 6B). One can see pseudopodial-like evaginations from cells of both sides protruding into this area (Fig. 9). Cells in the presumptive pars distalis are more rounded than the columnar marginal cells. They have the characteristic scalloped appearance and numerous mitotic figures are seen. Cytoplasmic organelles are unchanged in appearance from the preceding day (Figs. 10 & 11).

Rathke's pouch is closed at 11½ days of gestation, and the pocket has begun to rotate into a more horizontal position (Fig. 12A), although the gland is still connected to the stomodeum.
by a slender cord of cells (Fig. 12B). The pars nervosa is cellular, with an open infundibular recess (Fig. 12B). Blood vessels of the circle of Willis are evident (Fig. 12C), but there are still no internal vascular components. The marginal cells of the pocket now show well developed tight junctions and have filamentous projections into the lumen (Fig. 13). The palisade arrangement of these cells with point to point contact is still apparent; however, they have more Golgi, more REK, and many rosettes of ribosomes (Fig. 14). In the pars distalis anlage, cells are in closer contact with each other, more rounded, and some have a considerable amount of glycogen (Fig. 15).

Rathke's pocket becomes even more horizontal at 12½ days gestation (Fig. 16). The pars distalis is being invaded by mesenchymal components from the area of Atwell's recess and vascularization of the adenohypophysis is well underway (Fig. 16 & 17). Many more Golgi are evident in the cells of the pars distalis and secretory granules are just beginning to appear (Fig. 18). At the periphery of the pars distalis, cells are still in loose contact along the basal lamina. They have few organelles, and a few small granules are present (Fig. 19). Cells in the process of dividing were occasionally seen to contain granules (Fig. 20). The basisphenoid cartilage begins forming from condensations of mesenchyme (Fig. 16).

The pituitary at 13½ days gestation is still connected to the stomodeum by a thin cord of cells extending through the developing basisphenoid cartilage (Fig. 21A). Rathke's pocket is horizontal and
the marginal cells are still columnar (Fig. 21B). Vascularization has increased, and the pars tuberalis extends almost completely around the median eminence. Secretory granules are evident in the cytoplasm of some cells (Fig. 22), although the majority of cells are still chromophobes. The size of the granules is highly variable between cells as well as within cells (Fig. 22 & 23). Many more mitochondria are present, a necessary prerequisite for energy for protein synthesis.

At 14½ days gestation, the pars distalis is highly vascularized and the cells are becoming arranged in the typical anastomosing cords seen in the adult gland (Fig. 24). Rathke's pocket is lined with a single row of low columnar cells (Fig. 24A). A higher percentage of granulated cells is seen, and an unusual cell type appears which has secretory granules much larger than any seen in adult cells. They have a coarsely granular substructure (Fig. 25) and stain more lightly than other, mature granules. Other cells appear well differentiated and have dense granules which lie in close apposition to the cell membrane as though secretion could be taking place (Fig. 26).

The general morphology and histological appearance of the fetal pituitary at 15½ days gestation, is very much like that of the adult gland. The pars nervosa has taken on the appearance of neural tissue (Fig. 27A), and the cells lining Rathke's pocket are more flattened (Fig. 27B). Sinusoids are now well developed and one can see endothelial cells lining the vascular spaces (Fig. 27B). Elements of the developing portal system are evident around the pars tuberalis
in the connective tissue investment. The basisphenoid cartilage has begun to ossify (Fig. 27A). There are still many more chromophobes than granulated cells at this stage. Of those cells with granules, there are several types. Some contain a few small granules (Fig. 28), while other have many relatively large granules, but less electron dense than any seen in adult cells (Fig. 29). Some cells contain a large number of mitochondria and glycogen stores, but no secretory granules (Fig. 29). In contrast, there are a few cells with a very mature cytological appearance. They have well developed Golgi, many mitochondria, and large, dense secretory granules, some of which appear to be fusing with the cell membrane for possible exocytosis (Fig. 30).

For the first few days after parturition, there is no noticeable difference in the morphology or histology of the gland. A wide variety of cell types are seen at the electron microscope level. Some of these cells are obviously mature, with condensed granules ready for export (Fig. 31), while others appear still undifferentiated. Occasionally, secretory granules are seen forming in the Golgi region which are somewhat larger and less dense than the more mature granules at the cell membrane (Fig. 32). There are numerous cells with extremely large secretory granules which do not correspond in size or appearance to any seen in adult cells. Within these same cells are smaller, denser granules which may represent a mature product (Fig. 33). Many cells contain large deposits of glycogen (Fig. 34 & 35).

By 3½ days after birth, the cells of the neonate generally
have a more adult appearance (Figs. 36 & 37).

For comparison with the fetal and newborn pituitary cells, the different cell types of the adult pars distalis are illustrated in Figure 38.

The TSH cell (Fig. 38A) is polygonal with sparse endoplasmic reticulum and very small secretory granules, measuring 80-150 nm, which have a dense core and slight halo. The endoplasmic reticulum is smooth and poorly developed.

Granules of the ACTH cell (Fig. 38B) range in size from 100-200 nm in diameter. They are located at the poles of the cells and along the periphery beneath the plasma membrane. This cell is irregular in shape and has few mitochondria.

The FSH cell (Fig. 38D) is oval to round with an eccentric nucleus, and is always in association with vascular spaces. The granules are 100-250 nm in diameter and are less opaque than those of other granulated cells. The endoplasmic reticulum often appears vacuolated.

The cytoplasm of the LH cell (Figs. 38D, E & F) often has a characteristic, peculiar filigreed pattern of endoplasmic reticulum. The highly lobulated nucleus is smaller than that of the FSH cell. The granules are about the same average size as those of the FSH cell but have a wider range of measurements and are more uniformly dense. The Golgi is flattened and parallel to the outline of the nucleus.

The most common cell of the pars distalis is the STH cell (Fig. 38F). It has abundant spherical granules ranging in size from 300-500 nm in diameter. The endoplasmic reticulum may appear as
parallel waves in the actively secreting cell.

The prolactin cell (Figs. 38C & E) is relatively small and irregular but has the largest granules of any pituitary cell. They measure 350-800 nm and may be round, oval, or quite pleomorphic. During pregnancy, these cells proliferate and become conspicuous by their enormous amounts of dilated RER arranged in rows and whorls. The Golgi is hypertrophied, and there are many free ribosomes.

Chromophobes are identified by the absence of any secretory granules (Fig. 38C).

Although the cells of the fetal and neonatal pars distalis cannot be positively identified on a morphological basis, some cells, with a more mature appearance than others, can be tentatively identified using the criteria established for adult hamster pituitary cells.

A presumptive FSH cell, with granules measuring 200-250 nm, can be seen in close association with a sinusoidal space (Fig. 23). Cells with the morphological characteristics of adult prolactin cells with typical secretory granules measuring 450-500 nm, are illustrated in Figures 26, 30, and 31A and B. Many cells are present with very tiny granules. These could be TSH cells (Fig. 28), but the granules are not condensed and therefore, identification is uncertain. Cells with the appropriate morphology of STH cells are seen in Figures 32 and 35, and a cell with small granules lying closely beneath the plasma membrane is tentatively identified as an ACTH secreting cell (Figs. 32 & 36). However, there are many more mitochondria in these newborn animal pars distalis cells than are seen in adult ACTH cells.
DISCUSSION

The development of the hamster pituitary is described at 24 hour intervals, from the formation of Rathke's pouch at \( 8\frac{1}{2} \) days gestation, until \( 3\frac{1}{2} \) days after birth. Nidation is not completed until the 6th day of gestation, after which development proceeds at an unparalleled rate for the remainder of the very short, 16 day, gestation period. All hamster embryos in a litter are remarkably alike at any particular age of development (Boyer, 1968), but care was taken to select representative animals and to discard smaller or other obviously different fetuses before studies were initiated. As a reference point, the first embryos were taken at \( 7\frac{1}{2} \) days gestation and compared to a previous study for the hamster at this same age (Graves, 1945). The morphology and overall development of the embryo in this study was identical to established reports.

The sequence of events in the development of the hamster pituitary is similar to other laboratory rodents studied, but the rate of development is much faster. In the hamster, Rathke's pouch is formed as an evagination from the stomodeum at \( 8\frac{1}{2} \) days gestation, although it is not organized until 10 days gestation in the rabbit, or 12 days gestation in the mouse. Pouch closure occurs at \( 11\frac{1}{2} \) days gestation in the hamster, at 13 days in the mouse, 14 days in the rat, but is not mentioned in recent studies on the rabbit. The invasion of blood vascular elements from the vascular plexus via Atwell's recess into the body of the pars distalis takes place at \( 12\frac{1}{2} \) days gestation in the hamster and at 16 days gestation in the
rat, rabbit, and mouse. Vascularization is an important event coincident to the appearance of secretory granules in the case of each animal.

An interesting feature of the early hamster pituitary development is the extremely close association of the adenohypophysial anlage with the floor of the infundibulum during the first few days of its formation. In the rabbit, cell processes are seen extending from the neural tube contacting cells of Rathke's pouch (Schecter, 1970). This contact may be important as a site for the exchange of materials which may stimulate organogenesis in the developing pituitary, or as a method for the orientation of the rapidly growing cells in their environment (Schecter, 1970). Embryonic fibroblasts, seen in the ground substance around the outside edges of the growing pituitary anlage (Figs. 19 & 22), might also be suspected of having a similar inductive function. These cells have processes that reach out toward, and almost seem to support areas of the pituitary basal lamina. This interaction which appears to be taking place, may play some role in directing cell movement and in the organization of the pituitary as it differentiates.

In reports on the cytodifferentiation of the pars distalis of various mammals, an attempt has been made to identify and relate a certain cell type in the fetal pituitary to a functional cell type in the adult pituitary. Using criteria based on granule size determined for adult pituitary cells, Dupouy and Magre (1974) describe five cell types in the fetal rat pituitary. They gave
functional significance to ACTH and TSH cells based on the morphological changes seen after injection of drugs known to affect these cells. Other authors have reported various cell types in the fetus based only on granule size and morphology and have assigned a function to these cells. But little correlation has been demonstrated between bioassayable hormone levels and the presence or absence of a distinctive pituitary cell type in the fetus. STH cells are reported to be absent in the fetal rat pituitary (Pink and Smith, 1971), but are reported to be present in the fetal mouse pituitary (Sano and Sasaki, 1969). LTH and ACTH cells are reported to be absent in the fetal mouse (Sano and Sasaki, 1969), but another study describes the presence and secretion of LTH cells in the fetal mouse pituitary (Kohmoto, 1971).

In the hamster, the first granules appear at 12½ days gestation. These granules are very small and the cells for the most part are still morphologically undifferentiated. From 13½ days on, more cells of the pituitary become mature in appearance, but there is still a wide variation in the size of granules in the cells. There is a distinct cell type which appears late in embryonic development, 14½ days gestation, with extremely large granules, larger than any seen in the adult, but it also contains some small condensed granules. Another cell type is present having many intermediate size granules which stain lighter than the granules in a comparable cell of the adult. With few exceptions, it is impossible to use morphological criteria alone to ascribe function to the cells in the fetal or neonatal pars distalis.
Using Dekker's criteria of granule size and cell morphology for the identification of pituitary cells of the adult hamster, it is possible to tentatively identify a few cells of the fetal and newborn hamster which are morphologically mature enough in appearance. Using immunocytochemical procedures, prolactin cells have been localized in fetal hamster pars distalis at 13½ days gestation (Thompson and Trimble, unpublished), and Sétáló and Nakane (1972) report the presence of STH, LTH, ACTH, TSH and LH cells in the fetal rat at 16 or 17 days gestation. Klessen (1971) has demonstrated TSH cells at 14½ days gestation in hamsters using a histochemical staining procedure which takes advantage of the high cystine content of this hormone. It is apparent that there are functional hormone producing cells present in the fetal pituitary, but it is hazardous to attempt to make an identification based on morphological criteria established for adult cells and to assign a function to these cells in the developing pituitary using criteria gained from studies on adult animals.

This study of the developing hamster hypophysis illustrates several points of interest in common with other models of the developing pituitary: the close contact of the newly formed Rathke's pouch with the neural tube, the time of closure of Rathke's pouch, the 100% agreement in the time relationship between vascularization of the pars distalis and the appearance of cytoplasmic granules. These events are all normal, essential landmarks in the development of the various animal pituitary systems studied.
Although cause and effect is difficult to determine, one might speculate as to the relative importance of each of these events in the sequence of differentiation. Must one necessarily follow the other for normal pituitary development and differentiation to occur? Fink and Smith (1971) showed normal differentiation of rat pars distalis cells before the complete development of the portal system. Daikoku (1973) reports the appearance of differentiated pituitary cells with granules in rats without brains, although the number of granules was reduced and the number of granulated cells was less than normal. It is reasonable to assume that some event prior to the maturation of the portal system has already induced cytodifferentiation of the pituitary cells. The coincidence of vascular penetration of the pars distalis with the appearance of granules may indicate that this particular step is influenced by some part of the brain remote from the pituitary. It can be concluded that a series of sequential morphological events all contribute to normal pituitary development.
Figure 1

A-D) Hamster embryo at 7½ days gestation. Selected micrographs from serial sections showing the relatively undeveloped, last presomite stage of the primitive streak. Chorionic cyst (c), extraembryonic coelom (e), amnionic cavity (a), vitelline sac (v) are present. H & E (120X)

C) Note slight headfold (arrow) and allantois (a1).
Figure 2

Hamster embryo at 8½ days gestation.

A) Rathke's pouch (r) is formed and touches the infundibulum (i). H & E (185X)

B) Note the very close association between the neural and ectodermal components (open arrow). There are many mitotic figures (small arrows), especially on the neural side. H & E (670X)
Figure 3

Hamster embryo at 9½ days gestation illustrating the relationship of Rathke's pouch (r) to the rest of the embryo. Mesencephalon (m), diencephalon (d), stomodeum (st), heart (h). H & E (60X)

Inset) The lumen (r) is narrowed and cells of the pouch are beginning to proliferate. H & E (320X)
Figure 4

Rathke's pouch, near the stomodeum, is only a few cells high at 9½ days of gestation. Cells rest on a thin basal lamina (arrow), bordered by a thick amorphous ground substance. No well developed tight junctions are evident and the cells are still undifferentiated. Lumen of Rathke's pouch (r), nucleus (N).

(7700X)
Figure 5

Rathke's pouch at 9½ days gestation. Palisade arrangement of marginal cells is seen around the lumen (r). Cells have little RER, many free ribosomes, few mitochondria and Golgi. Nuclei (N) are finely granular with little heterochromatin. (10,500X)
Figure 6

Close association between the cells of Rathke’s pouch (r) and the neural tube (n).

A) At 9½ days gestation some cells appear to cross the space between the components of the pituitary anlagen (arrow).

B) At 10½ days gestation there is less close contact and a few mesenchymal cells (arrows) are interspersed in the ground substance between the neural tube and cells of Rathke’s pouch. 1 μm plastic sections. Methylene blue-Azure II (225X)
Figure 7

Rathke's pouch (r), 10½ days gestation. Sagittal sections near the midline.

A) The gland is essentially vertical and still connected to the stomodeum (st) by a cord of cells. The pars tuberalis is organizing (arrow) and is delineated from the future pars distalis by Atwell's recess (at). H & E (225X)

B) The pars nervosa (pn) has formed from an evagination of the diencephalon. H & E (225X)
Rathke's pocket at 10½ days gestation takes on a slightly inverted S shape due to a proliferation of cells on the anterior surface of the pocket. This mound of cells at the base of the lumen will become the pars distalis (pd). Numerous cells in mitosis (arrow) are seen in both neural and ectodermal components of the pituitary. H & E (895X)
Processes of cells (large arrow) from the neural tube (n) project into the ground substance toward the cells of the pars distalis (pd). 10½ days gestation. (11,200X)

Inset) Rathke's pouch (r) is still open to the stomodeum (small arrow). 1 μ plastic section Methylene blue-Azure II (210X)
Figure 10

Cells from the area of the future pars distalis are more rounded, have wide intercellular spaces with intermittent contacts with other cells, giving them a scalloped appearance. Note the dividing cell in anaphase. 10½ days gestation. (8400X)
Figure 11

Cells from the pars distalis anlage have more RER, Golgi (g) and mitochondria at 10½ days gestation. (8400)
Figure 12

Pituitary anlagen at 11½ days gestation.

A) A sagittal section illustrating the relationship of Rathke's pocket (r) to the stomodeum (st) and neural tube (n). H & E (35X)

B) Rathke's pocket (r) is closed and is becoming more horizontal although still connected to the stomodeum by a cord of cells (arrow). The pars nervosa (pn) has a hollow stalk open to the 3rd ventricle. Medial sagittal section, H & E (210X)

C) A transverse section through Rathke's pocket showing the lateral extent of the lumen and its relationship to the pars nervosa (pn). External vascular elements are well developed (arrows), but no internal vascularization has taken place. H & E (195X)
Cells lining Rathke’s pocket (r) at 11½ days gestation. Tight junctions (arrows) are now evident at the apical surface between cells. An autophagic vacuole is present, upper left. (11,200X)
Figure 14

Palisade arrangement of cells bordering Rathke's pocket. RER is dilated and more conspicuous, and many rosettes of free ribosomes are present. Cells still have characteristic scalloped appearance due to widely spaced contacts across intercellular spaces. 11½ days gestation. (8400X)
Figure 15

Section from the presumptive pars distalis at 11½ days gestation. Cells are more rounded and in much closer contact. Large deposits of glycogen (gl) are seen associated with empty vacuoles which may have contained lipid. (8400X)
Figure 16

Sagital sections of the pituitary near the midline at 12½ days gestation. Rathke's pocket is becoming more horizontally orientated.

A) Sinusoids (s) are forming within the pars distalis (pd). Section of the pars nervosa (pn), lateral to the stalk. Note the cellular nature. H & E (195X)

B) Invasion of mesenchymal tissue (ms) is seen preceding vascularization by the hypophysial portal system. The infundibular recess within the pars nervosa is now closed (arrow); the basisphenoid cartilage is forming (bs). H & E (195X)
Figure 17

Frontal section through the pars distalis anterior to the pars nervosa. 12½ days gestation. Many sinusoidal spaces (s) are apparent and blood cells are still nucleated.

A) H & E (145X)

B) H & E (785X)
Granules (arrows) are seen for the first time in cells of the pars distalis at 12.5 days gestation. Note the well developed Golgi (g) in close association with these granules. (8400X)
Figure 19

Section from lateral border of the growing pituitary gland. Few granules are evident (thin arrow). Mesenchymal cell processes (thick arrow) abut the basal lamina of the pituitary boundary. (8400X)
Figure 20

Pars distalis at 12½ days gestation. Cells which contain granules (arrows) are occasionally seen undergoing mitosis. (8400X)
Sagittal sections of pituitary at 13½ days gestation.

A) Section lateral to the midline. A slender cord of
cells still connects the pituitary to the stomodeum (st) through
an opening in the basisphenoid cartilage (bs). H & E (175X)

B) Rathke’s pocket is completely horizontal. The pars
nervosa (pn) is closely associated with the cells that will
become the pars intermedia. The pars distalis (pd) is highly
vascularized. The pars tuberalis (pt) extends almost completely
around the median eminence at this time. H & E (175X)
Figure 22

Cells of the developing border of the pars distalis at 13½ days gestation. Numerous well developed secretory granules and mitochondria with well developed cristae are present. Embryonic fibroblasts (f) in the ground substance have processes closely applied to the pituitary basal lamina (arrow). (8400X)
Cells of the pars distalis at 13½ days gestation. Note the vesiculated cell with dense granules (100-300 μm) in close apposition to a perisinusoidal space (ps). This cell generally fits the criteria used to describe adult FSH cells. (8400X)
Figure 24

Pars distalis, frontal section at 14½ days gestation.

A) Rathke's pocket is more attenuated. Marginal cells lining the lumen are lower and more cuboidal than seen on previous days. H & E (150X)

B) Sinusoids are well developed and lined with endothelium (arrow). H & E (735X)
Cells appear at 14½ days gestation, which have extremely large, lightly staining granules with a granular substructure. Many large mitochondria are also present. (19,500X)
Figure 26

Cell from the pars distalis of 14\(\frac{1}{2}\) day fetus. Granules are very electron dense, measure 460-510 nm in diameter. This cell could be classified as a prolactin cell based on morphological criteria used for adult pituitary cells. (19,500X)
The pituitary at 15½ days gestation appears very much like that of the adult. The hypophysial cleft (r) is narrow and lined with flattened cuboidal cells.

A) The pars nervosa (pn) has taken on the appearance of nervous tissue. The basisphenoid cartilage (bs) is beginning to ossify (o). Numerous blood vessels (arrow) permeate the connective tissue surrounding the gland. H & E (185X)

B) The sinusoids (s) are lined with endothelium (arrow), and divide the cells of the pars distalis into the typical anastomosing cords seen in the adult. H & E (735X)
Cells of the pars distalis at 15½ days gestation contain many small granules (arrows) similar in size to that seen in an adult TSH cell (cells 1 & 2). Note the large well developed Golgi (g) in cell (1). (8400X)
Figure 29

Cells are seen in the pars distalis at 15½ days gestation with a large accumulation of glycogen (gl) (cell 1). Another cell (2) is seen which contains a number of very lightly stained granules (arrow) of intermediate size. (8400X)
Cells of the pars distalis at 15½ days gestation. Some cells have a very mature appearance as noted by the well developed Golgi (g) and large, dense granules. Many granules near the cell membrane are ready for exocytosis or perhaps have already been secreted (arrows). This cell has all the characteristics of an adult prolactin cell. (19,500X)
Figure 31

A and B) Cells of the pars distalis at ½ day after birth.
Large granules at the plasma membrane indicate that secretion could be occurring. Both cells contain very large, dense granules (400-700 nm) which are characteristic of prolactin cells in the adult hamster. (19,500X)
Figure 32

Pars distalis of ½ day old neonate. Cells have well-developed RER (arrow) and Golgi (g). A cell (1) contains granules in the Golgi region (g) which are larger and less condensed than those near the plasma membrane. Except for the fact that there are more mitochondria in this cell, it could be identified as an ACTH secreting cell using criteria established for the adult pituitary. A cell at the right (2) has the characteristic granule size and RER pattern of the STH secreting cell of the adult. (8400X)
Figure 33

Cells of the pars distalis at 1½ days after birth. Some granules measure as large as 1200 nm (arrows); a size considerably larger than any found in adult pituitary cells. A wide variation in the size and density of granules is apparent. (8400X)
Figure 34

Pars distalis cell at 1½ days after birth contains glycogen, well developed Golgi and numerous mitochondria. Few secretory granules are present. (19,500X)
Figure 35

Cells of the pars distalis at 2½ days postnatal life. A cell (1) with the characteristics of an adult STH cell with granules 300-480 nm in diameter. These granules are less dense than those seen in mature STH cells. Two cells (2 and 3) with glycogen deposits, have very small granules, 100-150 nm. Other chromophobes (4) are evident. (8400X)
A cell with small granules (140-200 nm) at the plasma membrane could be characterized as an ACTH cell. 3½ days after birth. (14,000X)
Figure 37

Pars distalis, 3½ days after birth. A cell with pleomorphic granules which average 350 nm in diameter (range, 250-800 nm). (8400X)
Cells of the adult pars distalis are identified and illustrated for comparison with developing cells. See text for descriptive criteria.

A) TSH cell
B) ACTH cell
C) Chromophobes (C) and LTH cell
D) FSH and LH cells
E) LH and LTH cells
F) STH cell; probable LH cell (based on nuclear morphology).
Chapter 2

THE IMMUNOHISTOCHEMICAL LOCALIZATION OF THE PROLACTIN CELL IN EMBRYONIC AND NEONATAL HAMSTERS

The peroxidase-labeled antibody technique has been instrumental in the identification and localization of the different hormone producing cells of the anterior pituitary (Avrameas, 1970; Nakane, 1970; Moriarty, 1973). Prolactin is of particular interest because of the widespread implication of this hormone in the general scheme of metabolism and its interaction with other hormones in growth and reproduction (Horrobin, 1973). Most investigations to date have been directed toward the pituitary cytology of the adult animal with many reports of light level localization of prolactin in the adenohypophysis of various mammals, especially the laboratory rat, under normal and experimental conditions (Nakane, 1968, 1970, 1971; Baker et al., 1969, 1973; Baker, 1970; Merchant, 1974). There has been only one immunohistochemical study on prolactin cells at the electron microscope level (Parsons and Erlandsen, 1974).

In recent years, there has been a renewed interest in the embryogenesis of the anterior pituitary with emphasis on cytodifferentiation at the electron microscope level. In some cases, attempts have been made to tentatively identify the cells on the basis of granule size, as this criteria is an acceptable standard for the adult, or by observation of a cellular response to some experimental
manipulation (Sano and Sasaki, 1969; Fink and Smith, 1971; Daikoku et al., 1973; Dupouy and Magre, 1973). Few attempts have been made thus far to use the immunoperoxidase technique in the study of the developing hypophysis. Setaló and Nakane (1972) have detected the presence of functionally differentiated pituitary cells in the fetal rat adenohypophysis at the light microscope level using peroxidase-labeled antibodies.

Because of the sparcity of information available and because of the uncertain and conflicting evidence based on morphological identification of embryonic pituitary cells, it was the purpose of this investigation to examine the anterior pituitary in the developing hamster fetus and neonate using anti-ovine prolactin immunoperoxidase. This technique was used at the light and electron microscope level to determine the age at which immunoreactive prolactin cells could first be localized in the pituitary. Attempts were made to relate the morphology and distribution of the cells in the developing animal to that of the adult.

MATERIALS AND METHODS

Animals

Multiparous cyclic female hamsters were mated at 2100 hr on the night of estrous. When copulation was observed to be occurring, the animals were left undisturbed for a period of 2 hr, after which time the male was removed from the cage. This was determined to be "0" time of pregnancy.
Pregnant female hamsters were sacrificed by decapitation at 0900-1100 hr at 13½-15½ days of gestation and the fetuses rapidly removed from the uterus through a single longitudinal incision. For light microscopy, the entire head of each fetus was removed and fixed, whereas for electron microscopy, each pituitary was dissected out with as little surrounding tissue as possible to insure proper fixation and infiltration. Neonatal hamsters, ½-3½ days of age were decapitated and the pituitaries removed in a small block of surrounding tissue. Several pituitaries were taken from adult female hamsters to use as a positive control.

Light Microscopy

Fixation was in Bouin's, 24-48 hr, for immunocytochemistry or in formalin sublimate (1 part formalin to 9 parts saturated HgCl₂), 24 hr, for histological staining by Brookes' method (1968). Tissue was dehydrated in ethanol, cleared in benzene, and embedded in Paraplast. Sections, 4 μ, were cut and mounted with Mayer's albumin on glass slides.

Immunocytochemical staining was carried out using the indirect peroxidase-labeled antibody methods (Nakane and Pierce, 1967). Anti-ovine prolactin was produced in rabbits by the method of Vaitukaitis et al. (1971) and purified by precipitation of the globulin by the dropwise addition of 1 part saturated ammonium sulfate to 2 parts serum. The rabbit globulin was dissolved in a small amount of water, desalted on a Sephadex G-50 column and

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¹Ovine prolactin was a gift of NIAMDD, NIH.
dialyzed overnight against 0.01M phosphate buffered saline, pH 7.4, (PBS). The protein fraction was concentrated to the original volume of serum in a negative pressure membrane filter and adsorbed with acetone dried rat liver powder. The purified antibody was then filtered and stored in small aliquots at -12°C.

Sheep anti-rabbit globulin, purified in the same manner as the rabbit anti-prolactin, was coupled to horseradish peroxidase (Sigma Co., Type II) with 4,4'-difluoro-3,3' dinitrophenyl sulfone (FNPS) (Nakane and Pierce, 1967). Labeled globulin was removed from the unreacted peroxidase by reprecipitation with ammonium sulfate. Dialysis, adsorption, filtration and storage was carried out as previously described.

Immunocytochemical staining was carried out at room temperature in a high humidity chamber on sections which had been deparaffinized in xylene, hydrated, then rinsed with PBS. Tissue was covered with rabbit anti-prolactin (dil 1:8) and incubated for 1 1/2 hr, then washed in at least three changes of buffer for 15 min. This was followed by application of the peroxidase-labeled sheep anti-rabbit globulin (dil 1:4) and incubation for an additional hr. Another period of vigorous washing was followed by reaction of the tissue-bound peroxidase with its substrate, 3,3' dianinobenzidine (DAB) according to the method of Graham and Karnosky (1966). Slides were immersed in a coplin jar containing 0.005% H₂O₂ and 38 mg DAB in 50 ml Tris-HCl buffer, pH 7.6, for 20-30 min. Non-specific background staining was held to a minimum when this reaction was carried out in the dark. The dense brown precipitate which forms at the site of
the antigen-antibody reaction is stabilized by osmication for 1 hr in 2% OsO$_4$. The slides were then dehydrated and permanently mounted.

For histological differentiation of the two types of acidophils, formalin sublimate fixed tissue which had been treated with iodine to remove the HgCl$_2$ crystals, was stained according to Brookes' method (1968) using El Etreby and Tüshaus' (1973) staining times and counterstains. Carmoisine-L (C.I. 59643) and Aniline blue (C.I. 42755) were purchased from Gurr Ltd., Acid Alizarin Blue BB (C.I. 58610) was obtained from Matheson, Coleman and Bell, and the Orange G (C.I. 16230) from Fisher Scientific Co. The intensity of all colors was improved by adding a few drops of acetic acid to the distilled water rinses and by rapid dehydration of the tissue directly from 95% alcohol (a few dips) to 100% alcohol.

Electron Microscopy

For immunohistochemical study, tissue was fixed in picric acid-paraformaldehyde (PAF) (Stefanini et al., 1967) and embedded in polyethylene glycol (Fluka, Switzerland) using the alcohol dehydration sequence (Mazurkiewicz and Nakane, 1972). Tissue was stored desiccated at 4°C until sectioned and stained for prolactin, using the immunoperoxidase method described by Mazurkiewicz and Nakane (1972) with some modifications. Tissue was cut at 5 µm on a paraffin microtome using a new, disposable knife. A piece of dry ice can be used to chill the blade and dry the air in order to get ribbons when there is a problem of high humidity. Sections were floated on 5% glycerol for 3 hr, transferred to saline, then to
distilled water and onto a clean glass slide (Carolina Biological Co.) using a wire loop. The tissue was air dried at 40°C for at least 1/2 hr. After hydration in PBS for 15 min, sections were stained at room temperature in a high humidity chamber according to the following protocol.

The first antibody was applied and tissue washed as described for light microscopy. Sheep anti-rabbit globulin, purified according to the technique of Avrameas and Ternynck (1969) and coupled to peroxidase (Sigma, Type VI) with glutaraldehyde (Avrameas and Ternynck, 1971), was used as the second antibody. This was applied to the tissue directly, with no dilution, by adding a few drops to the small amount of PBS remaining over the tissue after washing. After 1 hr, slides were washed again thoroughly, then immersed in 20-30 mg DAB/100 ml Tris-HCl buffer, pH 7.6, without H₂O₂ and incubated for 60-90 min in the dark. Tissues were then transferred to a fresh DAB solution of the same concentration but with H₂O₂ added to a final concentration of 0.005%, and incubated for an additional 15-20 min. Slides can be examined and photographed at this point by coverslipping with buffered glycerol mounting fluid. The reaction product was then stabilized with 1% OsO₄ in phosphate buffer, 30 min, and dehydrated in ethanol. Osmication and dehydration were carried out in the cold. The tissue was embedded in Epon epoxy resin by inverting the filled capsule onto the tissue while still wet with 100% alcohol. After polymerization at 60°C, 24-48 hr, the blocks were removed from the slides by immersion in a dry ice-alcohol bath alternated with boiling water. Thin sections were cut on a Porter
Blum MT 2B ultramicrotome using a diamond knife and viewed without counterstaining with an Hitachi 11A electron microscope.

For cytological study, pituitary tissue was fixed in glutaraldehyde or PAF, post-fixed in OsO₄, dehydrated with ethanol and embedded in Spurr (1969) epoxy resin. Thin sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965).

**OBSERVATIONS**

Using the immunoperoxidase technique, prolactin cells were localized at the light level in adult cyclic female hamsters. Clusters of immunoreactive cells, located primarily around sinusoids, were observed to contain large concentrations of dark reaction product (Figs. 39A, 40A & B). Unreacted cells stand out in contrast. Several controls were run to test the specificity of the anti-prolactin antibody. When non-immune serum was used in place of the first antibody, no immunospecific staining occurred. However, a slight background coloration was imparted by OsO₄ (Fig. 39B). When antiserum which had been adsorbed with prolactin was used, staining was greatly reduced (Fig. 39C).

The first electron microscopic evidence of secretory granules was detected in the pars distalis of the hamster fetus at 12½ days of gestation (Fig. 41A), while at 13½ days there was definite evidence of mature secretory granules in a number of pituitary cells (Fig. 41B). At this time, a small number of prolactin cells, near invading vascular elements, could be localized with the immunoperoxidase technique (Figs. 42A & B). The granular nature of
the cytoplasm of one of these cells is illustrated in Figure 42B (insert). For the most part, staining of the gland was negative due to the sparse distribution of positive cells.

Little difference in the number or distribution of prolactin positive cells was noted in the pars distalis at 14½ days (Figs. 43A & B) or 15½ days gestation (Figs. 44A, B & 45) when compared to the gland at 13½ days of gestation. However, in the pars tuberalis, near its junction with the pars distalis, peroxidase positive cells were detected at 15½ days gestation (Figs. 44A & B). Numerous mitotic figures were also evident in the pars distalis at this time (Fig. 45).

At ½ day postpartum, the picture of the newborn hamster pituitary is strikingly different. Numerous immunoreactive prolactin cells are seen throughout a frontal section of the gland at the level of Rathke's pocket and the neurohypophysis (Fig. 46A). Cells with definite reaction product in the cytoplasm are clearly evident at higher magnification (Figs. 46B & C).

Comparable sections of the gland at 1½, 2½, and 3½ days of age, show little difference in the distribution and in the number of prolactin cells (Figs. 47A, 48A & 49A) when compared to the gland at ½ day after birth. Prolactin cells in the neonatal pars distalis (Figs. 47B, 48B & 49B) contain considerably less dense concentrations of reaction product than in adult female hamster cells (Fig. 40).

To corroborate immunochromal results, the histological stain described by Brookes (1968) and subsequently modified by El Etreby and Tűshaus (1973) was used to differentiate the two types of acidophils and to correlate these results with those of the antibody
procedure. Cells that stained with Carminorin-L were observed to have the same distribution as the prolactin cells localized with the immunoperoxidase technique. Few, if any, carminophils could be identified in the fetal pituitary.

Prolactin cells were localized at the electron microscope level in the pars distalis of the adult, pregnant hamster, at 13½ days gestation, using the peroxidase-labeled antibody technique. A section demonstrating prolactin positive cells was first photographed at the light level, before application of OsO₄ (Fig. 50A). This same section was then studied with the electron microscope. Prolactin cells could be identified by the presence of electron dense reaction product concentrated over the granules (Figs. 51A & B). Prolactin negative cells, containing granules with no immune precipitate, could be seen in the same field. These immunoreactive cells correspond in morphology to prolactin cells identified using regular electron microscope embedding and staining techniques (Fig. 52).

An adjacent section from the same gland was also stained using the peroxidase-labeled antibody technique except that saline was used instead of the first antibody. When seen at the light level, before treatment with OsO₄, a complete absence of staining is apparent (Fig. 50B). This section was also examined for immunoreactive cells at the electron microscope level, with negative results.

Fetal pituitaries at 15½ days of gestation and at ½ day postpartum were also reacted with immunoperoxidase for electron microscopic examination. Cells with immunoreactive granules were seen at both ages (Figs. 53A, 54A & B). Most granules measured
approximately 250 nm, with a range of 171-342 nm. Cells with very large secretory granules, approximating the size of prolactin granules of the adult pars distalis, were not stained with the anti-prolactin immunoperoxidase technique (Fig. 53C).

Using regular embedding and staining techniques, cells having the morphology and granule size analogous to the cells identified as prolactin cells by the immunoperoxidase technique were detected in the fetal and neonatal pars distalis (Figs. 55A & B).

DISCUSSION

The first electron microscopic evidence of what appears to be mature secretory granules in the cytoplasm of cells of the hamster pars distalis was detected at 13½ days gestation. At this same time, a few immunoreactive prolactin cells could be localized in the fetal pituitary at the light level using the peroxidase-labeled antibody technique. In the rat, functionally differentiated prolactin cells were reported as early as 16 days gestation using immunohistochemical methods (Sétaló and Nakane, 1972). However, mature secretory granules are not apparent in fetal rat pars distalis cells until the 17th or 18th day of gestation (Fink and Smith, 1971; Stoeckel et al., 1973). Information was not provided as to the timing of pregnancy or strain or rats used in the immunohistochemical report, therefore it is not known how great a discrepancy may exist. It is highly unlikely that prolactin could be detected at the light level unless concentrated into relatively mature secretory granules.
At 15½ days gestation, some cells of the pars tuberalis nearest the pars distalis appear to be staining immunohistochemically for prolactin. This observation was made in several different fetuses, in frontal as well as sagittal sections. Since the anterior pituitary of neonates was removed from the brain and the pars tuberalis lost, it is not known whether this is a transient observation or a developmental trend. It was not seen in earlier stages of development. It is difficult to explain this phenomenon on any known physiological basis. The cells in the pars tuberalis lie in close proximity to the developing portal system and are the first cells to become granulated in the rat (Stoeckle et al., 1973). If these immunoreactive cells are indeed producing prolactin, it is apparent that they must migrate to the pars distalis or atrophy, as the adult pars tuberalis is not known to secrete prolactin.

In the hamster, a dramatic increase in the number of obviously positive prolactin cells is apparent throughout the pars distalis on the first day after birth. Since prolactin is thought to be primarily under control of a hypothalamic inhibiting factor, the relative maturity of the portal system would have little to do with the cytodifferentiation of prolactin cells with regard to any effect from releasing factors. It has been shown that neither a mature portal system (Fink and Smith, 1971) nor the brain (Daikoku et al., 1973) of rats is necessary for cytodifferentiation and appearance of secretory granules. However, since prolactin has been measured in fetal hamster serum and shown to cross the placenta (Thompson and Terranova, unpublished), it is possible that maternal prolactin may
exert a negative feedback on the fetal prolactin cells which is released at parturition. The prolactin cells of the newborn animal could then assume full functional differentiation with synthesis and secretion of hormone.

Carmoisine-L staining was used to support immunochromic findings. In the adult, the distribution of carminophils corresponds to that of prolactin cells as seen using the immunoperoxidase technique. This histological stain is not as satisfactory in the hamster as in the pituitary cells of other animals as reported by El Etreby. The prolactin cells stain lightly and the STH cells are almost indistinguishable from the gonadotropes. However, the obvious increase in number of carminophils detected in newborn animals, parallels to a similar increase in the number of prolactin cells determined by immunoperoxidase.

Control staining procedures verified the specificity of the prolactin antibody technique and ruled out nonspecific background staining in the adult gland. However, there is some slight background staining due to OsO₄ fixation, and red blood cells always stain indiscriminantly with this technique. In the fetus, controls were similar to the adult, except for some staining of the brain and chondrocytes due to crossreactivity of the sheep anti-rabbit antibody with some factor in these tissues, perhaps a Forssman related antigen. In no case was there any nonspecific reaction within pituitary cells; therefore, no interference was encountered in the localization procedure.
The purified anti-ovine prolactin used in this study was further characterized by acetate strip electrophoresis using the Millipore PhoroSlide System. Only two protein bands were detected; a trace of albumin and a band having the migration characteristics of IgG. The antibody gave positive results in the ring precipitation test when reacted against high dilution of ovine prolactin antigen (1 mg/ml x 10^{-5}). Anti-ovine prolactin was found to cross react with rat prolactin, but did not cross react with rat growth hormone, rat luteinizing hormone, or rat follicle stimulating hormone either in the ring test or Ouchterlony double diffusion gels. On the basis of these observations it is felt that the antigen-antibody reaction in the immunoperoxidase technique was highly specific.

At the electron microscope level, prolactin cells were localized in the adult pars distalis especially near sinusoids. The specificity of the reaction is apparent when a positive cell is seen next to a cell containing negative granules. Cytology was poor, but the granules were well preserved although slightly smaller than those seen in regularly fixated and stained tissue. This size difference may be due to shrinkage, as PAF is not the best fixative. The long incubation times in antibody and the vigorous washings may also be detrimental to the preservation of cell architecture. However, it is necessary to use PAF in order to preserve the antigenicity of prolactin.

Immunoreactive cells of the fetus were examined to try to relate granule size to those seen in the fetus or adult which had been stained in the usual manner for morphology. Prolactin-positive
granules, localized with immunoperoxidase in the fetal pituitary cells, were about one half as large as prolactin granules in the adult pars distalis cells whether determined by immunoperoxidase staining or on a morphological basis. There was also a wide range in the size of these granules in both the fetus and neonate. Variation in granule size is not apparent in adult prolactin cells.

Other fetal and neonatal pituitary cells contain very large granules (1000 nm) which do not stain immunochemically for prolactin. It must be pointed out that to classify a hormone producing cell in the developing pituitary on the basis of relative granule size alone is very risky speculation, although in the adult, the prolactin cell has the largest granules (400-800 nm). If this particular cell with large granules, which has no exact counterpart in the adult adenohypophysis, is indeed synthesizing prolactin, then the secretory product is not in a form to act as an antigen, or it may be a precursor of some other cell type altogether.

The picture is still unclear as to whether prolactin has a function in the fetus or neonate. Sano and Sasaki (1969) report the absence of prolactin cells in the mouse fetal pituitary, while Kohmoto and Bern (1971) report their presence. The hamster has prolactin in its serum (Thompson and Terranova, unpublished) and peroxidase-positive cells in the pituitary as early as 13½ days gestation. The function of prolactin in the developing hamster has yet to be determined.
Cells of the adult cyclic female hamster. Immunoperoxidase technique.

A) Immunoreactive prolactin cells (arrows) contain large amounts of dark precipitate in the cytoplasm. (865X)

B) Control. Non-immune rabbit globulin used instead of specific, first antibody. Note the absence of any positive cells. (865X)

C) Control. Specific rabbit antibody adsorbed with prolactin was used instead of the first antibody. Staining is greatly reduced, but some slightly positive cells are seen (arrows). (865X)
Figure 40

A and B) Immunoreactive prolactin cells (P) of adult female hamster pars distalis. Note granular nature of the precipitate in the cytoplasm. (2160X)
Figure 41

Cells of the fetal pars distalis.

A) At 12½ days gestation, the first very small granules (arrows) are seen. (9800X)

B) At 13½ days gestation, larger, more mature granules are seen. (22,750X)
Figure 42

Sagittal section at the midline of a fetal pituitary at 13½ days gestation. Immunoperoxidase technique.

A) Pars distalis (pd) is essentially negative when stained with immunoperoxidase. Pars nervosa (pn), pars tuberalis (pt), Rathke's lumen (r). (205X)

B) A few immunoreactive cells (arrows) are seen near the sinusoids. (770X)

Inset) Granular reaction product is seen in the cytoplasm of a positive cell. (1840X)
Figure 43

The pars distalis (pd), sagittal section lateral to the midline at 14½ days gestation. Immunoperoxidase technique.

A) There are few positive cells. (205X)

B) At higher magnification, a few cells may be suspected of containing reaction product (arrows). (770X)
Figure 44

A sagittal section of the pituitary at 15½ days gestation. Immunoperoxidase technique.

A) Cells of the pars tuberalis (within lines) appear to be reacting with the immunoperoxidase. (160X)

B) Enlargement of the area within the lines. Note the darkly staining cells of the pars tuberalis. (640X)
Cells of the pars distalis at 15½ days gestation. Note many cells in mitosis (arrows), but there are no immunoreactive cells apparent. Immunoperoxidase technique. (800X)
Figure 46

The pars distalis at ½ day after birth. Immunoperoxidase technique.

A) Many immunoreactive cells are scattered throughout a frontal section at the level of Rathke's pocket and the neurohypophysis. (190X)

B) Prolactin positive cells. 7 μ section. (1920X)

C) Prolactin positive cells. Thinner, 4 μ section, gives better resolution of granular precipitate (arrow). (1920X)
The pars distalis at $1\frac{1}{2}$ days after birth. Immunoperoxidase technique.

A) Frontal section. Number and distribution of immunoreactive cells is the same as seen in Figure 46, $\frac{1}{2}$ day after birth. (190X)

B) Granular reaction product in the cytoplasm (arrow) is seen to be much less dense than that in adult prolactin cells. (1920X)
Figure 48

Neonatal pars distalis (pd), 2½ days of age. Immunoperoxidase technique.

A) Frontal section illustrating the same distribution of prolactin cells as noted on previous postnatal days (Figs. 46 & 47). Pars nervosa (pn), hypophysial cleft (r). (205X)

B) Higher magnification to show granular nature of the reaction product in the cytoplasm (arrow). (700X)
Figure 49

Pars distalis from neonate, 3½ days of age. Immunoperoxidase technique.

A) Immunoreactive cells in this particular frontal section seem to be congregated at the lateral borders. Otherwise, staining is similar to that seen in the previous three days (Figs. 46A & 48A). (210X)

B) Reaction product in the cytoplasm (arrow) is similar in density to previous postnatal pituitary cells illustrated in Figures 46B & 48B. (865X)
Figure 50

Pars distalis of adult pregnant hamster embedded in PEG, sectioned 5 μ. Immunoperoxidase technique.

A) Specific immunoperoxidase. Note numerous positive prolactin cells. Photographed before the application of osmium. (180X)

B) Control. Saline used in place of specific rabbit anti-prolactin. Note total lack of any staining. Photographed before the application of osmium. (180X)
Figure 51

A and B) Thin sections taken from reembedded 5 μ section illustrated in Figure 50A. Immunoperoxidase technique. Nuclei (N) are unstained. Note reaction product over prolactin granules measuring 300-600 nm. Cells with unreacted granules (*) are seen in the same field. (10,575X)
Figure 52

Prolactin cells (P) from adult female hamster pars distalis with granules measuring approximately 600 nm. Regular embedding and staining techniques to illustrate normal prolactin cell morphology. A TSH cell is seen in the upper left hand corner. (8400X)
Figure 53

A and B) Pars distalis cells from fetus at 15½ days gestation. Immunoperoxidase technique. Immunoreactive granules (arrows) are fewer than in adult cells and measure only 250 nm. (17,500X)
Figure 54

Cells of the pars distalis ½ day after birth. Immunoperoxidase technique.

A and B) Positive cells with granules (arrows) which measure up to 350 nm in diameter. (17,500X)

C) Cell with very large granules (970 nm) which do not stain with specific anti-prolactin antibody. (17,500X)
Cells of the fetal and neonatal pars distalis embedded and stained for electron microscopy in the usual manner. Granules are within the size range of those which react with the immunoperoxidase. (See Figures 53 & 54A, B)

A) 15½ days gestation. (9,800X)

B) ½ day newborn. (9,800X)
Chapter 3

THE DETERMINATION OF PROLACTIN SERUM LEVELS
OF FETAL AND NEONATAL HAMSTERS AND ITS
RELATIONSHIP TO MATERNAL LEVELS

Although much interest has centered around the presence and roles of pituitary hormones in development, it has only been since the development of radioimmunoassay (RIA) that a reliable quantitative tool has been available for studies in this area. Presently, very little information is available on hormone levels in fetal animals.

Prolactin has been identified in fetal rats by disc electrophoresis of pituitary homogenates (Ingleton et al., 1971) and has been measured in the plasma and pituitaries of fetal and neonatal rats by RIA (D'Angelo et al., 1975). In addition, prolactin cells were localized in fetal rat adenohypophyses using the peroxidase-labeled antibody technique (Sétáló and Nakane, 1971), and the pituitary of mouse embryos has been shown to contain as well as secrete this hormone (Kohmoto and Bern, 1971). Since iodinated prolactin (\(^{125}\text{I}-\text{PRL}\)) has been shown to cross the placenta in monkeys and enter amniotic fluid and fetal circulation (Josimovich et al., 1974), the question arises as to whether fetal serum prolactin levels are, in part, a contribution from a maternal source.

The present study was undertaken to determine whether fetal and neonatal hamster serum contains immunoassayable amounts of
prolactin, and to determine its relationship, if any, to maternal levels. The possibility of a maternal source for some of this hormone in fetal serum was also investigated.

MATERIALS AND METHODS

Animals

Multiparous cyclic female hamsters were maintained on a 12L:12D lighting schedule, 2400 hrs being the midpoint of the dark period. Each cyclic female hamster was mated on the night of estrous and at 0900 hrs the following morning, fetuses were considered to be ½ day of gestation. Pups were born during the dark hours at the end of 16 days of gestation, and these animals were considered to be one-half day old the morning following parturition. All animals were sacrificed and sera collections made at 0900-1100 hrs.

Maternal blood was collected in tubes after the animals had been sacrificed by decapitation. Serum was harvested from the clots after a period of 2 hrs and centrifuged to remove any residual blood cells.

For collection of fetal blood, the maternal uterus was opened by a single longitudinal incision and each fetus was carefully lifted from the uterus and blotted dry of amniotic fluid and maternal blood. Whenever possible, the umbilical cord was left intact and placental association with the uterus was maintained while each fetus was decapitated and blood collected in capillary tubes. Immediately after blood collection the samples were centrifuged for 15 min in a hematocrit centrifuge, after which sera from the fetuses of each
litter were pooled. Each sample represented pooled sera from a single litter of animals, except at 13½ days gestation, when sera of two litters were pooled to obtain a large enough sample.

All sera collections and centrifugations were carried out at room temperature and the samples were immediately frozen and stored at -12°C until assayed.

**Iodination of Prolactin**

Prolactin was iodinated by modification of the procedure provided by the Rat Pituitary Hormone Distribution Program, National Institute of Arthritis and Metabolic Diseases (NIAMD), National Institutes of Health (NIH). NIAMD Rat PRL-I-1 (30 IU/mg) was radiolabeled with $^{125}\text{I}$, New England Nuclear Co., NEZ 033. One μCi $^{125}\text{I}$ in 25 μl of 0.1 N NaOH was neutralized with an equal volume of 0.1 N HCl in a reaction vessel containing 25 μl of 0.1 M phosphate buffer, pH 7.6. Twenty μl (2 μg) of NIAMD Rat PRL-I-1, previously stored at -20°C in 0.01 M Na$_2$PO$_4$ buffer containing 0.15 M NaCl with 0.1% NaN$_3$ (PBS), pH 7.6, was added to the reaction vessel and immediately followed by the addition of 10 μl of chloramine-T (25 mg/ml PBS without NaN$_3$) that had been prepared immediately prior to use. The reaction vessel was gently agitated for 60 sec after which 25 μl of freshly prepared sodium metabisulfite (25 mg/ml PBS without NaN$_3$) was added, and the entire reaction mixture was applied to a Bio Gel P-60 gel filtration column, Bio Rad Labs., C.7 x 20 cm glass column, 100-200 mesh. The gel column was prepared for the reaction mixture by equilibrating with PBS. Then, 2 ml of 2% BSA in
PBS was added to the column, after which 5 ml of PBS was added. The reaction mixture was eluted from the column with PBS. Twenty 0.5 ml fractions were collected singly in culture tubes containing 50 μl of 2% BSA in PBS. Ten μl of each fraction was then placed in a liquid scintillation vial containing 10 ml of Aquasol, New England Nuclear Co. Each vial was gently agitated, then counted at 2% error in the full tritium window of a Beckman LS-100C liquid scintillation counter. Radioactivities of each vial were recorded as counts per minute (cpm).

Immunoassay of Prolactin

Immunoassay procedures were carried out in accordance with methods provided by NIAMD. In preparing a standard curve, NIAMD-Rat PRL-RP-1 (11 IU/mg) was employed as a reference preparation. Prolactin antiserum utilized in the assay was NIAMD-Anti-Rat PRL S-2. The minimum and maximum volume of serum used in the assay was 50 μl and 200 μl, and duplicate hormone determinations from each sample were employed using the same or different volumes of serum. The slope of the dose-response curve for pooled sera from pregnant hamsters was not significantly different from the slope of the standard curve. Samples were counted as described for the iodination curve samples.

Injection of $^{125}$I-PRL and Recovery from Mother and Fetus

Immediately after purification of the iodinated prolactin on Bio-Gel columns, 13½, 14½, and 15½ day pregnant hamsters were administered 0.1 ml $^{125}$I-PRL (620,000 cpm/0.1 ml PBS) at 0900-1100 hr
by a single intracardiac injection. Exactly 10 minutes after the
125I-PRL injection the pregnant animals were sacrificed by rapid
decapitation for collection of maternal and fetal blood. Serum was
harvested as described previously. All liquid scintillation vials
containing 10 ml Aquasol were precounted for background radioactivity.
One hundred µl of serum from each pregnant hamster was then placed in
a liquid scintillation vial, gently agitated, and counted at 3% error.
Radioactivities were recorded as cpm. One hundred µl of each fetal
serum sample was counted as described for maternal serum.

To determine whether radioactivity measured in serum was still
bound to prolactin, 200 µl of 15½ day maternal serum was then
subjected to gel filtration using the method previously described
for separation of 125I-PRL from other iodination products, except
that 0.5 ml fractions were collected directly in scintillation vials.

One hundred to 150 µl of each fetal serum sample was also
subjected to gel filtration. Twenty 0.5 ml fractions were collected
in scintillation vials from the gel filtration of the 15½ day fetal
samples, and six 0.5 ml fractions were collected for the remaining
fetal serum samples. Each vial was counted at 3% error and cpm
recorded.

Statistics

An analysis of variance with a completely randomized design
was conducted on hormonal data from maternal and fetal hamsters to
determine whether the two hormonal patterns were similar.
Results

Serum prolactin levels in fetal and neonatal hamsters are compared to the respective maternal serum levels in Figure 1. Fetal prolactin concentrations were found to be relatively high (approximately 4.0 ng) at 13½ days and 15½ days of gestation, whereas a drop in levels occurred on day 14½. Little or no prolactin was detected in the serum of newborn animals on the morning after parturition. Two of the five litters contained no measurable prolactin whatever at that time. Of particular interest in Figure 1 is the consistent manner in which the fetal prolactin levels reflect the rising and falling of maternal serum levels of the hormone before birth.

In order to determine whether prolactin in fetal serum could be attributed to placental transfer from maternal sources, \(^{125}\text{I}-\text{PRL}\) was injected into pregnant females. Ten min later a measurable quantity of radioactivity was detected in fetal serum (Table 1) and calculated to be an average of 3.9% of the radioactivity (cpm/ml) recorded in the maternal serum.

The amount of \(^{125}\text{I}\) still bound to protein was determined by columnning aliquots from each serum sample from mother or fetus. In each case, the higher level of radioactivity was found in fraction 4. Figure 2 shows the results of these determinations and their relationship to the purification curve for freshly iodinated prolactin. Note that there is little or no Na\(^{125}\text{I}\) in tubes 9-17 as recovered from the serum samples.
DISCUSSION

We have established by RIA that there are measurable quantities of prolactin hormone in the serum of fetal and neonatal hamsters. The fact that exogenous $^{125}\text{I}}$-PRL crossed hamster placentae and entered fetal circulation indicates the possibility that at least some of the endogenous hormone may have a maternal source in the unborn animal.

Josimovich et al. (1974) reported a similar observation when injecting pregnant monkeys with $^{125}\text{I}}$-PRL. The hormone was determined to cross the placenta in minute amounts and enter fetal circulation (0.5% of maternal serum levels) and amniotic fluid (1.0% of maternal serum levels). They were also able to show that the fetal pituitary released prolactin following an injection of exogenous TRF.

Immunohistochemical techniques have demonstrated the presence of a small number of pituitary prolactin cells in the fetal hamster as early as 13½ days gestation, and at the same age, electron micrographs first showed cells with some of the morphological characteristics of prolactin cells (Thompson and Trimble, unpublished). Thus, a fetal contribution of prolactin would be possible at 13½ days of gestation but not earlier. Because of the scarcity of immunoreactive cells and the small number of cytoplasmic granules in such cells, it does not seem likely that there are enough competent prolactin cells to account for the relatively high amount of circulating prolactin at this age.
At 14½ days there were considerably more granulated cells, yet the concentration of prolactin in the fetal serum drops.

An average of 3.9% of circulating maternal serum levels of $^{125}$I-PRL was measured in pooled fetal serum ten minutes after a single injection of $^{125}$I-PRL into pregnant hamsters on days 13½-15½, whereas on the same days endogenous fetal serum prolactin levels were, on the average, 37% of maternal levels as revealed by RIA. The difference between measured endogenous fetal-maternal ratio and the percentage of maternal $^{125}$I-PRL recovered in fetal serum, could be due to the limitations of the injection procedure and rapid serum hormone clearance rates (Koch et al., 1971). However, some of the difference may be an indication of the relative amount of prolactin contributed by fetal or maternal pituitary secretion.

Changes in serum prolactin levels of fetal hamsters coincided closely with the changes in maternal serum as revealed by analysis of variance. This suggests that maternal prolactin enters fetal circulation, thereby influencing fetal prolactin concentrations. Even after birth, the newborn prolactin levels continued to fluctuate synchronously with maternal prolactin levels. Since it has been suggested that serum prolactin in lactating rats may pass into milk in amounts proportional to maternal circulating levels (McMurty and Malven, 1972), and since large dietary proteins can be absorbed intact into the blood of neonatal rats (Daniels et al., 1972), it is reasonable to postulate that the same mechanism may be responsible for this observation in the neonatal hamster.
Although many more fetal and newborn serum samples were measured for prolactin than were adult samples, the adult values closely followed relative values measured in pregnant and lactating hamsters by Bast and Greenwald (1974), and in rats by Merchant (1974). Prolactin serum values in this study were considerably lower than Bast and Greenwald's, which may be due to several factors. The animals were multiparous vs nulliparous, from different colonies, and there was a difference in NIAMD antibody source, which may have contributed to the differences in absolute levels of prolactin. One of the shortcomings of the RIA technique is that actual values are seldom duplicated from one lab to another, whereas rises and falls in levels are accurately reproduced (Horrobin, 1973).

To determine whether radioactivity in the fetal and maternal serum samples was still bound to prolactin, the samples were columned as in the original purification of $^{125}\text{I-PRL}$. The location of the serum elution peak was similar to the $^{125}\text{I-PRL}$ purification curve, except that little or no Na$^{125}\text{I}$ was recorded in fractions 9-17. The increase in background counts for the serum samples could be duplicated by columning a similar amount of serum from non-injected hamsters, and is attributable to nonspecific chemiluminescence from the high concentration of serum used.

The presence of prolactin in pre- and postnatal hamsters makes it interesting to speculate concerning the possibility of some contribution to the growth and perhaps to the reproductive differentiation of the developing animal.
Table 1

Radioactivity Recovered from Fetal and Maternal Serum

10 Minutes Post Intracardiac Injection with $^{125}\text{I-PR}_{L}$ \(^{a}\)

<table>
<thead>
<tr>
<th>Day of Gestation</th>
<th>cpm/ml Serum (corrected for bkg)</th>
<th>% of Maternal Levels (Embryo/Adult)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
<td>Embryo</td>
</tr>
<tr>
<td>13½</td>
<td>1574.9(^b)</td>
<td>58.9(^c)</td>
</tr>
<tr>
<td>14½</td>
<td>1180.1</td>
<td>34.4</td>
</tr>
<tr>
<td>14½</td>
<td>681.3</td>
<td>36.5</td>
</tr>
<tr>
<td>15½</td>
<td>1081.2</td>
<td>53.6</td>
</tr>
<tr>
<td>15½</td>
<td>1938.5</td>
<td>50.4</td>
</tr>
</tbody>
</table>

\(^a\) 620,000 cpm/0.1 ml PBS

\(^b\) Average cpm of 2 adult serum samples

\(^c\) cpm of pooled sera from 2 litters
Figure 56

Serum prolactin (PRL) levels (ng/ml serum) of pregnant or lactating hamsters and fetal or neonatal hamsters. The number of samples assayed each day is in parentheses. For adults, one sample represents pooled sera from one litter, except at 13½ days gestation, where sera of 2 litters were pooled for one of the samples.
Gel filtration patterns. (●—●) products from the iodination of NIAMD-Rat PRL-I-1 in which a 1 μl aliquot of 0.5 ml fractions were counted. (○—○) maternal or (□—□) fetal serum containing 125I-PRL (mean of 4 samples, 6 litters, counted for fractions 1-6.); 0.5 ml fractions were collected and counted. (■—■) nonradioactive hamster serum; 0.5 ml fractions were collected and counted.
CONCLUSIONS

An interest in the possible functional roles of prolactin in fetal and neonatal animals has provoked these studies on the cytodifferentiation and localization of the prolactin cell and the determination of fetal and neonatal immunoassayable prolactin serum levels in the golden hamster.

The embryological development and cytodifferentiation of the pars distalis was described as this basic information was not already available in the literature. The normal chronological events in the development of the hamster pars distalis closely follows the pituitary organogenesis of other laboratory rodents. Rathke's pouch is formed and touches the infundibulum at 8½ days gestation, and is closed off from the stomodeum three days later. Penetration into the pars distalis of vascular elements of the developing hypophysial portal system occurs at 12½ days gestation which is the first day small secretory granules are seen in any cells. Further cytodifferentiation during the following prenatal and first few postnatal days of life reveal granulated cells which cannot be identified using morphological criteria or granule size as is done in the adult.

Using the immunoperoxidase technique, a few prolactin cells were localized in the pars distalis at 13½-15½ days gestation, and a dramatic increase in number of positive cells was demonstrated immediately after birth.
Concomitantly, prolactin was measured in fetal and neonatal serum using RIA. Since levels at 13½ days gestation were higher than expected from information based on the small number of immunoreactive cells, and since the fetal levels so accurately reflected maternal levels, studies were undertaken to determine whether fetal prolactin levels could have a maternal source. Radioactive prolactin, injected into maternal circulation, was shown to cross the placenta and enter fetal circulation.

Thus, prolactin cells have been demonstrated and the extracellular hormone has been measured in fetal and neonatal hamsters. It is probable that most of the prenatal hormone is furnished by the mother, whereas after birth, the dramatic increase in the number of peroxidase-positive cells indicate that the serum levels are now a product of the neonatal pituitary cells.

The unquestionable presence of prolactin in the hamster fetus and neonate strongly suggests that this hormone plays an important, though yet undetermined, role in the developing animal.


CURRICULUM VITAE

SUE ANN THOMPSON

Current Mailing Address:
Home: 448 W. Parker Blvd. Apt. 9
Baton Rouge, Louisiana 70808
Telephone: 504-766-4323

Business: Department of Zoology and Physiology
Louisiana State University
Baton Rouge, Louisiana 70803
Telephone: 504-388-1132

Birthplace and Date: Marital Status:
New Orleans, Louisiana, March 26, 1938 Single

Academic Training:

Louisiana State University
Baton Rouge, La. 70803
8/72 - 8/75 Physiology/Microbiology PhD

Louisiana State University Medical Center
New Orleans, La. 70112
9/68 - 1/71 Medical Microbiology MS

University of Alabama
Tuscaloosa, Ala.
9/57 - 6/59 Biology/Chemistry BS

Louisiana State University
Baton Rouge, La.
9/55 - 6/57

Louise S. McGehee High School
New Orleans, La.
9/51 - 6/55
Research Experience and Positions Held:

Current status: 8/72 - Present
PhD candidate in the Department of Zoology and Physiology, LSU-Baton Rouge.
Expected date of graduation, August, 1975.

7/65 - 8/72
Research biologist at Tulane Medical School, New Orleans, La.
Working with Dr. George Burch on various aspects of clinical and basic cardiac research. In addition to the general accumulation and analysis of numerous experimental cardiograms, my main duties involved the development of a fluorescent antibody technique for localizing Coxsackie and other cardiotropic viruses in experimental animal systems and in human autopsy and surgical specimens. Other responsibilities included coordinating experiments with the Electron Microscope and Histology Laboratories.

7/59 - 7/65
Associate biologist at Southern Research Institute, Birmingham, Ala.
Supervisor of a cancer chemotherapy research laboratory responsible for screening drugs vs L1210 leukemia in mice. Duties included full responsibility for routine administration, laboratory experiments, and the training, evaluation and rating of 8-10 laboratory technicians.

10/55 - 6/57
Technician for Dr. H. J. Werner, LSU-Baton Rouge.
Research in techniques to Xray the glomerulus of frog kidneys using perfusions of radiopaque thorotраст.

Teaching Experience:

Graduate Teaching Assistant in the following courses:
Histology 3 semesters
Embryology 2 semesters
Intro. Zool. 2 semesters
Histochemistry 1 semester
Physiology 1 semester

Professional Memberships:

Sigma Xi Society
Electron Microscope Society of America
Louisiana Society of Electron Microscopy
Honors:


LSU Graduate School Summer Research Fellowship. Summer, 1974.

American Association of University Women's Award for Outstanding Woman Graduate Student. LSU. May, 1974.


Publications:


EXAMINATION AND THESIS REPORT

Candidate: Sue Ann Thompson

Major Field: Physiology

Title of Thesis: The Embryological Development and Cytodifferentiation of the Pars Distalis of the Golden Hamster (Mesocricetus Auratus) with Special Reference to the Localization and Secretion of Prolactin.

Approved:

[Signatures]

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

Date of Examination: June 17, 1975