J. Anat. (1979), **128**, 1, pp. 121–133
With 11 *figures*
Printed in Great Britain

Cytology of the arcuate nucleus in newborn male and female rats*

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(*Accepted* 19 *January* 1978)

INTRODUCTION

The neural mechanisms controlling adult patterns of gonadotrophin secretion are programmed within the developing hypothalamus of the rat during the early postnatal period (Harris, 1964). This process is influenced by circulating levels of physiologically active oestrogens and aromatizable androgens (Gorski, 1963; McDonald & Doughty, 1974).

Anatomical data on hypothalamic development are sparse. Gonadal steroids have been shown to effect synaptogenesis within the medial preoptic region (Raisman & Field, 1973) and hypothalamus (Matsumoto & Arai, 1976*a*). Furthermore, Arai & Kusama (1968) report that high oestrone doses administered to female rats for 30 days post partum cause a decrease in neuronal nuclear size in the suprachiasmatic, paraventricular, supraoptic, arcuate, and ventromedial nuclei. Additionally, Reier & Rothchild (1973) report structural maturation of the medial preoptic region in the rat between birth and puberty, and Matsumoto & Arai (1976*b*) noted an increase in the number and maturity of synapses within the arcuate nuclei of female rats from day 5 to a plateau at day 45. Finally, the perivascular contact region of the external layer of the median eminence in neonatal rats closely resembles that of the adult (Monroe, Newman, & Schapiro, 1972).

Considering the influence of gonadal steroids in organizing adult patterns of gonadotrophin secretion, it is important to assess the state of development at birth of suspected hypophysiotrophic regions susceptible to the organizing action of gonadal steroids during the critical period of sexual differentiation. An evaluation of the stage of development at birth would indicate what structures could be susceptible to the developmental influences of gonadal steroids. Also, structural features should reflect physiological functions known to exist at birth, such as the production of gonadotrophin-releasing factor (Araki, Toran-Allerand, Ferin & Vande Wiele, 1975) and negative feedback of gonadal steroids to the hypophysiotrophic hypothalamus, which is also operative in the early postnatal period (Yaginuma *et al.* 1969; Gerall & Dunlop, 1971). Elements within the hypophysiotrophic system must be physiologically differentiated and developed sufficiently to account for these phenomena. Finally, a comprehensive study would serve as a morphological base

* This work was supported in part by a grant of the Charlton Fund of Tufts University School of Medicine, QRC Establishment Grant 291–96, MRC Scholarship 24783, and MRC Grant MA-5948. It was in partial fulfilment of the requirements for a doctorate degree by R. J. Walsh whose present address is Department of Anatomy, The George Washington University Medical Center, 2300 I Street N.W., Washington, D.C.

line to assess cytological changes induced by either neonatal androgenization or castration. This report details the cytological features of the arcuate nucleus in newborn male and female rats.

MATERIAL AND METHODS

Pregnant Wistar rats were obtained from the Charles River Breeding Laboratories. The arcuate nuclei of newborn male and female rats were examined with transmission electron microscopy (TEM). All animals were full term (i.e. born after 21 days of gestation). Four male and four female rats were examined. Animals at the time of killing were less than 16 hours old.

Neonates were anaesthetized by an intraperitoneal injection of 35 mg chloralhydrate per 100 g body weight. 0·1 ml of sodium heparin was administered concurrently. Brains were fixed by intracardial perfusion, using a 22 gauge hypodermic needle, of 1% formaldehyde–1% glutaraldehyde buffered to pH 7·4 with 0·12 molar phosphate buffer. The solution contained 0·5 mg of $CaCl_2$ per 100 ml. The perfusion was performed at room temperature and approximately 100 ml of fixative was perfused through the entire body of each animal. The animals were then placed in a jar of fixative and left overnight at 4 °C. The following day the hypothalamus was isolated, trimmed to include the entire extent of the arcuate nucleus, and then processed for TEM as described previously (Brawer, 1971). Tissue was embedded in Epon and oriented in the coronal plane. Sections were cut on a Porter–Blum MT2 ultramicrotome. Sections 1 μm thick were stained with toluidine blue 0. Thin sections were stained with uranyl acetate and lead citrate and examined in an RCA EMU-3 electron microscope.

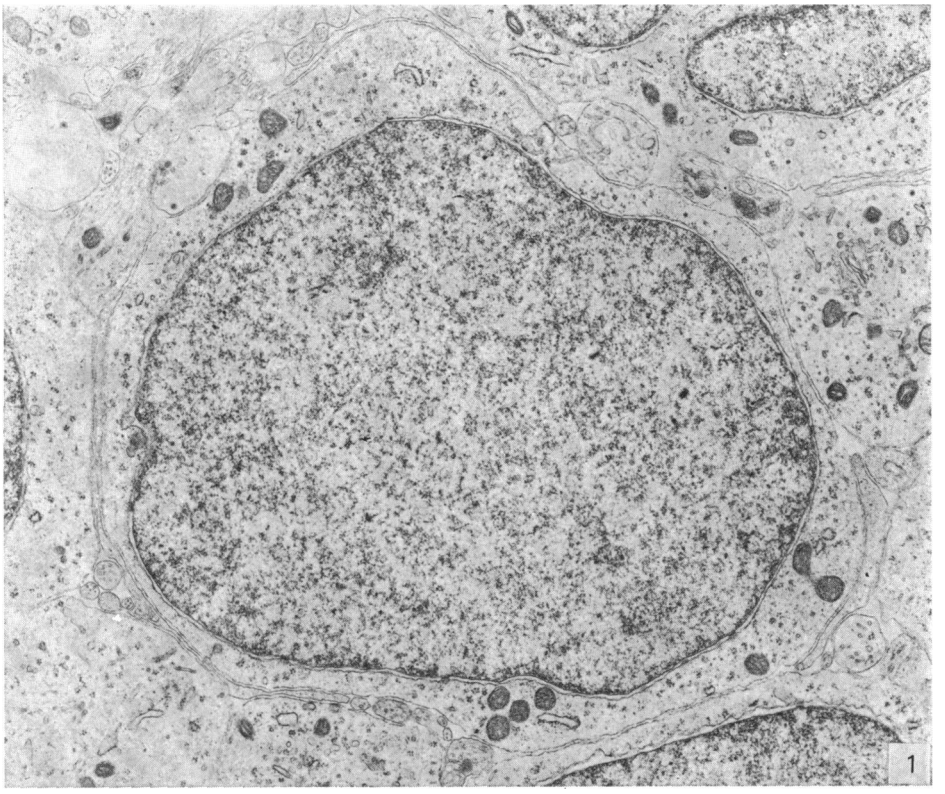
Five levels (A–E) of the arcuate nucleus were chosen for examination at ultrastructural level. Level A corresponds to the mid-anterior region where the arcuate nucleus appears as a single midline mass of cells directly beneath the floor of the third ventricle. Level B is approximately midway between the anterior single midline arcuate nucleus and the expansion of the floor of the third ventricle to form the lateral recesses. Level B exhibits two separate left and right arcuate nuclei lying on either side of the third ventricle, the floor of which has not yet expanded to form the lateral recess. Level C is at mid-lateral recess level and exhibits a very prominent median eminence and adjacent pars tuberalis. Level D includes the mid-infundibular recess where the pituitary stalk is starting to descend from the ventral surface of the diencephalon. Level E is the posterior arcuate nucleus region just posterior to the point where the pituitary stalk has descended from the base of the brain. Both left and right arcuate nuclei of levels B–E inclusive were examined.

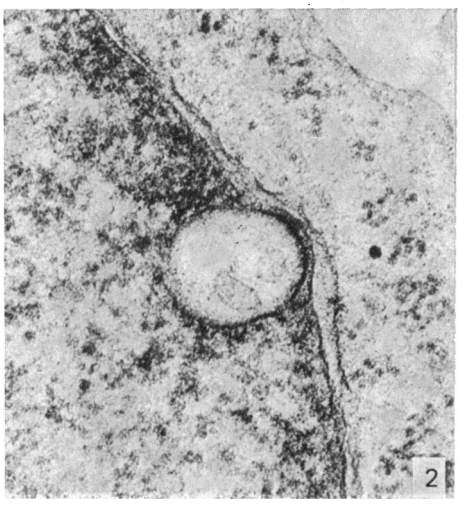
RESULTS

No qualitative differences were found to exist between the arcuate nuclei of newborn males and females, and no significant quantitative differences were apparent. Thus, the following descriptions pertain to both sexes.

Light microscopy

At level A the arcuate nucleus is a single, well circumscribed mass of cells located directly beneath the floor of the third ventricle in the midline. The paired arcuate nuclei of levels B and C exhibit less clearly defined boundaries, since the cell-poor zone between the arcuate and ventromedial nuclei, characteristic of the adult, is





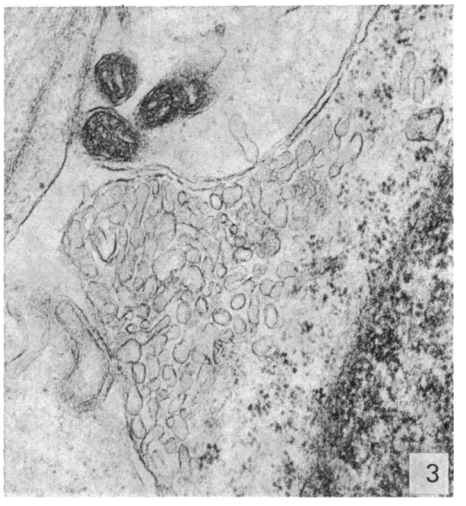


Fig. 1. An organelle-poor cell profile. The cell exhibits a large, centrally located nucleus and a thin perinuclear band of cytoplasm containing polysomes, mitochondria, and a few cisternae of RER. ×11700.

Fig. 2. Nuclear inclusion in an organelle-poor cell profile. The circular, membrane-bound body exhibits a pale interior with a vesicular structure within its core. The inclusion lies adjacent to the inner leaflet of the nuclear envelope and is bordered by a narrow condensation of heterochromatin. ×41540.

Fig. 3. Growth cone of a neuroblast. A large accumulation of clear to diaphanous vesicles and saccules lies directly beneath the cell membrane, creating a small bulge on the cell surface. ×20650.

densely populated with cells in the neonate. The neonatal arcuate nuclei at levels D and E lack clearly defined lateral and dorsolateral limits. At these levels the nucleus consists of a ventromedial condensation of cells which blends continuously with more peripherally located cell masses.

Regardless of level, the cells of the neonatal arcuate nucleus are primarily round to oval. Many exhibit a large centrally located nucleus and a narrow perinuclear rim of cytoplasm. Other cells exhibit a cytoplasmic bulge and an eccentric nucleus. Tear drop, triangular, and elongate perikaryal profiles are also present, but are not as numerous as the round to oval ones. Nuclei are also round to oval, and some exhibit varying degrees of invagination. The majority of nuclear profiles lack nucleoli. When present, however, they may occur either deep within the karyoplasm or adjacent to the nuclear envelope.

Transmission electron microscopy

The neonatal arcuate nucleus exhibits a wide variety of perikaryal profiles. They can be classified into two broad categories: those possessing abundant cytoplasmic organelles (organelle-rich) and those possessing sparse cytoplasmic organelles (organelle-poor). A spectrum of intermediates occurs at all levels.

Organelle-poor cell profiles

Organelle-poor cell profiles are prominent at all levels. These are round to oval with a large centrally placed nucleus and narrow rim of cytoplasm (Fig. 1). The nuclear chromatin consists of small, evenly distributed, heterochromatic aggregates. Nucleoli are usually absent. Occasionally, membrane-bound, circular, nuclear inclusions occur either deep within the karyoplasm or adjacent to the inner leaflet of the nuclear envelope (Fig. 2). These inclusions exhibit a pale interior containing a faint, evenly distributed flocculum as well as occasional irregular pale vesicles. Although the perimeters of some of these inclusions are coated with heterochromatin, others are bare. Interestingly, these nuclear inclusions resemble those occasionally observed in ependymal cells of the developing rat third ventricle (Walsh, Brawer & Lin, 1978).

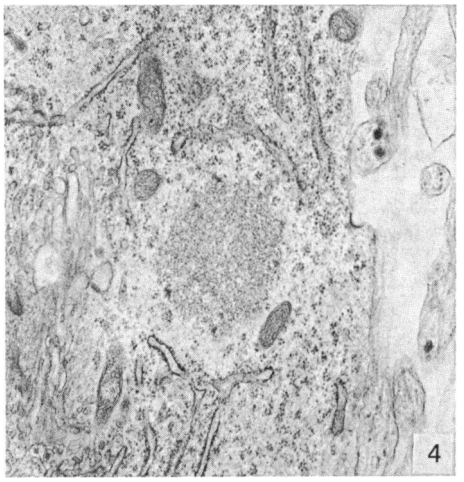
The cytoplasmic organelles of these cell profiles consist primarily of polysomes, mitochondria, and a few short, randomly arranged cisternae of rough endoplasmic reticulum (RER). Some profiles of this class exhibit a small Golgi complex which is often situated in a small expanse of cytoplasm at one pole of the cell. Profiles possessing a Golgi complex often exhibit an increased number of mitochondria and RER cisternae located mostly in the Golgi region.

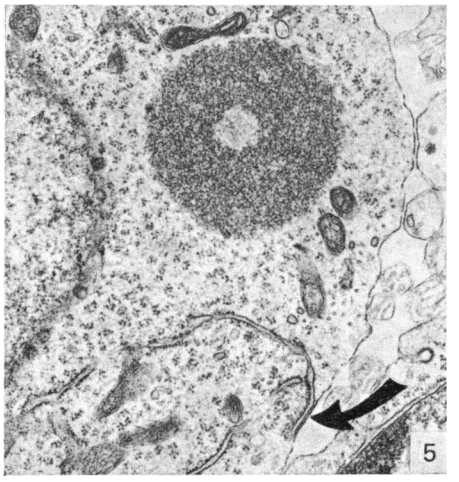
Occasionally, a dense accumulation of irregular, pale, diaphanous vesicles and saccules occurs immediately beneath the cell membrane. The overlying plasmalemma often appears to bulge from the cell, forming a stubby, vesicle-filled process (Fig. 3). The vesicle accumulations resemble growth cones described in other parts of the developing CNS (Del Cerro & Snider, 1968; Hannah & Nathaniel, 1975).

Organelle-rich cell profiles

All levels contain organelle-rich cell profiles which exhibit a much broader range of cytological variation than the organelle-poor cell class. Organelle-rich cell profiles are most often round to oval, but occasionally tear-drop, triangular, or elongated shapes are encountered.

Round to oval nuclei with varying degrees of invagination are often located





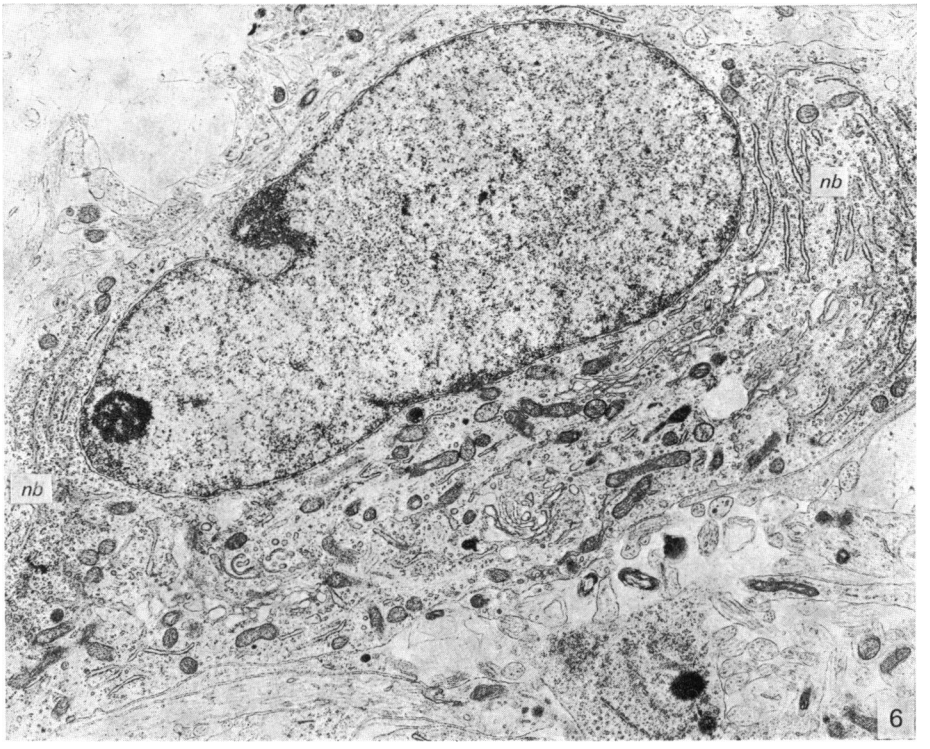


Fig. 4. Cytoplasmic filamentous body in a neuron. It is a uniformly pale structure, partially surrounded by cisternae of RER. × 11650.

Fig. 5. Cytoplasmic filamentous body in a neuron. This CFB consists of a dark matrix with a central spot of less densely packed material. Included in this micrograph is a strand of RER which approaches the cell membrane to lie in a subcisternal position (arrow). × 11850.

Fig. 6. Neuron in the neonatal arcuate nucleus. The eccentric nucleus exhibits a nucleolus and a small condensation of heterochromatin adjacent to the nuclear envelope. The cytoplasm contains two Nissl bodies (*nb*) at its periphery. The remaining cytoplasm contains numerous organelles and inclusions. × 8500.

eccentrically within neuronal profiles. Nuclei appear similar to those of mature neurons, and rarely exhibit membrane-bound inclusions.

The organelle-rich cell profile often displays multiple Golgi complexes, as well as one or two narrow coated cisternae and coated vesicles in close proximity to the Golgi apparatus. Furthermore, some cell profiles exhibit one or two long, narrow, smooth cisternae that are oriented parallel to the Golgi saccules. Cisternae of RER occasionally appear in close proximity to, and aligned in parallel with, the narrow cisternae. These various configurations of cisternae and adjacent smooth endoplasmic reticulum may represent components of GERL (Holtzman, Novikoff & Villaverde, 1967; Novikoff, Novikoff, Quintara & Hauw, 1971).

Short segments of RER often lay parallel and adjacent to the cell membrane (Fig. 5, arrow). This variety of subsurface cisternae is also seen in organelle-poor cell profiles, and has been observed in neuroblasts of developing rat spinal cord (Hannah & Nathaniel, 1975) and chick embryo spinal ganglia (Pannese, 1968) as well as in mature neurons (Rosenbluth, 1962). Unique to organelle-rich cell profiles are subsurface cisternae the lumina of which are collapsed or constricted over much of the length of the cisternae. These subsurface cisternae resemble the varieties reported in mature neurons by Rosenbluth (1962).

Rarely, organelle-rich cell profiles exhibit a lamellar body deep within the cytoplasm which is in continuity with rough endoplasmic reticulum. Similar structures occasionally occur in a subsurface position, as has been observed in mature neurons (Rosenbluth, 1962).

Two varieties of cytoplasmic filamentous bodies are occasionally seen in the organelle-rich cell profiles. One variety exhibits a fine, fibro-granulated texture of a faint uniform density (Fig. 4). The other displays a dark matrix spotted with small, lighter areas of less densely packed material (Fig. 5).

Abundant polysomes are scattered throughout the cytoplasm of organelle-rich cell profiles, and sometimes there are a few coated vesicles and dense core vesicles. Sparse microtubules course singly or in small bundles in an apparently random fashion. Occasional centrioles and myelin figures are seen in organelle-rich cell profiles. Growth cones, similar to those described in the organelle-poor cell profiles, also occur on the surface of organelle-rich cell profiles. Multivesicular bodies and dense bodies are also common to organelle-rich cell profiles.

The proximal portions of many dendritic trunks contain the cytoplasmic organelles characteristic of the adult (Peters, Palay & Webster, 1970). In some cell profiles the majority of the cell cytoplasm and organelles are in the proximal dendritic trunk.

Pronounced differences occur between the organelle-rich cell profiles with regard to their quantity and organization of RER. For example, some organelle-rich cell profiles exhibit peripherally located Nissl blocks (Fig. 6) which vary in size and extent from cell to cell. Other perikarya exhibit a prominent peripheral band of RER oriented parallel to the cell surface, with cisternae varying widely in length, and frequently branched. Numerous at all levels are cell profiles with a very random arrangement of RER, the cisternae of which vary widely in length and quantity and intermix with the other cytoplasmic organelles.

Glia and neuropil

Mature oligodendrocytes and astrocytes are not present in the newborn arcuate nucleus. A variety of cell profiles occurs that probably corresponds to glioblasts or immature glia.

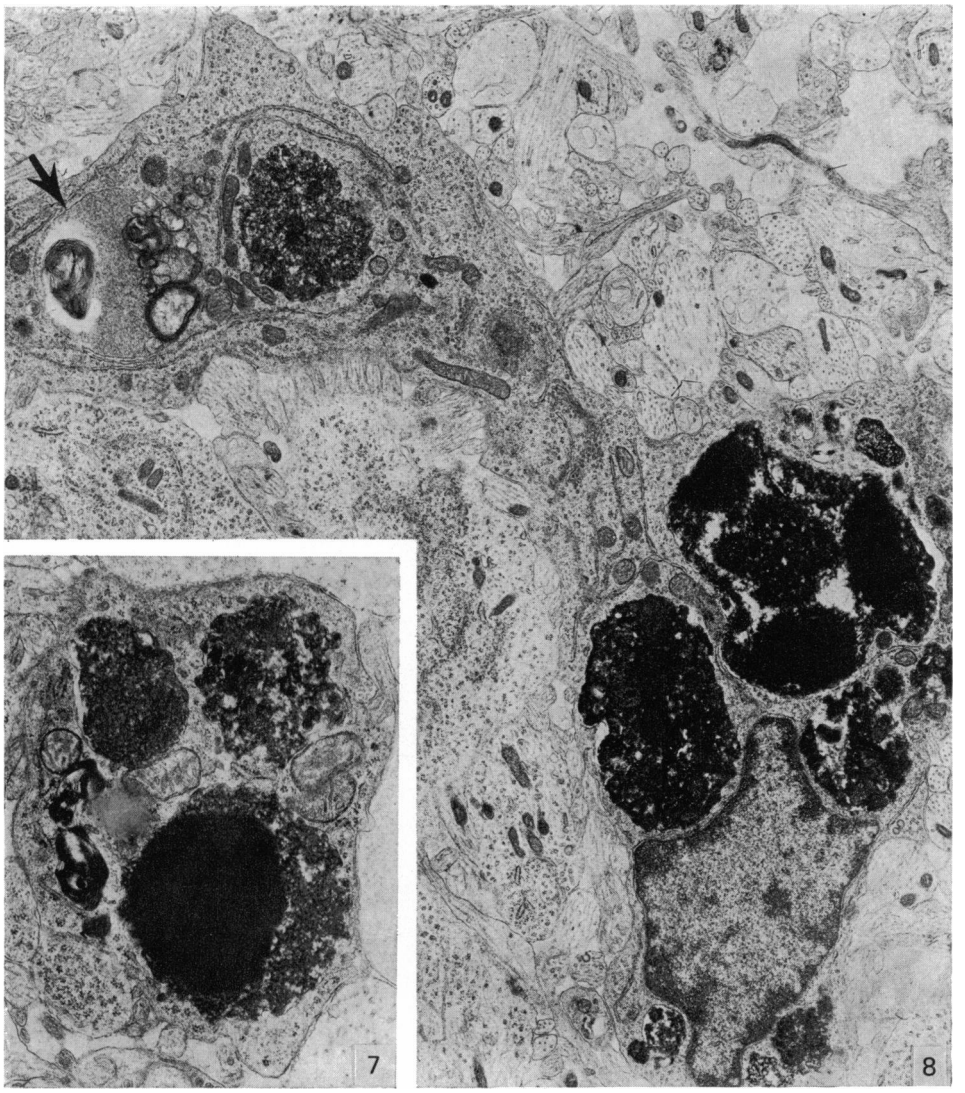
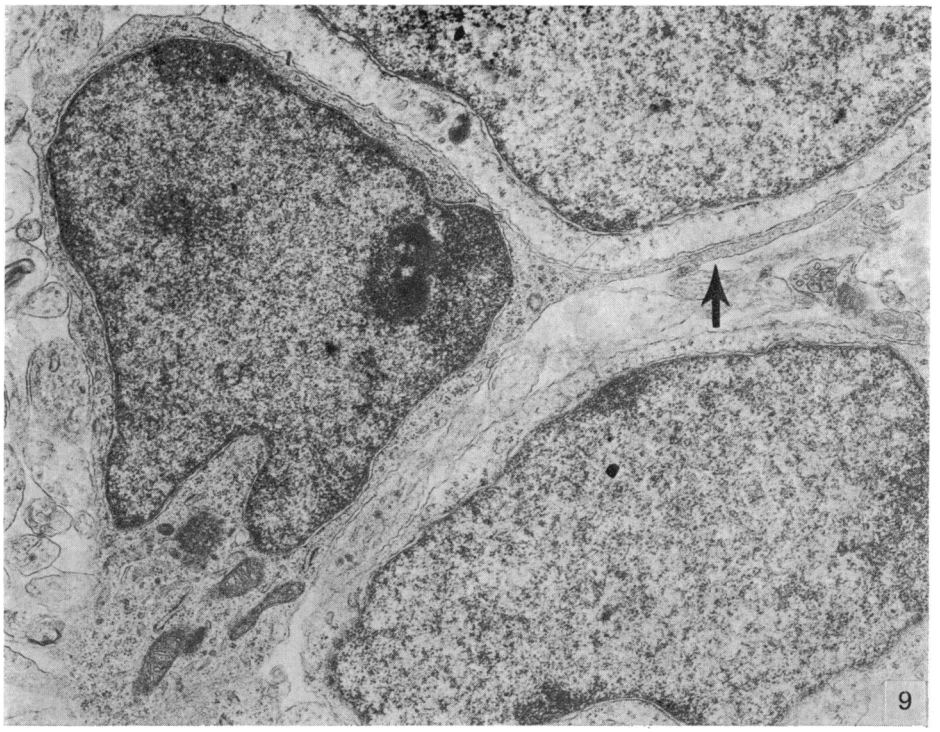


Fig. 7. Dense aggregates of a course, membrane-bound material within a dense cytoplasm. ×9600.

Fig. 8. Reactive microglial cell with dense cytoplasm containing varying sized, membrane-bound accumulations of a dark, pyknotic-looking material. One inclusion exhibits a lighter matrix (arrow) within which are myelin figures. ×5400.

Rarely, the neonatal arcuate nucleus exhibits membrane-bound accumulations of dense, coarse material which are encapsulated by a rim of dark cytoplasm containing a few cisternae of RER, mitochondria, and ribosomes (Fig. 7). Occasionally the processes encompassing such bodies show continuity with a cell profile (Fig. 8) which exhibits cytological characteristics associated with microglia (Mori & Leblond, 1969).

Other cell profiles with a dark cytoplasm exhibit a homogeneously heterochromatic nucleus with an occasional nucleolus (Fig. 9). The cytoplasm contains primarily polysomes, varying length cisternae of RER, and mitochondria.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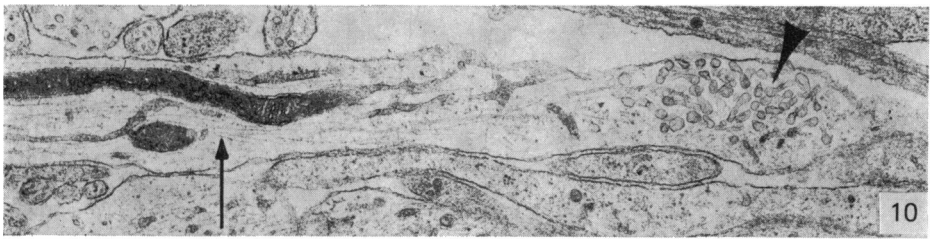
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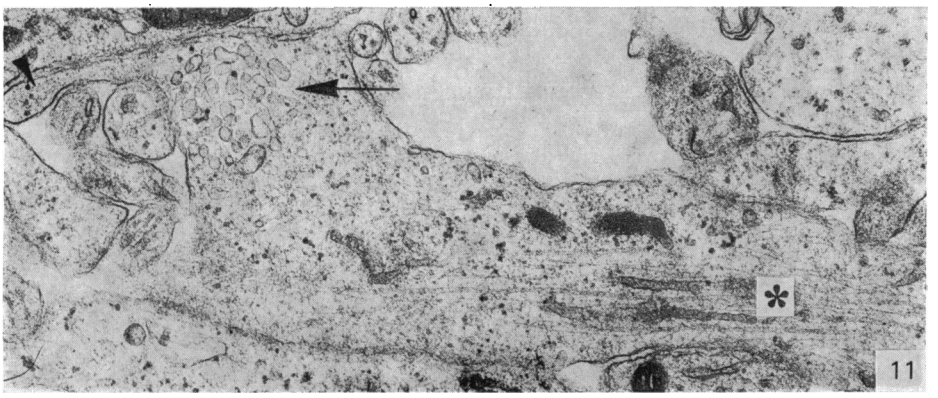
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Occasionally, these profiles exhibit a dense body, lipid droplet, or a few microtubules. The nuclear envelope may exhibit deep invaginations, and cell shape varies widely. Thin processes occasionally extend from these cells and meander between elements of the neuropil, or over the surface of neighbouring cells (Fig. 9 – arrow). These profiles may represent immature macroglia, but due to the lack of more distinguishing features their identity is uncertain.

The neonatal arcuate nucleus exhibits variable amounts of extracellular space, which is characteristic of developing nervous tissue (Sumi, 1964). It also displays small, unmyelinated axons and dendritic profiles characteristic of the adult arcuate nucleus (Brawer, 1971). The axons course primarily in an anterior to posterior direction. Although the adult arcuate nucleus exhibits myelinated axons, none are present in the neonatal arcuate nucleus. In addition, the neuropil of the neonatal arcuate nucleus contains processes that exhibit cytological features characteristic of growth cones described in other regions of the developing central nervous system. Some of these contain primarily loosely packed, paraxial filaments, agranular endoplasmic reticulum, and mitochondria (Fig. 10). Others additionally display sparse, paraxial microtubules and an occasional dense body (Fig. 11). The organelles, microtubules, and most of the filaments usually terminate prior to the distal ends of these processes which expand into variably shaped bulbs with amorphous ground substance. The terminus often contains an accumulation of clear to slightly electron-dense vesicles and saccules (Fig. 10). Frequently projecting from the terminal region are narrow filapodia containing only the flocculent ground substance and occasional filaments (Fig. 11, arrowhead). Detached profiles of these filapodia are seen throughout the neuropil.

Focal accumulations of subsurface vesicles and saccules, similar to those described in the perikarya, often occur on the periphery of dendrites and processes containing primarily mitochondria, microtubules, agranular endoplasmic reticulum and ribosomes. These clusters of growth vesicles usually occupy a bulge or small distension of the process plasmalemma.

Synapses are sparse throughout the neonatal arcuate nucleus. Although several typical adult synapses are observable, most synapses exhibit a paucity of vesicles, and the pre- and post-synaptic specializations are often poorly developed. Dense core vesicles, variable shaped saccules and vesicles, and tubular SER are occasionally present in these synaptic structures.

Fig. 9. Dark cell profile, possibly a glioblast. The nucleus exhibits a relatively homogeneous distribution of chromatin. The dark cytoplasm contains primarily mitochondria and polysomes. A long, thin process (arrow) projects from the cell body and courses over the surface of a neighbouring cell. × 13800.

Fig. 10. Growth cone. The proximal portion contains fine filaments (arrow), mitochondria, and agranular endoplasmic reticulum. The distal end is a vesicle and saccule-filled bulb (arrowhead) with a flocculent background and a few short fine filaments. × 22200.

Fig. 11. Growth cone. The proximal stalk (*) contains fine filaments, microtubules, mitochondria, and agranular endoplasmic reticulum. The distal end expands into a broad process with a flocculent background and a few short, fine filaments. A collection of vesicles and saccules (arrow) occurs in one corner of the distal expanse from which projects a narrow filapodium (arrowhead). × 18500.

DISCUSSION

The arcuate nuclei of newborn male and female rats exhibit cytological features indicative of a very early stage of development. These features include (1) a significant population of organelle-poor cell profiles, suggestive of immature or undifferentiated cells, (2) a paucity of synapses, (3) the immature appearance of synapses that are present, (4) a lack of definitive macroglia, and (5) the presence of growth cones.

The organelle-poor cell profiles observed in the present study resemble the early neuroblasts of neonatal rat spinal cord (Hannah & Nathaniel, 1975), cerebral cortex (Caley & Maxwell, 1968*a*), and cerebellum (Nosal & Radouco-Thomas, 1971). Furthermore, within neuroblasts of the developing rat spinal cord, cerebrum, and cerebellum there is a progressive increase in the quantity and complexity of organization of cytoplasmic organelles as neuronal differentiation proceeds. Organelle-poor perikarya are sparse in the arcuate nucleus of the adult rat, and thus the majority of organelle-poor cells observed in the present study will undoubtedly develop further. Thus, the varieties of neuronal profiles observed in the present study may in part reflect a developmental continuum, the extremes of which are represented by the organelle-rich and organelle-poor profiles.

The overall structural immaturity of the neonatal arcuate nucleus is indicated by its paucity of synapses. Furthermore, axon terminals present at birth appear immature as suggested by scant synaptic vesicles, the presence of occasional growth vesicles, and usually poorly developed pre- and post-synaptic specializations. Matsumoto & Arai (1976*b*) quantified synapses in the arcuate nucleus of female rats 5, 20, 45, and 90 days of age. On day 5 the arcuate nuclei contained a very small number of synapses, and these exhibited small numbers of synaptic vesicles and thin pre- and post-synaptic specializations. Up to day 45 the number of synapses in the arcuate nucleus increased, and there was a progressive maturation in synaptic cytology.

The arcuate nuclei of newborn rats lack mature macroglia, as does the neonatal rat cerebral cortex (Caley & Maxwell, 1968*b*), spinal cord (Hannah & Nathaniel, 1975), the cerebellum (Woodward, Hoffer, Siggins & Bloom, 1971). The dark cell profiles observed in the present study, apart from those suggestive of microglia, closely resemble the small glioblasts of the mouse corpus callosum (Sturrock, 1976), and those depicted in the newborn rat spinal cord (Hannah & Nathaniel, 1975). Furthermore, the lack of differentiated oligodendroglia in the neonatal arcuate nucleus correlates with the absence in neonates of myelinated axons, which do exist in the arcuate nucleus of adult rats (Brawer, 1971).

The large, dense masses within apparent microglial cells are not characteristic of the arcuate nucleus in adult male rats (Brawer, 1971). However, similar inclusions appear in phagocytes of the neonatal rat corpus callosum (Leblond, personal communication) and within the neuropil of chick embryonic spinal cord (Wechsler, 1966). These inclusions resemble phagocytized products of cell degeneration as seen in the mutant 'staggerer' mouse cerebellum (Sotelo & Changeux, 1974). Inasmuch as cell degeneration was not observed in the neonatal arcuate nucleus, the presence of reactive microglia may reflect a terminal phase of prenatal degeneration, for there is evidence to suggest that degeneration accompanies normal development of the central nervous system (Jacobson, 1970).

The presence of growth cones further indicates the morphological immaturity of the neonatal arcuate nucleus. The subplasmalemma accumulations of growth

vesicles, such as in Figure 3, resemble growth cones observed in the developing rat spinal cord (Hannah & Nathaniel, 1975), cerebral cortex (Johnson & Armstrong-James, 1970), and cerebellum (Del Cerro & Snider, 1968). Additionally, fine filaments, agranular endoplasmic reticulum, and filapodia are prominent components of growth cones described by other investigators both *in vivo* (Kawana, Sandri & Akert, 1971; Hinds & Hinds, 1972) and *in vitro* (Yamada, Spooner & Wessells, 1971). The presence of growth cones in the neonatal arcuate nucleus correlates with the observed paucity and immaturity of synapses.

It appears from the preceding account that the arcuate nucleus is largely undeveloped and undifferentiated in newborn male and female rats. However, the arcuate nucleus of newborn rats also exhibits some neuronal profiles with the variety and quantity of organelles characteristic of the adult (Brawer, 1971). Furthermore, the arcuate nuclei of adult male rats exhibit some neuronal profiles with a paucity of cell organelles. Thus, the neonatal arcuate nucleus exhibits cytological features suggesting developmental immaturity in combination with some structurally mature cells. Those elements of this neonatal mosaic which resemble adult components of the medial basal hypothalamus may play a role in the early postnatal regulation of gonadotrophin secretion. The hypophysiotropic area is sufficiently developed at birth to produce gonadotrophin-releasing factors as determined by radioimmunoassay (Araki *et al.* 1975). Also, steroid feedback mechanisms are operable in the early postnatal period, as evidenced by significant ovarian compensatory (Gerall & Dunlop, 1971) and testicular hypertrophy (Yaginuma *et al.* 1969) within the first 5–10 postnatal days in male and female rats unilaterally castrated at birth. The structurally mature elements seen on the day of birth in the arcuate nucleus may play some role in early hypophysiotropic function. Changes in hypothalamic regulation of gonadotrophin secretion with age may in part reflect the progressive structural maturation of the undeveloped portion of this system. For example, the paucity of synapses in the neonatal arcuate nucleus suggests minimal afferent connexions, such as those from the medial preoptic–anterior hypothalamic region (Dyer & Cross, 1972). Since the number of synapses in the arcuate nucleus increases with age (Matsumoto & Arai, 1976*b*), the activity of arcuate neurons will be progressively influenced by other regions with increasing age. Furthermore, subsequent development of neuronal circuitry probably involves, at least in part, establishment of intrinsic connections. Additionally, increases in hypothalamic content of gonadotrophin-releasing factor with age (Araki *et al.* 1975) may in part reflect the progressive maturation of poorly differentiated arcuate neurons into organelle-rich cells typical of the adult.

The structural maturation of the poorly differentiated components of the neonatal arcuate nucleus may be under control of gonadal steroids (Sheridan, Sar & Stumpf, 1974*a*, *b*; Toran-Allerand, 1976; Matsumoto & Arai, 1976*a*). The lack of apparent sexual dimorphism in the neonatal arcuate nuclei may well reflect the sexually undifferentiated state of both the male and female hypothalamus.

SUMMARY

Five coronal levels of the arcuate nuclei in newborn male and female rats were examined with the transmission electron microscope. The nuclei from male and female neonates appear similar in all respects. All levels exhibit a significant population of round to oval cell profiles with large centrally located nuclei and scant cytoplasm which contains predominantly ribosomes, sparse mitochondria, and a

few short cisternae of rough endoplasmic reticulum. These organelle-poor cell profiles resemble neuroblasts in other parts of the developing CNS. The arcuate nuclei of neonates also exhibit some cell profiles with the variety and quantity of organelles characteristic of mature neurons in the arcuate nuclei of adult rats. In addition, the neonatal arcuate nuclei show a paucity of synapses with apparent immaturity of those present, and numerous structures identified as growth cones. Definitive macroglia are not present in the arcuate nuclei of newborn rats.

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