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A MORPHOLOGICAL INVESTIGATION OF THE EPENDYMAL SURFACE OF THE THIRD CEREBRAL VENTRICLE

by

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Submitted in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

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ABSTRACT

The morphology of the ependymal lining of the third cerebral ventricle in the rabbit was studied with the light microscope, transmission and scanning electron microscopes.

The walls of the third ventricle are lined for the most part by ciliated-cuboidal ependyma. At least two types of ciliated ependymal cells were identified on the basis of their morphology. A third and distinct population of ependymal cells - the tanycyte ependymal cells however, represented the main cell type encountered in only a narrowly circumscribed region of the ventricular wall in the rabbit especially in the infundibular recess of the third ventricle. Tanycytes could be distinguished easily from the common mural form of ependymal cell by virtue of their radially directed basal processes which projected for variable distances into the hypothalamic neuropil. Such processes frequently terminated on blood vessels in a manner reminiscent of perivascular astrocytes. The luminal surface of the tanycyte is not ciliated.

Examination of the surface of the ventricle with the scanning electron microscope revealed a pattern of regional differences in surface morphology that was consistent with the distribution of common mural and tanycyte ependymal cells in the rabbit seen with the light and electron microscopes. On the basis of this regional variation, the ventricle could be divided into three distinct regions; dorsolateral wall, transitional zone and ventrolateral wall and floor. The dorsolateral wall is distinguished by the presence of a homogeneous population of densely-ciliated cells, whereas the luminal ependymal

surface of the ventrolateral wall and floor of the ventricle, corresponding to the area of greatest tanycyte concentration is non-ciliated and exhibits a rather more distinctive morphology. With minor interspecies differences a similar organization was seen in the third ventricle of the rat, mouse, rat kangaroo and human. It is compatible with the view that morphologically different regions may possess different functional capacities.

The efficacy of synthetic LH-RH in inducing ovulation in the rabbit was determined for the intraventricular and intravenous routes of administration and compared. The minimum effective dose of LH-RH required to induce ovulation in 100% of the rabbits when injected into the marginal ear vein was 400 ng/kg body weight. The equivalent dose in terms of its effectiveness when injected into the lateral cerebral ventricle was only 100 ng/kg body weight. The results suggest that when systemically ineffective amounts of LH-RH are injected intraventricularly they are able to induce ovulation in the rabbit and that intraventricular administration may be 4-5 times as potent as intravenous administration in this respect. The possible reasons for this are discussed. The efficacy of synthetic LH-RH in inducing ovulation in conscious and urethane anesthetized rabbits when administered intravenously was also determined. The results showed that ovulation in the rabbit was neither delayed nor blocked by urethane anesthesia. The response of ependymal cells within the lateral walls and floor of the rabbit third ventricle to ovariectomy and to ovulation, induced by intravenous or by intraventricular administration of LH-RH was determined. No significant changes in ependymal morphology were found that could be ascribed to either of these two commonly accepted procedures

for determining endocrine interrelationships.

In contrast, pronounced changes in the morphology of the ependymal surface of the ventrolateral wall and floor of the third ventricle were observed in rabbits sacrificed 5 minutes after intraventricular injection of LH-RH. These changes which took the form of microvillous eruptions on the luminal surface of the ependymal cells were not seen in rabbits sacrificed at 15 minutes after LH-RH injection nor in saline controls. The nature of the changes suggested that they are transient eruptions of the surface which may signify increased absorptive activity. These observations provide some morphological basis for the hypothesis that the ependyma of the ventrolateral wall and floor of the third ventricle may be implicated in the uptake and possibly the transfer of LH-RH from the CSF to either the hypothalamic neuropil or to the pituitary portal vascular plexus.

This investigation was supported by a three year-term research grant to Dr. D. G. Montemurro, Department of Anatomy, The University of Western Ontario, from the Medical Research Council of Canada. The author wishes to express his appreciation to this organization for their support.

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LIST OF ABBREVIATIONS

AC	Anterior commissure
ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
AP	Area postrema
A-P	Anterior-posterior plane
ARC	Arcuate nucleus
As	Astrocytic
AsP	Astrocytic process
CAHP	Chrome alum hematoxylin phloxine
Cap	Capillary
CC	Corpus callosum
CD	Caudate nucleus
CH	Cerebral hemisphere
CL	Capillary lumen
CNS	Central nervous system
CO_2	Carbon dioxide
CP	Choroid plexus
CRF	Corticotropin-releasing factor
CSF	Cerebrospinal fluid
CVO	Circumventricular organ
D	Dendrite
DB	Diagonal band
DCV	Dense core vesicle

E	Ependymal cell
Ep	Ependymal process
EpT	Tanycyte ependymal cell
ER	Rough endoplasmic reticulum
FSH	Follicle stimulating hormone
FSH-RH	Follicle stimulating hormone - releasing hormone
Fx	Fornix
G	Golgi complex
GH	Growth hormone
HCG	Human chorionic gonadotropin
HDM	Dorsomedial hypothalamic nucleus
HRP	Horseradish peroxidase
HVM	Ventromedial hypothalamic nucleus
IR	Infundibular recess
LH	Luteinizing hormone
LH-RH	Luteinizing hormone - releasing hormone
LM	Light microscope (microscopy, microscopic)
LPO	Lateral preoptic nucleus
LSN	Lateral septal nuclei
LV	Lateral cerebral ventricle
Ly	Lysosome
M	Microglia
MD	Medulla oblongata
ME	Median eminence of tuber cinereum
MED_{100}	Minimum effective dose 100
MIF	Melanocyte stimulating hormone release - inhibitory hormone

MM	Mammillary body
MPO	Medial preoptic nucleus
MR	Mammillary recess
MSN	Medial septal nuclei
MTN	Midline thalamic nuclei
N	Neuron
N=	Sample size (in tables)
NaCl	Sodium chloride
Nuc	Nucleus
OB	Olfactory bulb
OCh	Optic chiasma
ON	Optic nerve
OsO_4	Osmium tetroxide
OT	Optic tract
OVLT	Organum vasculosum of the lamina terminalis
P	Pericyte
Pb	Polar body
PAS	Periodic acid-Schiff
PC	Posterior commissure
PF	Parafloccular lobule
PL	Pyriform lobe
PM	Cerebellar hemisphere
PO	Pons
PV	Paraventricular nucleus
PVO	Paraventricular organ
SCO	Subcommissural organ
S.D.	Standard deviation

SEM	Scanning electron microscope (microscopy, microscopic)
SFO	Subfornical organ
SO	Supraoptic nucleus
Tc	Tuber cinereum
TEM	Transmission electron microscope (microscopy, microscopic)
TFP	Tanycyte terminal process
Tp	Tanycyte process
TRH	Thyrotropin-releasing hormone
TS	Tanycyte soma
TSH	Thyroid stimulating hormone
V	Cerebral ventricle
Ve	Vermis
V_{III}	Third cerebral ventricle
Za	Zonula (puncta) adherens
bb	basal body
cil	cilia
dH_2O	distilled water
f	filaments
fm	foramen magnum
hp	hard palate
i	island of ependymal cytoplasm
m	maxillary and mandibular archs
min	minute
mit	mitochondria
mv	microvilli
mvb	multivesicular body

n	neuronal processes (dendritic and/or axonal)
nc	nose clamp
no	nasal cavity
o	occipital bone
oc	orbital cavity
orc	oral cavity
ps	perivitelline space
r	ribosome
sem	standard error of mean
vm	vitelline memrane
wk	week
$\bar{x}$	mean
z	zygoma
za	zygomatic arch
zp	zona pellucida
III	oculomotor nerve

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I. INTRODUCTION

The ependymal lining of the cerebral ventricles has received comparatively little attention from investigators over the past several decades. Purkinje (1836) is generally credited with being the first to document the epithelial character of the lining of the ventricles. Reviews of the early literature on the topic of ependyma are contained in the papers of Studnička (1900), Agduhr (1932) and Wislocki (1932). They indicate that variation in ependymal cell morphology was recognized by the early investigators. The presence of some ependymal cells for example with basal processes extending into the neuropil had been noted. Some uniformity in the terminology applied to these morphologically distinct cells was provided by Horstmann (1954) when he applied the descriptive term "tanycyte" to elongated ependymal cells with a single long peripherally directed process in Selachians. The presence of such distinct ependymal elements have been restricted largely to non-mammalian vertebrates, to developmental stages of mammalian forms and to rather circumscribed regions of the ventricular system in mature mammals.

The revival of interest in ependyma in recent years is due mainly to the tanycyte which is conceived by some as a specialized form of ependymal cell. Attention has focussed primarily on the tanycyte ependymal cells of the mammalian third ventricle. Interest in these cells derives largely from the anatomical connection

they have been reported to establish between the cerebrospinal fluid on the one hand and the neurons and capillaries of the hypothalamic neuropil on the other. The intriguing relationship established by tanycytes of the infundibular recess, between the CSF and the hypophysial-portal vasculature led Löfgren (1959, 1960) to postulate a CSF to pituitary transport system with a possible involvement in the neuro-endocrine regulation of adenohypophysial function. A great deal of evidence, albeit largely speculative, for such an involvement has subsequently been generated and this has given rise to a plethora of current hypotheses.

Although there is a growing awareness that the ependyma may be more than just an inert epithelial lining and less of a barrier than was once suspected, our present information on both the structural and functional diversities of the ventricular walls is still fragmentary. The precise cytological features of tanycytes, their distribution, their interrelationships within the ventricular walls and the neuropil and their function remain poorly understood. As a basis for determining function, I have examined regional differences in the structure of the ventricular walls in the rabbit with the light microscope, transmission and scanning electron microscopes. Attention was focussed principally on the third ventricle and on the resident population of tanycyte ependymal cells.

Prior to 1970, almost all available data on ependyma had been gathered from conventional light microscopic and transmission electron microscopic studies. Knigge and Scott's (1970) abbreviated report of regional differences in surface morphology within the infundibular

recess of the rat was among the first reports employing scanning electron microscopy. The scanning electron microscope seemed to be particularly well suited for the study of ependyma because it afforded a convenient means of visualizing and comparing broad regions of the ventricular walls. This had been previously impossible with the more conventional methods of investigation and thus represented a distinct advantage. In recent years, the scanning electron microscope has been used rather more extensively for this purpose.

Since Löfgren's initial suggestion that specialized forms of ependyma may subserve some unknown neuroendocrine function, a number of reports have emerged which have encouraged this belief. A number of correlations between pituitary hormone secretion and changes in ependymal cell morphology have been documented, the evidence that tanycytes are responsive in some obscure way to endocrinological changes within the animal however is confusing, largely inconsistent and somewhat tenuous. We have therefore attempted in this study to characterize morphologically the responsiveness of these cells to ovariectomy and ovulation, two commonly accepted procedures for determining brain-pituitary-gonadal endocrine interrelationships.

Lastly, several lines of evidence have led to speculation that the cerebrospinal fluid may serve as a vehicle for the transport of hypothalamic neurohormones to the pituitary. It is envisaged that this occurs by traversing the median eminence, and the ependymal tanycyte components of this tissue have been implicated in this translocation. Although a number of investigators have suggested

that tanycytes may be involved in the selective absorption and/or transport of a variety of active principles from the CSF, the relationship of these ependymal cells to the movement of such substances is obscure. In this investigation I have examined in a preliminary way the feasibility of such a transport system by determining: 1) whether synthetic LH-RH intraventricularly administered can exert an effect on the pituitary, 2) the relative effectiveness with which this is accomplished (i.e. the effectiveness of LH-RH in inducing ovulation by the intraventricular route of administration as compared with intravenous administration) and 3) whether any anatomic changes could be found that might suggest an ependymal involvement.

2. REVIEW OF THE LITERATURE

2.1 Morphology of the Walls of the Cerebral Ventricles

Surprisingly little is known about regional differences in the structure and function of the ventricular lining. Most reports dealing with the subject indicate that the lining of the ventricles consists of a continuous epithelial-like layer of ependymal cells that varies from flattened squamous-like to cuboidal-columnar in form, with and without cilia and cell processes. However, considerable variation in the morphology and stratification of the ependyma and hypendyma occurs with age, species and location within the ventricular system. For example, a pseudostratified or multilayered ependymal lining, while typical in embryonic and neonatal stages, is rarely found in the adult (Tennyson and Pappas, 1962, 1968).

Some attention has been given to structural variation in the ventricular wall as a basis for understanding uptake and exchange of substances between the CSF and the neuropil. Monakow (1921) expressed the view that the CSF may pass from the ventricles through the whole mass of the brain and in so doing convey a number of hormonal substances to nuclear regions. In anesthetized cats, uptake of the acidic dye bromophenol blue from the perfused ventricles was found to vary in different regions of the ventricular wall (Feldberg and Fleischhauer, 1960). Very little penetration of dye was observed in those regions of the ventricular walls underlain by white matter, while particularly intense penetration occurred in regions that

consisted mainly of grey matter, such as the wall of the third ventricle and infundibulum. Similar observations were made in cats with histamine perfused intraventricularly (Dráskoci, Feldberg, Fleischhauer and Haranath, 1960) and acetazolamide injected intravenously (Roth, Schoolar and Barlow, 1959). The transfer of bromophenol blue from the CSF to brain parenchyma was considered to be an active process influenced by regional differences in the cytology of the ependyma and inherent cytological differences in the neuroglia of grey and white matter (Feldberg and Fleischhauer, 1960).

Characteristic regional differences in ependymal and subependymal structure have been reported in several species. In the walls of the lateral ventricles three more or less distinct layers are generally distinguishable: 1) the ependymal lining, 2) a subependymal fiber layer, and 3) a mitotically active subependymal cell layer. The composition and function of the mitotically active layer is not fully known. It has been given a variety of designations in the literature. Globus and Kuhlenbeck (1944) referred to it as the "subependymal cell plate" and, because of its complement of undifferentiated cells, considered it to be a potential source of neoplasms of neuroectodermal origin. Lewis (1968) reported that the subependymal plate persists in the adult rat and gives rise to subcortical neuroglia of different types, astrocytes, oligodendroglia and microglia. Smart (1961) however, concluded that the fate of the cells of this layer in the adult mouse was not to migrate into the brain and differentiate, thereby contributing to the cell population, but rather to degenerate within the functionless layer. In adult mice and rats, the walls of the lateral ventricles are composed of a

columnar ependymal cell layer and subependymal cell plate which are variable rostro-caudally (Smart, 1961; Westergaard, 1970). The subependymal cell plate, varies in thickness from one to several cells and is most pronounced in the lateral wall of the anterior horn. It becomes gradually less prominent caudally through the anterior horn and body of the ventricle. The subependymal cell plate is absent in the caudal part of the body of the ventricle and in the inferior horn, and is generally not found where the wall overlies white matter. The ependymal cells overlying the subependymal cell plate are tallest in the rostral part of the anterior horn, decreasing in height through the body and inferior horn, and are generally very low in the region of the wall overlying white matter.

In the cat, a well defined subependymal glial fiber layer is also lacking in the rostral part of the lateral ventricle and in practically all regions of the ventricles bordered by white matter (Fleischhauer, 1961). The ependyma is tall and ciliated in the upper region of the cat hypothalamus with a thick, ordered layer of glial fibers beneath. In contrast, ependymal cells were reported to be smaller, and the subependymal glial fiber layer absent, near the floor of the hypothalamus, septum pellucidum and caudate nucleus. The ventricular wall of the hypophysis is characterized by flat nonciliated ependyma with no subependymal glial fiber layer, whereas a layer of tall ependymal cells and a dense network of subependymal glial fibers are found in the pineal recess.

The presence of fusions (coarctations) of the ventricular walls has been described in the anterior horn, body and inferior horn of the lateral ventricles in a number of species, both neonatal and

adult (Westergaard, 1970). Contact between ventricular walls is believed to be a prerequisite for their formation. In the human, coarctations were observed infrequently, a fact attributed by Westergaard to the large size of the ventricular cavities where the walls are less likely to be in contact. Histological examination of the sites of fusion indicate that the ependymal cells of adjacent walls become closely applied and fuse, obliterating the ventricular lumen. Most of the ependymal cells disappear, while some persist to form islands, sometimes surrounding a small lumen. No histological evidence to suggest why walls fuse in certain areas was apparent.

Circumscribed regions of folded ependyma (sulcated areas) of unknown significance are found consistently throughout the ventricular walls in a number of species (Friede, 1961). In the human, sulcations were found principally in the thalamic, mammillary and supraoptic areas of the third ventricle. Sulci showed regular plicae covered by normal ependyma of medium height. The plicae contained fibrous glial tissue with very few nuclei. The subependymal cell plate was frequently thickened beneath the sulcated areas. Sulcations were believed to result from active growth rather than passive narrowing or mechanical folding of the ventricular lumen. The development of both the sulcated areas and the subependymal cell plate appeared to be related to the size of the brain or its ventricles. In an earlier paper, Friede (1951) suggested that the increased surface area of these sulcations may facilitate exchange of substances between the CSF and brain tissue. Although all cavities of the central nervous system are generally lined with a continuous ependymal epithelium, areas in which the ependymal lining is lacking are not uncommon in man

and other vertebrates (Agduhr, 1932; Friede, 1961; Schmrigk, 1966), particularly following various inflammatory conditions (Johnson and Johnson, 1972; Betty, 1977).

(i) Common Mural Ependyma

Although the morphological structure of ependymal cells varies greatly, four principal components of the ventricular wall (cuboidal ependymal cell, ependymal tanycyte, ependymal astrocyte and "glandular" ependyma) have been distinguished. Knowles (1972) pointed out that a morphological classification of this type is merely intended to facilitate description and cautioned against applying the criteria too precisely. In its simplest and most common form, the lining of the ventricular wall consists of a single layer of cuboidal ependymal cells, although flattened and columnar varieties are also recognized. Reviews of the earlier literature on this subject are provided by Studnička (1900), Wislocki (1932) and Agduhr (1932). More recent comprehensive reviews on ependyma have been provided by Tennyson and Pappas (1968) and Knowles (1972).

A number of recent transmission electron microscopic studies have contributed to the accumulated knowledge on the morphology of the ependyma lining the lateral ventricles in several mammalian species. Tennyson and Pappas (1962) described the ultrastructure of the ependymal lining of the cerebral aqueduct in the fetal, neonatal and adult rabbit. In the feline lateral ventricle the ependyma has been described as a ciliated, cuboidal to columnar epithelium rarely more than one cell thick; similar to descriptions of ependyma lining the lateral ventricles of other mammals (Klinkerfuss, 1964). The luminal plasma

membrane is complex, being organized into numerous microvilli. The ependymal cytoplasm is pale, containing many vesicles with and without dense cores, a network of fibrils, scant vesicular endoplasmic reticullum, a prominent Golgi apparatus and numerous dense mitochondria. A large ovoid nucleus with a well defined nucleolus is located near the base of the cell. Ependymal cilia contain the usual two central filaments surrounded by nine paired peripheral filaments radially arranged. Membrane specializations between contiguous cells have been described by Brightman and Palay (1963) in the rat. They have reported extensively on the fine structure of the ependyma in the third ventricle, cerebral aqueduct and the fourth ventricle of the rat brain with particular emphasis on specialization of the apical and lateral cell surfaces. In the Rhesus monkey, Knowles and Anand Kumar (1969) distinguished three areas of ependyma within the third ventricle which differed in their gross structure, ultrastructure and staining affinities (see Chapter 2.iii, iv). Cells designated "type A" were termed typical unmodified ependyma and resembled characteristic ependyma found elsewhere in the ventricles of the monkey brain. "Type A" ependymal cells were described as being columnar in form with both cilia and microvilli on their juxtaventricular border. Basal processes extending into the neuropil beneath were notably absent. While they did not stain with conventional silver methods, the cells were demonstrable with connective tissue stains. The authors considered these cells to be capable of some synthesis, secretion and absorption, though not actively engaged in these functions. In the Coturnix quail, "typical" ependymal cells in the dorsal wall of the third ventricle have been similarly described (Sharp, 1972).

Relatively little information is available regarding the morphology of the ventricular wall in the human. Contributions, however, have been made by the following investigators: Opalski (1933); Jahn (1940); Globus and Kuhlenbeck (1942, 1944); Beckett, Netsky and Zimmerman (1950); and Friede (1961). The structure of the walls in the lateral and third ventricles in man varies from a single layer of ependyma to three layers composed of ependyma, a subependymal layer of glial fibers and a layer of subependymal glial cells (Schimrigk, 1966). According to Malinsky (1968), the cuboidal ependymal cells of the lateral ventricles are provided with numerous microvilli and a few cilia. The lateral walls of these cells are without plication and are joined by zonulae occludentes and adhaerentes while their basal membrane runs out into cytoplasmic processes in places.

Various functions have been attributed to the ciliated cuboidal ependymal cells. Constituting a boundary between the CSF and brain tissue, they have been purported to act as a semipermeable barrier regulating the passive transport of substances, as a layer for absorptive or secretory processes, as a vibratory epithelium, and as a mechanically resistant boundary (Malinsky, 1968). Histochemical observations reveal that they are cells of high metabolic activity which may be related to secretion, absorption or transport of substances from the vasculature into the ventricle or vice versa (Nandy and Bourne, 1964). In contrast, their enzymatic content has also been regarded as an expression of energy transformation associated with ciliary motility (Schachenmayr, 1967). Histochemical findings in cats, dogs, guinea pigs, rats and rabbits indicate that the ependyma has a very active oxidative metabolism while the subependymal glial layer

in contrast, generally lacks oxidative enzymes (Friede, 1961). The distribution of phosphorylase activity is inverse, indicating an active glycolysis in the subependymal cell plate (Shimizu, Morikawa and Ishii, 1957; Friede, 1959).

(ii) Ependymal Tanycyte

The other principal cell type identified within circumscribed areas of the ventricular wall is the ependymal tanycyte. It is apparent from reviews of the earliest literature (Studnička, 1900; Agduhr, 1932; Wislocki, 1932) that the wide variation in ependymal cell morphology was recognized, with some cells having long basal extension that extend into the brain parenchyma. There was little consensus in the terminology applied to these extensions until Horstmann (1954) introduced the descriptive term "tanycyte" for the elongated ependymal cells of selachians that stained with the Cajal gold sublimate method. They were distinguishable from common mural ependyma by virtue of their long basal processes extending into the subjacent neuropil. Their presence has since been demonstrated by a number of histological and histochemical procedures. In the Rhesus monkey, Anand Kumar and Knowles (1967) distinguished an area of specialized ependymal cells with long basal processes extending to the region of the pars tuberalis, situated anterolaterally in the tuber cinereum. These cells stain deeply with the Gomori-chrome alum hematoxylin phloxine (CAHP) technique (Anand Kumar, 1968b). They are not evident, however, after silver impregnation techniques, nor are they detectable after performic acid-alcian blue staining, but can be demonstrated by cresol fuchsin, aldehyde fuchsin and the periodic acid-Schiff (PAS) method.

The non-specific reaction to CAHP of ependymal cells and even subependymal glial cells in all parts of the lateral ventricles of mice and rats prompted Westergaard (1970) to suggest that lipofuscin rather than an ependymal secretion may be responsible for the staining. Tanycytes are said to comprise more than one layer of cells, to be non-ciliated, to have processes extending into the white matter to varying depths and to show considerable variation with age, sex and reproductive activity (Knowles and Anand Kumar, 1969). They have been described throughout the ventricular system in nonmammalian vertebrate forms (Horstmann, 1954; Oksche, 1958, 1968; Paul, 1967, 1968). In newborn and adult mammals, their presence has been demonstrated throughout the hypothalamic portion of the third ventricle (Fleischhauer, 1964, 1966; Colmant, 1967; Bleier, 1972), but not in the lateral ventricles (Klinkerfuss, 1964; Schachenmayr, 1967). Tennyson and Pappas (1962) found tanycytes among the ependyma of the midbrain aqueduct in the early neonatal rabbit, but not in the adult. Their occurrence, however, is not limited to the floor of the third ventricle (Schachenmayr, 1967). Clusters of tanycytes are found throughout the dorsoventral extent of the third ventricle, although they are most numerous and easily demonstrated along the lateroventral walls and floor (Knowles and Anand Kumar, 1969; Millhouse, 1971; Sharp, 1972; Bleier, 1972).

Ultrastructural studies (Tennyson and Pappas, 1962, 1968; Leonhardt, 1966; Brawer, 1972; Millhouse, 1972) confirmed that the tanycyte is a fundamentally distinct cell with a single dense peripheral process that is generally unbranched except at its termination. Tanycyte processes are of variable form, length and caliber; they

appear to encircle cells and to form complex networks in relationship to neurons and capillaries (Bleier, 1971, 1972). Recently, different forms of tanycytes have been identified on the basis of their structure (particularly of their basal processes), distribution and staining affinities (Millhouse, 1971; Sharp, 1972). In addition to morphological data, some cytochemical observations (Schachenmayr, 1967; Luppa and Feustel, 1971) also indicate structural and possibly functional differences among tanycytes themselves.

Although regional differences in the structure of the ependymal lining are generally regarded as a possible expression of differences in function, the precise nature or function of the component cells is as yet undetermined. Because of the resemblance of tanycytes to spongioblasts, Millhouse (1971) has suggested that tanycytes may be undifferentiated cells awaiting a signal to differentiate into glial or ependymal cells or that they may in fact be spongioblasts differentiated _in situ_ without having lost their ventricular and pial or vascular connections. To some, the morphology of tanycytes suggested a transport capacity (Leonhardt, 1966); to others, their enzymatic pattern indicated a synthesizing activity (Schachenmayr, 1967). Ignoring for the moment any potential neuroendocrine involvement (see Chapter 2.2i, 2.3), tanycytes have also been suggested to subserve a purely supportive role, both structurally and metabolically, for the delicate meshwork of subependymal astroglia or unmyelinated periventricular fibers (Millhouse, 1971, 1972). Their unique structural features have encouraged the belief that they may form a diffusion barrier between the ventricle and neuropil, thereby effectively segregating functional groups of neurons (Millhouse, 1971). The selective absorption by

tanycytes of certain substances from the CSF and transport of these to capillaries or specific hypothalamic neurons and vice versa have also been proposed (Bleier, 1971; Millhouse, 1972). From this concept derives the notion that tanycytes, acting as sensing devices in the systemic and ventricular circulations, may function as modulators of neuronal activity (Millhouse, 1971, 1972; Bleier, 1972). According to Goslar and Bock (1970) the esterase content of tanycytes recommends them as modulators of synaptic activity. On the other hand, their primary role, as postulated by Bleier (1972), may be to regulate the composition of the CSF by selective absorption and transport of substances from the CSF to the systemic circulation or vice versa.

(iii) Ependymal Astrocyte

In addition to the ependymal cell types thus far described, a third cell, the ependymal astrocyte, has been distinguished as a component of the ependymal lining. Tennyson and Pappas (1962, 1968) identified it within the ependymal lining surrounding the aqueduct of the neonatal rabbit midbrain as a columnar cell with a branched peripheral process of light density. This cell corresponds closely to a similar cell described by Horstmann (1954) in a light microscopic study of selachians. In addition to its large peripheral branching processes, other characteristics such as a sparcity of mitochondria, endoplasmic reticulum and filaments, an elaborate plication of the lateral cell membrane, and multiple thin projections from the cell body and main process are its main distinguishing features. The complex infoldings of the lateral cell membrane have been suggested as representative of a stage in the formation and storage of its extensive

processes (Tennyson and Pappas, 1962). In the adult feline lateral ventricle, the presence of a similar cell type was distinguished in the subependymal neuropil (Klinkerfuss, 1964), leaving the ependymal nature of these cells open to question. Although they are not necessarily associated with the neuroglial astrocyte, as the name might suggest (Tennyson and Pappas, 1962), the possibility that they may be astroblasts has not been excluded (Tennyson and Pappas, 1968).

(iv) "Glandular" Ependyma

"Glandular" cells located among the ependymal cells lining the floor of the infundibular recess cannot be categorized with certainty. They appear to be concentrated in the midline region being found less frequently laterally (Knowles, 1967, 1972). Sterzi (1907) reported that the floor of the infundibular recess of the shark was characterized by two forms of ependyma, a non-glandular type, resembling tanycytes, and a glandular variety with few or no basal processes, but filled with secretory granules. Knowles (1971) identified these "Sterzi cells" in the floor of the dogfish infundibular recess and distinguished them from neighbouring ependymal cells by their vacuolated cytoplasm, extensive agranular reticulum and content of dense-core secretory granules (1400-2000 Å diameter). It has been suggested that their morphology more closely resembles neurosecretory cells. In a recent publication (Knowles, 1972), the presence in the dogfish of secretory cells with the same distribution and morphology as described in his earlier paper (Knowles, 1971) were referred to as "cerebrospinal fluid contacting neurons." The presence of similar "specialized" ependymal cells (designated type C^1 cells) has been

activity in the mouse arcuate nucleus and ME following ovariectomy. In contrast, striking regressive changes were observed in the ventricular border of tanycytes and in their contents two months following ovariectomy in the Rhesus monkey (Knowles and Anand Kumar, 1969). Subsequent estrogen administration reversed this appearance by increasing the number of cytoplasmic protrusions and bulbous projections on the juxtaventricular surface (Knowles and Anand Kumar, 1969; Coates, 1974). The changes in tanycytes occurring in response to estrogen treatment argues in favour of a role for them, possibly as receptors sensitive to levels of estrogen in the CSF (Anand Kumar and Knowles, 1967; Knowles and Anand Kumar, 1969).

(i) Immunohistochemical Localization of Releasing Hormones Within the Hypothalamus

The recent availability of synthetic LH-RH has made possible the production of antisera against the peptide for use in immunohistochemical investigations. Kozlowski and Zimmerman (1974) and Zimmerman et al. (1974) localized LH-RH within the perikarya of arcuate (mouse) and infundibular (sheep) neurons in contact with the ependyma of the third ventricle, as well as in the zona externa of the ME and throughout the tanycytes in both species. Naik (1974) similarly localized LH-RH within both the tanycyte ependymal cell bodies near the arcuate nuclei of the rat third ventricle and their processes descending toward the portal plexus. Silverman (1976), however, confirmed the localization of LH-RH within ependymal tanycytes of the mouse ME but not in the guinea pig, suggesting species differences in the presence of detectable quantities of LH-RH in tanycytes. Leonardelli, Barry

Based on observations in the rat, Millhouse (1972) concluded that tanycytes along the walls of the ventricle did not exhibit any of the cytological features of active secretory cells. It is quite apparent that the literature is confused regarding "glandular" ependyma. The precise nature and distribution of these cells is ill understood and often obscure from most descriptions. As a consequence, comparing the results of different studies is difficult and unavoidably imprecise.

Relevant to a discussion of secretory ependyma are other, more circumscribed regions of the ventricular wall that are also recognized as deviating structurally and functionally from the regular ependymal lining. They are collectively designated "circumventricular organs" (CVO) or "neurohaemal organs" and include the subfornical organ, organum vasculosum of the lamina terminalis, subcommissural organ and paraventricular organ. All are located in the wall of the third ventricle and are similar with regard to their unique vascularization and cellular organization. For completeness the area postrema in the caudal portion of the fourth ventricle and the newly described (Stumpf, Lamb, Hellreich and Aumuller, 1977) collicular recess organ of the caudal midbrain aqueduct-fourth ventricle may be added to this list. Although their functions are unknown, special ependymal and hypendymal organization, barrier properties and some indications of neuroendocrine activity have been found. A brief discussion of the third ventricle related CVO is provided in the Appendix.

2.2 Neurovascular Regulation of the Adenohypophysis

There is now incontrovertible evidence that the secretory activity of the anterior pituitary is regulated by the central nervous

system through a neurovascular mechanism. The development of the "neurohumoral hypothesis" began with the gradual awareness by early observers of the peculiar system of vessels in the hypophysial stalk. The portal nature of these "hypophysio-portal" venules was first recognized by Popa and Fielding (1930, 1933). They maintained that blood from the hypophysis was drained by these vessels to the capillary network extending into the hypothalamus. In independent studies of these venules, which were presumed to be systemic, Pietsch (1930) and Basir (1932) adopted the same concept of direction of flow from the anterior lobe. A complete description of their extent and arrangement was provided by Wislocki and King (1936) and Wislocki (1937, 1938), who reported that the upper extent of the portal venules was the capillary bed of the infundibular stalk and the median eminence, and that they conveyed blood from the plexus of the stalk downwards to the sinusoids of the anterior lobe. The suitability of this structural arrangement for the transfer of substances from the brain to the anterior lobe and an appreciation of its functional implications were soon recognized. The concept of humoral transmission was advanced in a number of reports as a natural consequence of attempts to explain available experimental evidence. There was little doubt at this time, for example, that the secretory activity of the adenohypophysis was to some extent under the control of the central nervous system, that its innervation was sparse, and that the hypothalamus was involved in the nervous reflex pathway. Credit for the notion of neurohumoral control of anterior lobe function is given to Hinsey and Markee (1933), who postulated a humoral transmission of stimuli from the neurohypophysis to the pars distalis. This hypothesis was repeatedly

and Dubois (1973), using an immunofluorescence technique to detect LH-RH, showed that specific fluorescence is found within certain hypothalamic axons and their pericapillary endings on the portal vasculature in the mouse, rat, hamster and the guinea pig ME. In normal, castrated, colchicine and testosterone treated guinea pigs, immunoreactive LH-RH was found within the perikarya of neurons scattered throughout a large area extending from the preoptic and septal area to the caudal part of the tuber cinereum. Localization of specific fluorescence was restricted mainly to the pre- and suprachiasmatic region, although a pre- and post-anterior commissural and a parolfactory localization was also distinguished (Barry, Dubois, Poulain and Leonardelli, 1973; Barry, Dubois and Carette, 1974). Pelltier, Labrie, Puviani, Arimura and Schally (1974) reported the immunohistochemical localization of the neurohormone in the secretory granules (750-950 Å) of some nerve terminals situated in the palisade layer of the rat ME. Curiously, however, no mention was made of localization within ependyma in any of these reports. Employing similar procedures (Baker, Dermody and Reel, 1974), LH-RH was localized with variable distribution in the ME of the mouse, rat and guinea pig. However, it was not detected in the perikarya of suprachiasmatic, ventromedial, ventrolateral, arcuate, paraventricular or supraoptic nuclei or more particularly in the ependyma of any of these species. Sétälö et al. (1975) determined that LH-RH staining of ependymal cells of the rat third ventricle in their experiments was non-specific. A recent light and electron microscopic autoradiographic investigation of the medial basal hypothalamus of rat brains following in vitro incubation in ^{3}H-LH-RH revealed the releasing factor within tanycytes and perivascular glia in the palisade zone of

recognized, however, that the capillaries in the median eminence were surrounded by nerve fibers derived from various hypothalamic nuclei that could not be identified with certainty. Although there was little information available as to the nature of the hypothetical humoral substances transmitted via the portal vessels, a large body of experimental evidence demonstrating the dependence of the pituitary gland on hypothalamic neurohumoral secretion gradually accumulated. In recent years, attention has been directed toward elucidating the nature, source and mechanism of action of these hypophysiotrophic principles. Older designations for this group of neurohumoral factors have been largely supplanted by the designation "hypothalamic releasing factor," coined by Saffran, Schally and Benfey (1955) to signify the stimulatory or inhibitory effects that substances of hypothalamic origin exert on the release of hormones from cells of the anterior pituitary. Schally, Arimura, Bowers, Kastin, Swano and Redding (1968) proposed the term "hypothalamic releasing hormones" for fully identified substances on the basis that some satisfied the criteria for designation as hormones. The current concept, now reasonably well established, is that there is at least one releasing factor for each of the adenohypophysial hormones, and for at least three (growth hormone, prolactin and melanocyte stimulating hormone) there is a dual system of hypothalamic control--inhibition and stimulation. The first direct evidence in support of the neurohumoral theory of adenohypophysial regulation has come only with the recent isolation and characterization of such active components from crude hypothalamic extracts and their synthesis. Although corticotropin-releasing factor (CRF) was the first hypothalamic principle to be demonstrated on the basis of

physiological evidence (Saffran and Schally, 1955; Saffran et al., 1955; Guillemin and Rosenberg, 1955; Guillemin, Hearn, Cheek and Housholder, 1957), its structure has yet to be elucidated. The first hypothalamic hormone to be isolated was the tripeptide, thyrotropin-releasing hormone (TRH). Its amino acid sequence and structure have been determined and its synthesis achieved (Bøler, Enzman, Folkers, Bowers and Schally, 1969; Burgus, Dunn, Desiderio and Guillemin, 1969). The presence of a luteinizing hormone-releasing hormone in hypothalamic extracts of rats was first demonstrated by McCann, Taleisnik and Friedman (1960). It was thought initially that LH-RH and follicle stimulating hormone-releasing hormone (FSH-RH) activities were properties of two different substances but this belief has since been altered although not entirely abandoned. The isolation from porcine hypothalami of a decapeptide with both LH-RH and FSH-RH activity has been accomplished; its amino acid composition and sequence has been determined (Schally et al., 1971; Matsuo, Baba, Nair, Arimura and Schally, 1971) and its synthesis (Matsuo, Arimura, Nair and Schally, 1971) achieved. Growth hormone release-inhibitory hormone, a cyclic tetradecapeptide isolated from ovine hypothalami, is the most recently described and synthetized hypothalamic regulatory hormone (Brazeau, Vale, Burgus, Ling, Butcher, Rivier and Guillemin, 1973). The structure of a fourth hormone, melanocyte stimulating hormone release-inhibitory hormone (MIF) has also been elucidated following its purification from bovine hypothalami (Nair, Kastin and Schally, 1971, 1972). In synthetic form, it has been shown to be effective in several animal models, but appears to be ineffective in man. Although it is now generally accepted that hormonally active agents secreted by the brain

diffuse into the portal vasculature and either stimulate or inhibit the release of trophic hormones from the adenohypophysis, many aspects of this neurohumoral system are still not understood. The control of their discharge, how they reach the portal vasculature, and their cellular mechanism of action have still not been satisfactorily ascertained (Porter, Kamberi and Ondo, 1972). There is, however, some recent evidence to indicate that cyclic AMP may be a mediator of their action on anterior pituitary cells (Jutisz, 1971; Labrie, Barden, Poirier and DeLean, 1972; Schally, Arimura and Kastin, 1973.

(i) Hypothesis Relating Ependyma to the Neuroendocrine Control of Adenohypophysial Function

Neurosecretory neurons within the hypothalamus are commonly viewed as the cellular source of releasing and inhibiting hormones. Although there is evidence to indicate that they are synthetized and packaged in granules within the perikarya of these neurons, transported to the median eminence by axoplasmic flow, and there stored and/or released into the primary plexus of hypophysial portal vessels (Clattenburg et al., 1971, 1972a; Clattenburg, 1972, 1974; Clattenburg, Montemurro, Bruni and Singh, 1973), no clear demonstration of their site(s) of origin within discrete neurons of the hypothalamus has been obtained. In part, the paucity of significant data on this subject has contributed to the current belief that cells in the hypothalamus other than neurons together with the cerebrospinal fluid may constitute important pathways integrating the endocrine functions of the adenohypophysis. Indeed, it has been suggested that ependymal cells might be involved in the production and/or transport of releasing

factors (Knigge and Scott, 1970; Scott and Knigge, 1970; Anand Kumar, 1972; Knowles, 1972; Lévêque, 1972). Morphological and functional observations have led a number of investigators to speculate that hypothalamic factors may be secreted into the CSF and then transported to the hypophysial portal vessels (Kendall, Grimm and Shimshak, 1969; Knowles and Anand Kumar, 1969; Knigge and Scott, 1970; Porter, Kamberi and Ondo, 1972; Anand Kumar, 1973). In support of this concept is recent evidence favouring the diffuse distribution of releasing hormone producing cells throughout the hypothalamus. Jackson and Reichlin (1974) have found that while the highest concentration of TRH in the brain occurs within the hypothalamus and/or pituitary complex, small concentrations are present in most extrahypothalamic brain tissues of the rat and chicken, and unexpectedly large amounts are found in extrahypothalamic brain tissue of most submammalian forms. TRH synthesizing cells in the mink hypothalamus (identified as such by their in vitro biosynthetic capacity), while concentrated in one discrete region (arcuate nucleus) were also present in a more diffuse dorsomedial zone throughout the entire anterior-posterior extent of the hypothalamus (Knigge et al., 1974). Endogenous hormone was similarly detectable in all regions of the hypothalamus, although it was particularly concentrated in a dorsal anterior region and in a ventral middle zone. This wide topograhical distribution of TRH-producing cells throughout the hypothalamus suggested the prospect of a dual route of delivery. Remotely lying neurons might conceivably deliver their hormones to the ME via the CSF. Krulich, Quijada, Hefco and Sundberg (1974), in contrast, reported that TRH activity in the rat was not dispersed throughout the hypothalamus, but rather was concentrated in the ME, dorsomedial

hypothalamus and in the preoptic region. Nevertheless, they did not discount the possibility that TRH produced at sites distant from the ME was released into the CSF and subsequently taken up by the ME. Whether this may be applicable to other releasing factors is not certain at this time. An ependymal-ventricular transport route for adenohypophysial regulation is predicated in part, on the observation that tanycyte ependymal cells situated along the floor and lateral walls of the infundibular recess establish a morphological link between the cerebrospinal fluid on the one hand and the pars tuberalis and vasculature of the median eminence on the other. It was this intriguing structural relationship that led Löfgren (1959, 1960) to first suggest their possible involvement in the neuroendocrine control of pituitary function. Such a mechanism, as yet a matter of speculation, does not necessarily preclude the more conventional hypothesis of axonal transport. Harris (cited in Knigge and Scott, 1970), however, stated that "teleologically based, it is unlikely that such a fast-reacting system as the central nervous system should employ such a slowly reacting system as fluid currents in the cerebrospinal fluid to control such fast-reacting systems as, for example, the discharge of corticotrophic hormone in response to acute stress." Other investigators have concurred with the view that a CSF to pituitary route of transport is probably not of physiological importance.

In addition to the hypothesis that hypophysiotropic substances conveyed via the CSF are actively transported by specialized ependymal cells across the ME into the affluent blood of the pars distalis, the view has also been expressed that target gland hormones might pass from the general circulation by way of the choroid plexus into the CSF

and eventually be transported to the brain for metabolic effects or to the ME for feedback suppressive effects (Kendall, Jacobs and Kramer, 1972). In view of the relationship of tanycyte processes with neurons and capillaries, Bleier (1971, 1972) found it reasonable to propose that they may serve as a communication system between the CSF and neurons and capillaries within the hypothalamus, thereby influencing particular neurons that exert a regulatory influence on adenohypophysial secretion.

2.3 Evidence for Neurosecretory Ependymal Function

There is morphological evidence to suggest apical secretion by specialized ependymal cells into the CSF of the third ventricle (Lévêque and Hofkin, 1961; Vigh, Aros, Wenger, Koritsánsky and Ceglédi, 1963; Vigh, 1964; Lévêque *et al.*, 1966; Knowles, 1967; Matsui and Kobayashi, 1968; Wittkowski, 1969; Kobayashi, Matsui and Ishii, 1970). On the other hand, some investigators have postulated that tanycytes may absorb substances from the CSF and/or subserve a transport capacity (Anand Kumar and Knowles, 1967; Knowles and Anand Kumar, 1969; Knigge and Scott, 1970; Kobayashi *et al.*, 1970; Kobayashi, 1972; Kobayashi, Wada and Uemura, 1972).

A number of correlations between pituitary hormone secretion and changes in ependymal cell morphology have been documented. Lévêque and Hofkin (1962) observed that gonadectomy, adrenalectomy, hypophysectomy and cortisone treatment had no effect on the content of PAS-positive material in the ependymal cells of the rat infundibular recess. Cold-stress and propylthiouracil-treatment, however, increased and decreased their content, respectively. In the Rhesus monkey,

Anand Kumar (1968a) and Knowles and Anand Kumar (1969) correlated structural differences in the tanycyte ependyma of the anterior hypothalamus with the age and sex of the animal. Bulbous projections on the ventricular surface of these cells in the female were observed to vary in relation to the menstrual cycle, being well developed during mid-cycle and regressing during menstruation. Similarly, seasonal changes in ependymal morphology associated with sexual activity have been demonstrated in the skunk (Hagedoorn, 1965). In contrast, ultrastructural studies on the "glandular" ependyma in the medial basal hypothalamus of the Coturnix quail failed to show any obvious differences among castrated, estrogen or testosterone treated or sexually mature or immature birds (Sharp, 1972). In a recent SEM investigation of the rat third ventricle, Brawer, Lin and Sonnenschein (1974) reported that the apical surfaces of non-ciliated tanycytes in the ventral wall and floor undergo profound changes during different stages of the estrous cycle. At mid-diestrus, the apical surface of tanycytes appears devoid of microvilli and other surface eruptions. At late diestrus, a marked change occurs, during which microvilli and other surface irregularities appear. They increase in number during proestrus, persist through estrus, and then disappear in early to mid-diestrus.

Kobayashi and Matsui (1969) and Kobayashi et al. (1970) reported that three weeks after ovariectomy most ependymal and hypendymal cells of the rat ME were enlarged and became cylindrical; their nuclei were also enlarged and cytoplasmic organelles showed a marked increase in number. Comparable results were obtained by Oksche, Zimmerman and Oehmke (1972) in a morphometric analysis of ependymal

was found to be no more effective than intravenous injection, it was concluded that, although the transport of TRH from the CSF to the pituitary could not be excluded, it was not supported by the findings. A similar study (Gordon, Bollinger and Reichlin, 1972) demonstrated that although TRH can reach the anterior pituitary from the third ventricle, this route of administration was less effective in stimulating TSH secretion than injections into the median eminence, anterior pituitary or systemic circulation. The availability of synthetic LH-RH has also enabled investigators to test its efficacy in promoting LH release following injection into the CSF. Ondo, Eskay, Mical and Porter (1973) concluded that LH-RH administered via the CSF of the third ventricle reaches the hypophysial portal vasculature directly by diffusion or active transport across the median eminence. They also concluded that LH-RH in the CSF of the cisterna magna can influence the release of LH from the anterior pituitary by bulk flow transport of the CSF to systemic blood by way of the subdural sinuses. In a more recent report, Ben-Jonathan, Mical and Porter (1974) concluded that LH-RH can be transported from the CSF to hypophysial portal blood in significant quantity and that LH-RH given intraventricularly is more effective on a prolonged basis in stimulating LH release than when given intravenously. Weiner, Blake and Sawyer (1971b) and Weiner *et al.* (1972) observed that administration of LH-RH by the intraventricular route to ovariectomized estrogen-progesterone primed rats, was less effective in causing an increase in serum LH than intravenous administration. Attempts to demonstrate systemic uptake of intraventricularly injected LH-RH were unsuccessful (Weiner *et al.*, 1972). Furthermore, direct uptake from the ventricle was considered to be of

and Dubois (1973), using an immunofluorescence technique to detect LH-RH, showed that specific fluorescence is found within certain hypothalamic axons and their pericapillary endings on the portal vasculature in the mouse, rat, hamster and the guinea pig ME. In normal, castrated, colchicine and testosterone treated guinea pigs, immunoreactive LH-RH was found within the perikarya of neurons scattered throughout a large area extending from the preoptic and septal area to the caudal part of the tuber cinereum. Localization of specific fluorescence was restricted mainly to the pre- and suprachiasmatic region, although a pre- and post-anterior commissural and a parolfactory localization was also distinguished (Barry, Dubois, Poulain and Leonardelli, 1973; Barry, Dubois and Carette, 1974). Pelltier, Labrie, Puviani, Arimura and Schally (1974) reported the immunohistochemical localization of the neurohormone in the secretory granules (750-950 Å) of some nerve terminals situated in the palisade layer of the rat ME. Curiously, however, no mention was made of localization within ependyma in any of these reports. Employing similar procedures (Baker, Dermody and Reel, 1974), LH-RH was localized with variable distribution in the ME of the mouse, rat and guinea pig. However, it was not detected in the perikarya of suprachiasmatic, ventromedial, ventrolateral, arcuate, paraventricular or supraoptic nuclei or more particularly in the ependyma of any of these species. Sétáló et al. (1975) determined that LH-RH staining of ependymal cells of the rat third ventricle in their experiments was non-specific. A recent light and electron microscopic autoradiographic investigation of the medial basal hypothalamus of rat brains following in vitro incubation in ^{3}H-LH-RH revealed the releasing factor within tanycytes and perivascular glia in the palisade zone of

the ME (Scott et al., 1974a), supporting the belief that these cells possess an affinity for LH-RH.

2.4 Scanning Electron Microscopy of the Third Cerebral Ventricle

Scanning electron microscopy has been employed increasingly in recent years to examine the morphological features of the ependyma lining the third ventricle in several mammalian species (Scott, Krobisch Dudley, Gibbs and Brown, 1972a; Scott, Kozlowski and Dudley, 1973; Kozlowski, Scott and Krobisch Dudley, 1973). Its application to neuroendocrine research has a very brief history. Knigge and Scott (1970) indicated that ependymal cells lining the infundibular recess of the third ventricle judging from their morphology and architectural arrangement, subserve some functional relationship between CSF of the third ventricle and blood of the pituitary portal plexus. In pursuit of data to support this view, they were the first to examine regional differences within the area of the infundibular recess with the SEM. Their study revealed that within the area of the rat IR, there were ependymal specializations (at the ventricular surface) which they believed were consistent with evidence of intense transfer activity, thus suggesting that the role of these cells may be the extraction and transport of some releasing hormones placed in the CSF at sites distant from the ME. The scope of subsequent SEM investigations has been extended to include topographical analyses of the lateral ventricles and choroid plexus in the cat (Clementi and Marini, 1972; Noack, Dumitrescu and Schweichel, 1972), rat (Peters, 1974), sheep (Kozlowski, Scott and Murphy, 1972) and dog (Allen and Low, 1973); the fourth ventricle and circumventricular organs in the rat (Torack and Finke,

1971), rabbit, cat and squirrel monkey (Weindl and Joynt, 1972a, b) as well as normal and developmental abnormalities in rat ependyma and choroid plexus (Chamberlain, 1972, 1973). The morphological features of the ependymal surface of the rabbit, rat, mouse and human third ventricle have also been described (Bruni, Montemurro, Clattenburg and Singh, 1972; Scott, Paull and Krobisch Dudley, 1972b; Bruni, 1974; Bruni, Clattenburg and Montemurro, 1974; Mestres, Breipohl and Bijvank, 1974; Scott, Kozlowski and Sheridan, 1974b). Within certain areas of the ventricle in all species examined, specializations of the ependymal surface were consistently found which were suggestive of a possible secretory or absorptive capacity. To avoid repetition, further discussion of this topic is provided in subsequent sections of this dissertation and will therefore not be elaborated upon further at this time.

2.5 Transport of Hormones by the CSF and Ependyma

Recent attempts to further elucidate the involvement of the ependyma and the CSF in pituitary regulation include both qualitative and quantitative data on the transport of various hormones to the portal vasculature and pituitary following systemic and/or ventricular administration. Evidence for the possible existence of a CSF to ME-pituitary transport route for both cortisol and thyroxine, either for the purpose of metabolic or feedback suppressive effects, is provided by Kendall et al. (1969, 1972). Following injection of radiolabelled ACTH, thyroxine corticosterone and TRH into the lateral ventricle of rats selective concentration in the ME occurs (Kendall et al., 1972). Ondo, Mical and Porter (1972) and Porter et al. (1972) have demonstrated

that a variety of labelled compounds when placed in the third ventricle transfer in significant quantities from the CSF across the ME to the hypophysial portal system and are present there in concentrations greater than in the blood of peripheral vessels. In considering the ME as the site of exchange between the CSF and the portal blood, the possibility that ependymal cells may constitute a potential link between the two cannot be excluded. Recent _in vitro_ and _in vivo_ evidence has demonstrated the ability of median eminence tissue to engage in active transport of amino acids and thyroxine by an energy dependent process and suggests that the ependymal component of the ME may be responsible for this metabolic ability (Silverman, Knigge and Peck, 1972; Silverman and Knigge, 1972; Knigge and Silverman, 1972). Twelve hours following intramuscular injection of tritium-labelled 17-β estradiol in the Rhesus monkey, Anand Kumar and Knowles (1967) and Anand Kumar and Thomas (1968) found radioactivity in samples of CSF at a level one-eighth of that found in the plasma. Following similar injections in ovariectomized monkeys, a strong concentration of activity was found in the specialized elongated ependymal cells situated anterolaterally in the tuber cinereum.

Significant free movement of the proteins horseradish peroxidase (HRP) and ferritin from the ventricle into the neuropil underlying ciliated ependyma has been documented. Ogata, Hochwald, Cravioto and Ransohoff (1972) observed the transventricular absorption of HRP to occur mainly intercellularly and perivascularly in the lateral ventricle of both normal and hydrocephalic cats. Only some ependymal cells were found to contain vacuoles filled with quantities of the enzyme, a situation indicative of intracellular diffusion. In rats, appreciable

amounts of ferritin injected into the CSF similarly traverse the ependymal epithelium by intercellular diffusion (Brightman, 1965). A larger fraction of the protein, however, enters the ependyma by pinocytosis and is eventually segregated within membrane enclosed organelles. The ependymal epithelium overlying the ME, in contrast, is impermeable to the free passage of HRP injected into the CSF (Reese and Brightman, 1968; Brightman and Reese, 1969). This finding peculiar to ependyma in this region necessitates that active mechanisms of uptake rather than passive diffusion be invoked. The uptake of HRP by ependymal perikarya and their processes observed in the ME of the mouse, rat and quail, following injection into either the third ventricle or subarachnoid space, was viewed by Kobayashi (1972) and Kobayashi et al. (1972) as direct evidence of the capacity of these cells to absorb ventricular fluid and transport it to the terminals of their processes. No indication, however, of uptake and transport of either peroxidase or the amino acid ^{3}H-phenylalanine by the ependyma of the feline ME could be detected after their intraventricular injection (Weindl and Joynt, 1972a).

Catecholamines administered intraventricularly exert a significant influence on the secretion of hormones from the anterior pituitary. Injection of epinephrine into the third ventricle of the estrous rabbit or the pentobarbital-blocked proestrous rat induced ovulation (Sawyer, 1952; Rubinstein and Sawyer, 1970). Studies of Schneider and McCann (1969, 1970a), Kamberi et al. (1970a, b; 1971a, b, c) and Porter et al. (1972, 1973) demonstrated that intraventricular injection of dopamine, epinephrine or norepinephrine caused an appreciable release of luteinizing hormone (LH) and follicle stimulating hormones (FSH) in the

male rat, presumably by regulating the concentration in hypophsial stalk blood of releasing factor activity. This stimulatory effect of dopamine on LH-RH release in the rat is blocked by estradiol injected into the third ventricle (Schneider and McCann, 1970b) and is consistent with the report that a dose of estradiol which is systemically ineffective can inhibit LH release when injected into the third ventricle of ovariectomized rats (Orias, Negro-Vilar, Libertun and McCann, 1974). Furthermore, injection of catecholamines into the third ventricle has also been shown to cause changes in electrical activity of the ME and arcuate nucleus--areas known to be involved in the regulation of the secretion of anterior pituitary hormones (Weiner, Blake, Rubinstein and Sawyer, 1971a). Changes in electrical activity in these areas have been correlated with an ovulatory surge of LH (Terasawa and Sawyer, 1969). These findings correspond with morphological changes in the ependymal lining of the third ventricle of the male rat following intraventricular administration of catecholamines, in amounts known to stimulate the secretion of releasing factors (Schechter and Weiner, 1972). Their observations reveal an alteration in the morphology of the ependymal surface of the floor and lateral recess of the third ventricle immediately following epinephrine or dopamine administration. This increased surface activity was interpreted to represent a secretory process of unknown nature in which fragments of ependymal cell cytoplasm were released into the CSF. The pituitary responsiveness to intraventricular administration of hormones is not necessarily explained by transependymal transport through the ME. Duvernoy and Koritké (1968) described subependymal capillary nets that anastomose with the primary plexus and long capillary loops from the primary plexus in close

contact with the CSF of the third ventricle. Thus the possibility of direct uptake albeit a remote one (Weiner, Terkel, Blake, Schally and Sawyer, 1972) into the capillary plexus of the portal system cannot be excluded. An alternative explanation for the efficacy of substances such as dopamine has been advanced by Weindl and Joynt (1972a). A free exchange between the extracellular fluid of the arcuate nucleus and the CSF was postulated whereby, by a process akin to iontophoresis, action potentials generated in these neurons leads to the discharge of releasing hormones from their axon terminals into the portal vasculature with subsequent adenohypophysial hormone release.

There is little information regarding the involvement of the CSF and ependyma in the transfer of releasing hormones from their origin in the brain to the hypophysial portal blood. Attempts have recently been made, however, to demonstrate that releasing hormones are transported from the ventricular system to the portal circulation. ^{3}H-TRH infused into the lateral ventricles resulted in rapid localization of the tripeptide in cells of the arcuate nucleus and in the ependyma of the ME (Knigge _et al._, 1974). Similarly, _in vitro_ incubation of the ME with ^{3}H-TRH also revealed concentration in the ependyma with virtually no evidence of localization in nerve terminals. Kendall _et al._ (1972) observed a selective concentration of ^{14}C-TRH in the median eminence and paradoxically in the posterior pituitary after microinjection into the lateral ventricles. The functional significance of this observation was tested by comparing the effects of synthetic TRH injected systemically and into the lateral ventricle of rats on thyroid stimulating hormone (TSH) secretion (Kendall, Rees and Kramer, 1971; Kendall _et al._, 1972). Since TRH injection into the ventricle

was found to be no more effective than intravenous injection, it was concluded that, although the transport of TRH from the CSF to the pituitary could not be excluded, it was not supported by the findings. A similar study (Gordon, Bollinger and Reichlin, 1972) demonstrated that although TRH can reach the anterior pituitary from the third ventricle, this route of administration was less effective in stimulating TSH secretion than injections into the median eminence, anterior pituitary or systemic circulation. The availability of synthetic LH-RH has also enabled investigators to test its efficacy in promoting LH release following injection into the CSF. Ondo, Eskay, Mical and Porter (1973) concluded that LH-RH administered via the CSF of the third ventricle reaches the hypophysial portal vasculature directly by diffusion or active transport across the median eminence. They also concluded that LH-RH in the CSF of the cisterna magna can influence the release of LH from the anterior pituitary by bulk flow transport of the CSF to systemic blood by way of the subdural sinuses. In a more recent report, Ben-Jonathan, Mical and Porter (1974) concluded that LH-RH can be transported from the CSF to hypophysial portal blood in significant quantity and that LH-RH given intraventricularly is more effective on a prolonged basis in stimulating LH release than when given intravenously. Weiner, Blake and Sawyer (1971b) and Weiner et al. (1972) observed that administration of LH-RH by the intraventricular route to ovariectomized estrogen-progesterone primed rats, was less effective in causing an increase in serum LH than intravenous administration. Attempts to demonstrate systemic uptake of intraventricularly injected LH-RH were unsuccessful (Weiner et al., 1972). Furthermore, direct uptake from the ventricle was considered to be of

questionable physiological significance since the "equi-effective" intraventricular dose was considerably higher than the intravenous dosage. Spies and Norman (1974) recently demonstrated that LH-RH infusion (30 minutes) directly into either the pituitary or third ventricle of ovariectomized Rhesus females resulted in a significant rise in serum LH over saline infused control levels. Pretreatment with 17-β estradiol potentiates the LH response to ventricular but not pituitary LH-RH administration.

Although the possibility that hypophysiotrophins, whatever their source, reach the ME via the CSF of the third ventricle is most attractive, supportive data is clearly indirect and the need for much additional work apparent. In order to establish the physiological significance of such a system unequivocally, Ondo _et al._ (1973) have stressed the importance of demonstrating both the presence of hypophysiotrophins in the CSF and also the relationship between their concentration in the CSF and the rate of release of hormones from the anterior pituitary. The presence of releasing factors in the CSF has been postulated by a few investigators. The studies of Eik-Nes, Brown, Brizzee and Smith (1961) have indicated that a "corticotrophin influencing factor" may be present while Averill and Kennedy (1967) consider that there are low concentrations of TSH-releasing substances in the CSF of the rat. The presence of releasing factors within the CSF has recently been demonstrated in a few laboratories. Knigge _et al._ (1974) and Knigge and Joseph (1974) measured TRH activity by _in vitro_ pituitary assay in the CSF of the rat third ventricle and in the lateral and fourth ventricles of the monkey under normal conditions. They found that conditions known to alter hypothalamo-

pituitary-thyroid relationships in the rat such as cold exposure or thyroxine treatment markedly decreased the concentration of TRF in the CSF. Morris, Tandy, Sundberg and Knigge (1975), Morris and Knigge (1976) and Asai and Uemura (cited in Uemura et al., 1975) have identified the presence of LH-RH in the ventricular fluid. Significant increases in the endogenous LH-RH concentration of third ventricular CSF were produced in rats following hypothalamic deafferentation (Morris et al., 1975) and in response to ether anesthesia (Morris and Knigge, 1976). Not all results, however, have been positive. The observation that ectopic adenohypophysial implants in contact with the CSF of the third ventricle were not preserved, prompted Halasz, Pupp and Uhlarik (1962) to conclude that the CSF did not contain gonadotrophin-releasing factor in physiologically significant amounts. Grazia, Kamberi and Porter (cited in Porter et al., 1973) were unable to demonstrate releasing factor activity in artificial CSF that had been collected after ventricular perfusion with or without dopamine presumably in amounts known to elicit releasing factor secretion. Cramer and Barraclough (1974, 1975) not only failed to find detectable levels of LH-RH in the CSF of the rat third ventricle but also found that neither intra-arterial administration of the peptide nor electrochemical stimulation of the medial preoptic area altered this concentration even though both procedures resulted in significant release of pituitary LH.

3. MATERIALS AND METHODS

3.1 Experimental Animals

The rabbit (Oryctolagus cuniculus L.), was selected as the principal experimental animal for this investigation primarily because ovulation in this species normally occurs either following coitus (Heape, 1905) or as a result of some form of stimulation[1] other than coitus. Since ovulation therefore occurs reflexly, there is a definite time interval between mating and follicular rupture that can be determined accurately. This has been used to advantage in certain of our experiments in which the ventricular ependyma was examined following ovulation induced by the administration of synthetic LH-RH. Furthermore, in the absence of sophisticated immunoassay procedures, examining the ovaries for rupture sites affords a convenient and reliable method of confirming ovulation.

Inasmuch as the period of gestation in the rabbit averages approximately 31 days (Friedman and Lapham, 1931) and the corpora lutea of pseudo-pregnancy remain active for approximately 17-20 days (Hammond and Marshall, 1925; Friedman and Lapham, 1931) during which time ovulation cannot recur, sexually mature female rabbits used in

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[1]Ovulation may be induced by mechanical stimulation of the vagina artificially applied (Hammond and Marshall, 1925; Bishop, 1933), by the behaviour of the buck in the absence of vaginal penetration or when mounted by another doe (Hammond and Marshall, 1914), by electrical stimulation of the whole CNS (Marshall and Verney, 1936), or ventral hypothalamus (Markee, Sawyer and Hollinshead, 1946; Harris, 1948), or by injecting extract of anterior pituitary or urinary gonadotrophin (Bellerby, 1929; Friedman, 1929; Friedman and Lapham, 1931).

this investigation were isolated by individual caging from males and other females for a minimum of 25 days prior to use to avoid the possible complication of pseudo-pregnancy (Hammond and Marshall, 1925) or ovulation induced by another female (Staples, 1967). All animals were maintained under conditions of constant lighting (12 hours light, 12 hours darkness) and temperature (22 ± 2°C). They were fed <u>ad libitum</u> with a standard diet (Rabbit Pellets, Master Feeds) and allowed free access to drinking water.

Virgin female Sprague-Dawley rats (200-300 gm body weight) and CR_1 mice (25-30 gm body weight) were also used in a comparative study during the course of this investigation (Chapter 4.2). They were anesthetized with 25% urethane (0.7 ml/100 gm body weight) and 5% Evipan (0.006 ml/gm body weight) respectively. The brains were fixed by vascular perfusion as detailed below. The volume of fixative used was 250 ml for each rat and 100 ml for each mouse. Human post-mortem material, both male and female, was obtained shortly after death. At autopsy, the brain was removed, the diencephalon excised, washed in balanced salt solution and fixed by immersion at 4°C for 24 hours. Fixation as well as the subsequent preparation of the tissue for SEM was identical to that described in Chapter 3.5 for the rabbit.

3.2 Procedure for Fixation by Perfusion

All rabbits used in this study, were anesthetized in preparation for perfusion fixation with an aqueous solution of 25% urethane (ethyl carbamate) administered intravenously (1.25 g/kg body weight) in the marginal ear vein.

The abdominal cavity was opened by a midline incision through

the linea alba and then the descending aorta clamped at the level of the renal arteries. The thorax was opened by a longitudinal midline incision through the sternum. The pericardium was carefully dissected to expose the heart, and the cannula carrying the perfusate inserted into the ascending aorta through a small incision made in the apex of the left ventricle. A similar incision in the right atrium served as an outlet for the blood and perfusate. Once the cannula was positioned, the ventricular myocardium was clamped with a hemostat around the point of insertion of the cannula to prevent reflux of the fixative.

3.3 Preparation of Tissues for Light Microscopy

Regional differences in the structure of the ependymal lining throughout the entire ventricular wall were systematically investigated in brains from rabbits prepared for serial light microscopy. Because of our particular interest in tracing the distribution of tanycytes, suitable histological methods applicable to the staining of these cells were necessary. In view of the difficulties inherent in all histological methods for the staining of neuroglia, it was not surprising to find little information in this regard on the staining of ependyma in general and tanycytes in particular. The methodology was selected primarily on the basis of its reported suitability for demonstrating tanycytes. In one series of preliminary studies, the brains of rabbits were fixed *in situ* by vascular perfusion of either 4% formalin or 10% neutral buffered formalin and following dissection of the hypothalami, fixation was continued overnight by immersion in a volume of the same fixative at 4°C. Frozen sections (15-20 μm thick) were subsequently cut on a cryostat and individually impregnated with

uranyl nitrate according to the silver staining technique described by Gallyas (1970) for fibrous neuroglia. The brains from an additional series of rabbits were fixed by perfusion with Bouin's fluid, subsequently dehydrated and cleared in dioxane (Luna, 1968) and embedded in paraffin. Six to twelve μm thick serial sections were then cut and stained with chromium hematoxylin-phloxine (Gomori, 1941; Luna, 1968). Finally, the brains from an additional series of rabbits were prepared according to Ramón-Moliner's tungstate modification of the Golgi-Cox technique (Ramón-Moliner, 1958, 1970) and celloidin embedded.

All light micrographs were taken using a Zeiss photomicroscope and Kodak Panatomic-X 35 mm film with or without appropriate filters. Negatives were developed in Kodak Microdol-X (undiluted) at development times appropriate to the termperature of the developer solution and yielding negatives of average contrast.

Photographs of gross specimens were taken with Kodak Panatomic-X film and developed as above or Kodachrome 11 (daylight) colour transparency film. Optimal results were obtained using a close-up bellows with a 105 mm lens and attached ring flash (medium intensity) at F 8 or 11 for black and white and F 5.6 - 8 for colour film.

3.4 Preparation of Tissues for Transmission Electron Microscopy

(i) Fixation

Specimens prepared for TEM were fixed *in situ* by vascular perfusion of 1500 ml of a modified Karnovsky's paraformaldehyde-glutaraldehyde fixative (Karnovsky, 1965) in 0.1M phosphate buffer (Karlsson and Schultz, 1965). The perfusion apparatus employed in this investigation was an open regulatable gravity-fed system located

150 cm above the level of the cannulated aorta. It thereby provided a perfusion pressure equivalent to 150 cm H_2O (110 mm Hg) and a flow rate of 35-40 ml/min. A more detailed description of this apparatus has been previously reported (Clattenburg, 1972). The Karnovsky-Karlsson and Schultz method of fixation was modified by Clattenburg (1972), Clattenburg et al. (1971, 1972a, b) to provide for a final fixative concentration of 2.5% glutaraldehyde-1.3% formaldehyde and an osmolarity of approximately 1200 mOsM.

All fixatives were freshly prepared just prior to perfusion, heated to 39°C and oxygenated before being introduced into the vascular system. Exsanguination with 35-40 ml of phosphate buffer alone preceded perfusion with the above fixative.

(ii) Excision of Ventricular Ependymal Blocks

Following perfusion fixation, the intact brain and attached infundibular stalk were carefully removed from the cranium (Plate 1a, b). A block of tissue containing the diencephalon was then excised (Plate 1c), carefully split in the mid-sagittal plane (Plate 2), washed with 0.1M phosphate buffer (mammalian Ringer-Locke or Hank's balanced salt solution) and further fixed by immersion in a volume of the same fixative for 2-14 hours at 4°C. Following initial fixation, small pieces of hypothalamic tissue (1 mm^3) containing ventricular ependyma were excised from the hemisected diencephalic blocks using a finely sharpened cataract needle.[2] All tissues were removed with the aid of a Zeiss binocular dissecting microscope. A photomicrograph of the

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[2]Miltex Instrument Co., New York, N.Y., U.S.A.

Plate 1 a, Dorsal view of the intact rabbit brain removed from the cranium following *in situ* fixation by vascular perfusion. OB, olfactory bulb; CH, cerebral hemisphere; VE, vermis; PM, cerebellar hemisphere (paramedial lobule); PF, parafloccular lobule; MO, medulla oblongata. X 2.

b, Ventral view. MO, medulla oblongata; PO, pons; PL, pyriform lobe; OT, olfactory tract; OB, olfactory bulb. X 2.

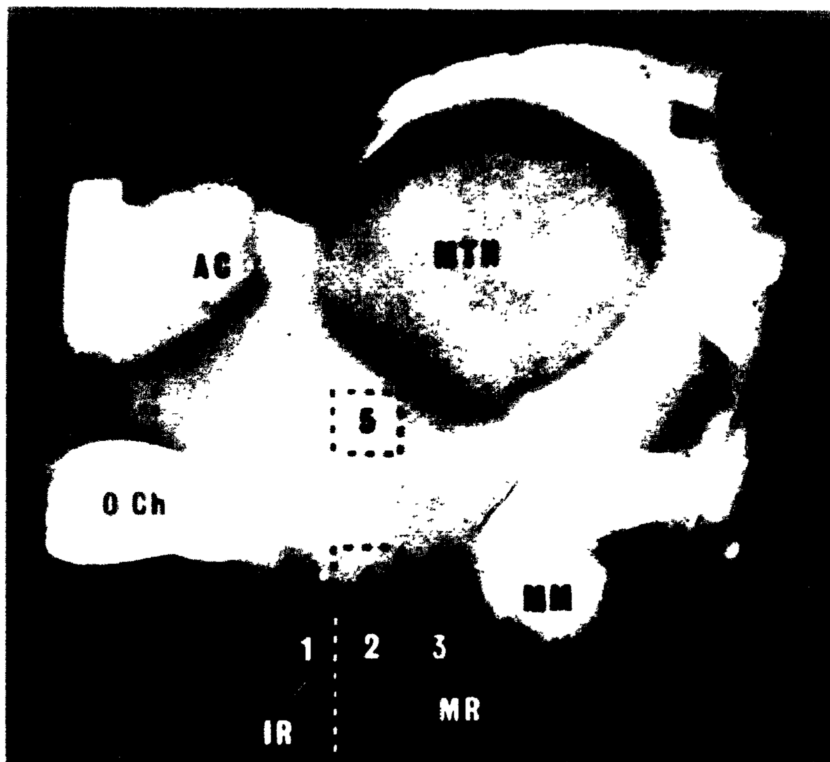
c, Diencephalic block excised from the intact rabbit brain shown above (ventral view). The infundibular stalk has been removed in this preparation to reveal the overlying third ventricle. ON, optic nerve; OCh, optic chiasma; OT, optic tract; Tc, tuber cinereum; VIII, third ventricle; MM, mammillary body; III, oculomotor nerve. X 8.

VE
CN
OB
PM
MO
PF
a

OT
PO
MO
b

ON
OCh
c

Plate 2 Mid-sagittal section through the hemisected rabbit diencephalic block illustrating the location (numbers 1 through 6) on the ventricular surface from which small representative samples of hypothalamic tissue containing ependyma were removed for TEM examination. AC, anterior commissure; OCh, optic chiasma; MTN, midline thalamic nuclei; MM, mammillary body; PC, posterior commissure; IR, infundibular recess; MR, mammillary recess. X 8.


AC
O Ch
1
2
3
IR
MR

diencephalic block in sagittal section (Plate 2) illustrates the locations on the ventricular surface from which the ependymal blocks were excised.

(iii) Post-fixation, Dehydration, Embedding and Sectioning

The small blocks of hypothalamic tissue with ventricular ependyma attached were washed thoroughly in 5.4% phosphate buffered sucrose and post-fixed in 1% osmium tetroxide (OsO_4) in the same buffer at 4°C. The tissues were then rinsed in distilled water (dH_2O), stained "en block" with a saturated aqueous solution of uranyl acetate, and subsequently dehydrated in graded ethyl alcohol and propylene oxide. All specimens were placed in flat embedding molds and oriented with the aid of a Zeiss dissecting microscope. The embedding medium used was Epon 812. For orientation, thick epon sections (0.5-1.0 µm) were cut on a Reichert RU2 automatic microtome. Sections were mounted on glass slides and stained with a 1% solution of Mallory's azur 11-methelene blue (Richardson, Jarett and Finke, 1960) for 30 seconds or less at 50°C.

Ultrathin sections were also cut on a Reichert RU2 automatic microtome. These were placed on 75 x 75 or 100 x 100 mesh copper grids coated with 0.25-0.3% formvar and stained with lead citrate (Reynolds, 1963; Venable and Coggeshall, 1965). Sections were viewed with an AEI EM 801 electron microscope. All electron micrographs were taken on Ilford type E N4E50 70 mm roll film and developed in Kodak D-19 developer for six minutes at 20°C.

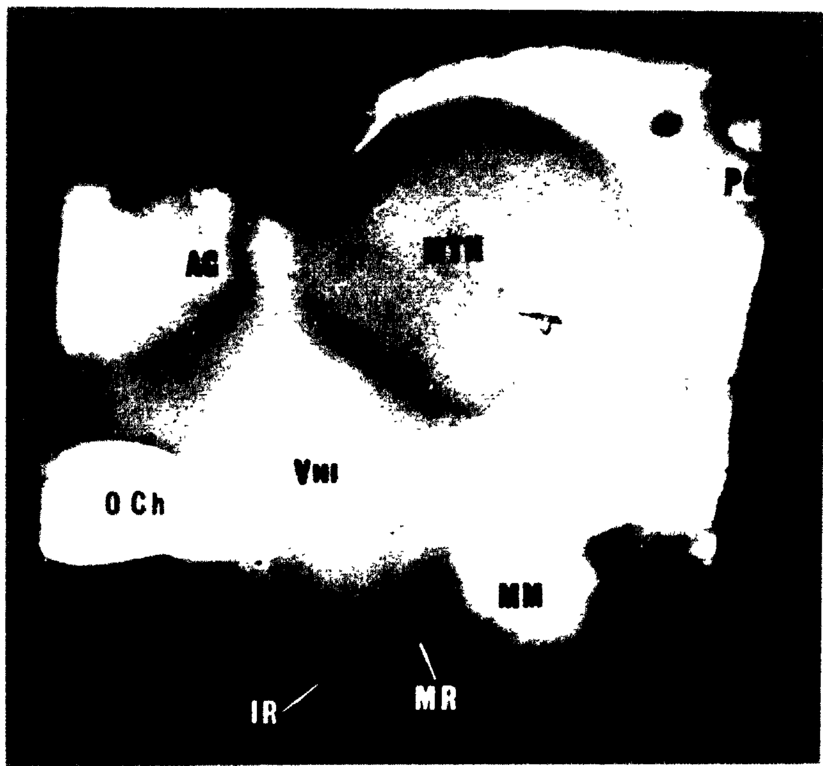
3.5 Preparation of Tissues for Scanning Electron Microscopy

Specimens prepared for SEM were fixed *in situ* by vascular

perfusion of 1500 ml of 1.3% formaldehyde-2.5% glutaraldehyde in 0.1M phosphate buffer as previously described for TEM (Chapter 3.4i). The procedure employed for fixation by perfusion has already been outlined in Chapter 3.2. After the brain was removed from the cranium, a block of tissue containing the diencephalon was excised, split in the mid-sagittal plane and then pinned to a thin piece of cork so as to expose the ventricular surface to be examined (Plate 3). The preparation was quickly rinsed in Hank's balanced salt solution (0.1M phosphate buffer or mammalian Ringer-Locke solution) and then further fixed by immersion in a volume of the same fixative for two hours at 4°C. These steps and all subsequent steps are carried out with constant gentle agitation. Hemisected pieces of the diencephalon were subsequently washed in 5.4% sucrose in 0.1M phosphate buffer, post-fixed in 1% phosphate buffered OsO_4, dehydrated in graded alcohols and then placed in acetone for critical point drying using liquid CO_2 without prior infiltration with amyl acetate. Alternatively, the treatment of some specimens subsequent to alcohol dehydration was according to an improved technique of specimen preparation for SEM described by Watters and Buck (1971). The principal features of this procedure involve the further dehydration in acetone and propylene oxide, extraction of lipids in benzene, and finally drying by sublimation of camphene at room temperature. The advantage afforded by this procedure as with the critical point method is to minimize tissue surface damage that ordinarily occurs as a consequence of surface tension forces when air drying from a liquid.

Dried specimens were mounted on 1/2 inch aluminiumstubs, coated under vacuum with gold, gold-palladium or silver (approximately

Plate 3 Mid-sagittal section through the hemisected rabbit diencephalic block showing the exposed surface of third cerebral ventricle precisely as examined with the SEM. AC, anterior commissure; OCh, optic chiasma; MTN, midline thalamic nuclei; MM, mammillary body; PC, posterior commissure; IR, infundibular recess; MR, mammillary recess; V_{III} , third ventricle. X 8.


O Ch
MM
IR
MR

20 nm) and viewed with a Cambridge Stereoscan or Hitachi HHS-2R scanning electron microscope. All scanning electron micrographs were taken on Polaroid Land film (200 speed, type 42 or 3000 speed type 107) or Kodak Plus-X panchromatic 35 mm film developed in Microdol-X or microphen (undiluted) at times appropriate to the temperature of the developer solution yet yielding negatives of average contrast.

3.6 Procedure for Intraventricular and Systemic Administration of Luteinizing Hormone-Releasing Hormone (LH-RH)

Female white New Zealand rabbits ranging in weight from 3.0 - 6.3 kg ($\bar{x}$ 4.4, S.D. 0.8 kg), were used in this investigation. All does were initially anesthetized with either light ether or a single unsupplemented dose of an aqueous solution (25% w/v) of urethane injected intravenously (1.25 g/kg). Following anesthesia, prior to LH-RH administration, all animals were laparotomized to ascertain that the ovaries showed no evidence of recent ovulation or luteal formation and to confirm the presence of mature follicles. Clear cystic follicles bulging from the surface of the ovary were considered mature (Virutamasen, Hickok and Wallach, 1971). Urethane anesthetized rabbits were also subsequently fitted with a tracheal cannula through a small longitudinal tracheostomy incision. In the first series of experiments, synthetic LH-RH dissolved in 0.15M NaCl, was adminsitered in doses ranging from 0.1 - 6.0 μg/kg body weight, as a single acute injection in the marginal ear vein of randomly selected conscious rabbits. In a second series of similar experiments, LH-RH in doses ranging from 0.016 - 3.0 μg/kg body weight was injected intravenously in urethane anesthetized rabbits.

The efficacy of intraventricularly administered LH-RH in inducing ovulation was examined by injecting varying doses into the lateral cerebral ventricle. Urethane anesthetized animals were secured in a Kopf stereotaxic frame adapted for use with the rabbit as illustrated in Plate 4. The tip of a 25 µl Hamilton syringe was inserted acutely into the lateral cerebral ventricle with the aid of a modified system of coordinates[3] (Sawyer, Everett and Green, 1954) and verified in preliminary experiments by X-ray.[4] Although accurate placement was subsequently found to be invariably associated with a positive reflux of CSF prior to injection, the in situ locus of the needle tip in the ventricle was routinely confirmed at the end of each experiment by tracing the path of the needle tract under the dissecting microscope. Synthetic LH-RH was injected in doses ranging from 0.05 - 0.4 µg/kg body weight. The volume of solution injected was 10 µl or less. In all animals, the ovaries were excised 13-16 hours after injection and their surfaces examined under a binocular microscope for the presence of

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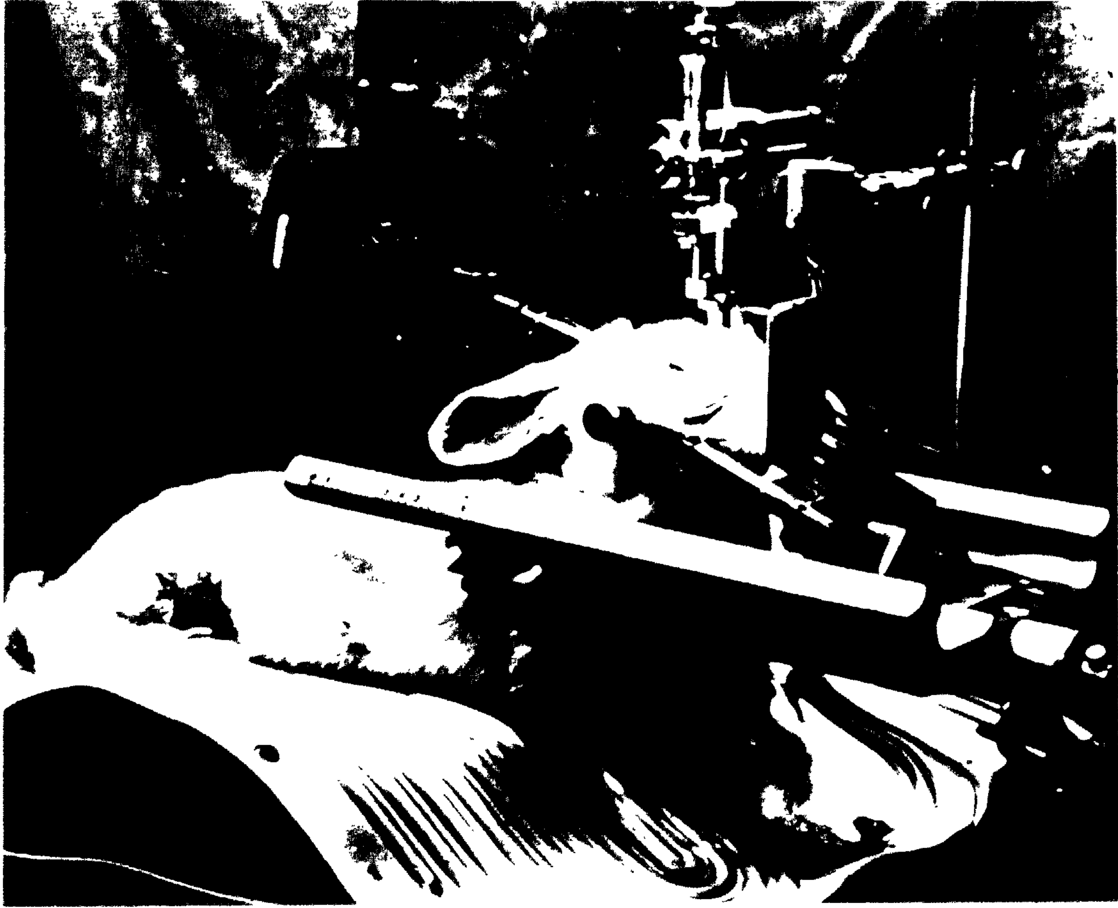
[3]With a special rabbit head holder in a standard Kopf frame, the head was leveled such that bregma was 1.5 mm higher than lambda. Using bregma as the reference point, the frontal zero plane passed through the coronal suture, the sagittal zero plane passed through the sagittal suture and the horizontal zero plane was arbitrarily taken at the surface of the brain below the top of the skull. In preliminary experiments employed in the tentative determination of coordinates, the third cerebral ventricle was dismissed as an injection site because of an inability to repeatedly place our cannula in this potential space with precision and accuracy. In its place was substituted the lateral cerebral ventricle. Our coordinates were selected such that placement of the cannula was consistently in the body of the left lateral ventricle at the level of the interventricular foramen of Monro.

[4]X-rays were taken with a Philips Oralix X-ray unit using Kodak occlusal ultra-speed dental X-ray film DF-45, 2 1/4 x 3" A.N. standard speed group D and developed in Kodak D-19 developer for 5 minutes at 20°C.

Plate 4 Apparatus employed for the intraventricular administration of synthetic LH-RH in the urethane anesthetized rabbit secured in a Kopf stereotaxic frame.



ruptured follicles. The number of rupture sites and the time of ovulation were recorded. The ovaries were then fixed in buffered formali
for histologic examination. If ovulation had occurred, as evidenced by presence of ruptured follicles, ova were then recovered by saline (0.9% NaCl) flushings of the excised uteri and oviducts, and counted under the dissecting microscope. The brains were also preserved by *in situ* fixation by vascular perfusion of 1.3% formaldehyde-2.5% glutaraldehyde in 0.1M phosphate buffer (Chapter 3.2, 3.4i). Following initial fixation, one-half of the diencephalic block which included the intact ME and adjacent periventricular ependymal surface was removed and prepared for TEM examination in the conventional manner (Chapter 3.4ii, iii). For comparative SEM, subsequent treatment of the other one-half of the diencephalic block was as described in Chapter 3.5.

In an additional series of experiments, twelve female New Zealand rabbits ranging in weight from 3.8 - 5.2 kg ($\bar{x}$ 4.4, S.D. 0.4 kg) were used. A 200 ng/kg dose of synthetic LH-RH (twice the predetermined minimum effective ovulatory dose by this route of administration dissolved in 0.15M NaCl was administered intraventricularly in six of the urethane anesthetized animals. The volume of solution injected was approximately 10 μl. The animals were sacrificed 5 minute and 15 minutes following injection. An equal number of identically treated controls received 10 μl of vehicle (0.15M NaCl) only and were sacrificed at the same time intervals. At the time of sacrifice, diencephalic blocks were prepared for TEM and SEM examination in the conventional manner as already described.

3.7 Surgical Procedure Employed for Laparotomy and Ovariectomy

Sexually mature rabbits ranging in weight from 3.7 to 5.8 kg ($\bar{x}$ 4.5, S.D. 0.6 kg) were used in this aspect of the investigation. Each rabbit was randomly allocated to either a control laparotomized or an ovariectomized group. Ovariectomy was performed under aseptic conditions through small bilateral flank incisions under 2.5% Surital (sodium thiamylal) anesthesia administered by intermittent intravenous injection. With the rabbit in a lateral recumbant position, an adequate area was shaved, cleansed with zephiran chloride and incised. The line of incision was situated at the apex of the triangle formed by the last rib and the crest of iliac bone. The ovary was then gently withdrawn through the incision, ligated above and below and then dissected free. The wound was closed by suturing with fine chromic catgut. The procedure followed for laparotomy was identical in all respects excepting excision of the ovaries.

Laparotomized animals were sacrificed at the following postoperative intervals: 3 days, 1 week (wk), 6 wks and 8 wks. Similarly, a minimum of 2 rabbits were sacrificed at 3 days, 1 wk, 2 wks, 3 wks, 4 wks, 6 wks, 8 wks, 16 wks and 34 wks following chronic ovariectomy. At the time of sacrifice, the brains of all animals were fixed by perfusion (Chapter 3.2, 3.4i) and appropriately selected tissue from one hemisected diencephalic block prepared for TEM as described in Chapter 3.4ii, iii. The remaining intact half ventricle was prepared for examination with the SEM (Chapter 3.5).

4. RESULTS AND DISCUSSION

4.1. Morphology of the Walls of the Rabbit Lateral and Third Cerebral Ventricles

i) Light Microscopic Observations

Lining of the Lateral Ventricles

In the rabbit, the walls of the lateral ventricles for the most part are lined by a single, continuous layer of cuboidal ependymal cells (Plates 5b, d, e; 8e). The pseudostratified or stratified variety of ependymal epithelium, typically found in embryonic and neonatal rabbits by Tennyson and Pappas (1962, 1968a) was not evident within the lateral ventricles of the adult rabbit. The most conspicuous feature of ependymal cells in the adult rabbit lateral ventricle was their large prominent vesicular nuclei. They were generally distinct in outline, regularly spheroid or ovoid in shape and uniformly positioned with respect to one another. The apical surface of most, if not all, cells was provided with numerous cilia discernible even at the light microscopic level while their basal surfaces were frequently irregular and indistinct. The ependymal lining as a whole nevertheless was readily demarcated from the subjacent neuropil. These findings are in substantial agreement with descriptions provided of typical ependyma at various loci within the ventricular system of a variety of species (Ferraz De Carvalho, Costacurta and De Carvalho Filho, 1975; Fleischhauer, 1961; Feldberg and Fleischhauer, 1960).

As in other species, variations in the characteristics of

Plate 5 Coronal sections taken through the rabbit telencephalon at the level of the anterior horns of the lateral ventricles. The diagram at the upper left shows the approximate regions of the ventricular wall represented in the photographs a - e. LV, lateral ventricle; CC, corpus callosum; CD, caudate nucleus; DB, diagonal band; AC, anterior commissure; LSN, lateral septal nuclei; MSN, medial septal nuclei; OCh, optic chiasma.

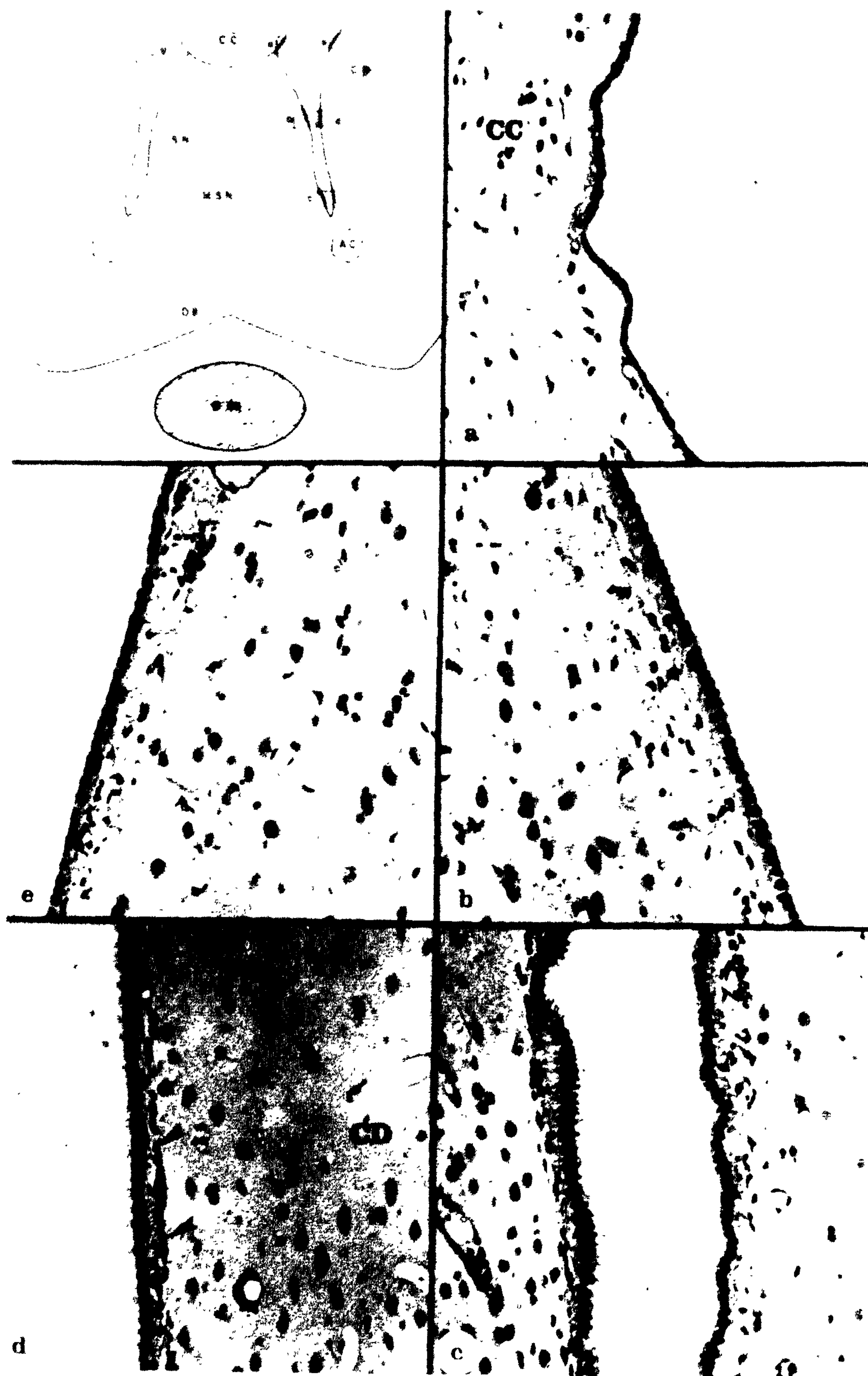
a, A thin sheet-like layer of flattened ependymal cells overlying the corpus callosum (CC). The nuclei of these ciliated ependymal cells are widely separated and flattened. There is not a well-defined layer of subependymal cells. CAHP. X 365.

b, The walls of the lateral ventricles for the most part are lined by a single layer of cuboidal ependymal cells as seen here along the medial wall of the anterior horn. Cilia invariably project from their apical surface while the basal surface, adjoining the neuropil, appears to be drawn out into short processes. CAHP. X 350.

c, Opposing ventral walls of the ventricle lined by taller more cylindrical ependymal cells with closely packed elongated nuclei. There is a conspicuous accumulation of subependymal cells many of which appear, from Golgi-Cox preparations, to be astrocytes. CAHP. X 355.

d, Lateral wall of the ventricle bordering the caudate (CD). Note the characteristically well defined layer of nuclei (arrowheads) which are found under the ependyma. CAHP. X 360.

e, Dorsolateral wall of the ventricle. Common cuboidal ependyma overlies the neuropil. Cilia are seen on their luminal surface which is clearly demarcated by a thin cuticle of ciliary basal bodies and rootlets. CAHP. X 400.



CC
a
e
b
CD
d
c

ependymal cells were distinguished within different regions of the rabbit lateral ventricles. Two such locations were 1) in regions of the ventricles directly adjoining white matter such as the corpus callosum (Plate 5a) and 2) along the ventral walls of the anterior horns of the lateral ventricles (Plate 5c). In the former location, the ependymal epithelium was reduced to a thin, flattened or squamous layer of predominantly ciliated cells. The nuclei of these cells were correspondingly flattened along the long axis of the cells and widely separated. The basal poles of these cells were distinct, relatively straight and roughly parallel to the ventricular surface. In the latter location, however, taller more columnar ependymal cells predominated. The nuclei of these cells were elongated and lay along an axis perpendicular to the ventricle. They were closely packed and found at different levels within the cells. The luminal cell surfaces appeared ciliated for the most part while basal surfaces were indistinct, often appearing to be drawn out into short processes which were frequently directed toward subjacent blood vessels.

In Golgi-Cox preparations it was possible to distinguish within the walls of the lateral ventricle, a small percentage of randomly impregnated ependymal cells against a relatively clear background (Plate 6 and 7). Ependymal cells in such preparations were stained selectively but in a rather scattered fashion. They often appeared in small groups or clusters with outlines of individual cells indistinctly defined. Confined to the outermost epithelial layer bordering upon the ventricle, there can be little doubt that they correspond in position and in distribution to the ubiquitous common mural variety of ependymal cell identified in routine histological preparations. Although the cellular

outlines were irregular and their shape somewhat variable, their size (8-14 μm) approximated that of typical mural cells. Assuming that such cells were stained in their entirety and were accurately represented in Golgi-Cox preparations then it may be hypothesized that not all ependymal cells within the walls of the lateral ventricles are identical in their morphology. Based on the structure of their basal poles at least three seemingly distinct populations of ependymal cells may be identified. For descriptive purposes these cells have been classified as type I or type II ependymal cells or ependymal tanycytes although the first two may perhaps be more appropriately thought of as subtypes of the common mural cells that line the major part of the ventricular wall.

The basal surface of the type I ependymal cell terminated bluntly along the interface with the adjoining neuropil (Plate 6b, c; 7c). Ependymal cells exhibiting this distinct characteristic were the most numerous and too ubiquitous in their distribution to be delimited to any one region of the ventricle. The somata of the second cell type (type II), were drawn out into single or multiple short basal processes (Plate 6c, d; 7a, b, c); they too were randomly distributed within the walls of the ventricle and could not be assigned to any one particular region. Not only were the numbers of basal processes inconstant, but their morphology and length were also highly variable suggesting that type II cells although distinct, may not represent an homogeneous population.

The bases of some type II cells tapered into single stout prolongations which were found to branch occasionally (Plate 6c, d; 7b, c) while in other cells multiple delicate, undivided processes (Plate 6d, 7a)

Plate 6 Photographs of 100 um thick Golgi-Cox sections taken at various rostro-caudal levels through the rabbit lateral ventricles.

a, The medial wall of the anterior horn equivalent to the level shown in Plate 5b. Dendritic processes (arrowheads) of impregnated neurons extend to the ventricle and terminate immediately beneath the ependymal lining. X 166.

b, Ependymal cells within the lateral ventricles representative of those most frequently impregnated by the Golgi-Cox technique. Most of the cells in this field lack basal processes. X 165.

c, Two selectively impregnated ependymal cells observed within the wall of the lateral ventricle. A single branching process extends from the cell on the right for only a short distance from its basal surface parallel to and immediately beneath the ependymal layer. No processes emerge from the cell on the left. X 675.

d, Selectively impregnated ependymal cells in the body of the lateral ventricle overlying the thalamus. The basal pole of some cells taper into a single stout prolongation while multiple delicate processes project from the base of others. X 700.

a

b

c

d

Plate 7 Photomicrographs of 100 um thick Golgi-Cox sections taken at various levels through the adult rabbit lateral ventricle.

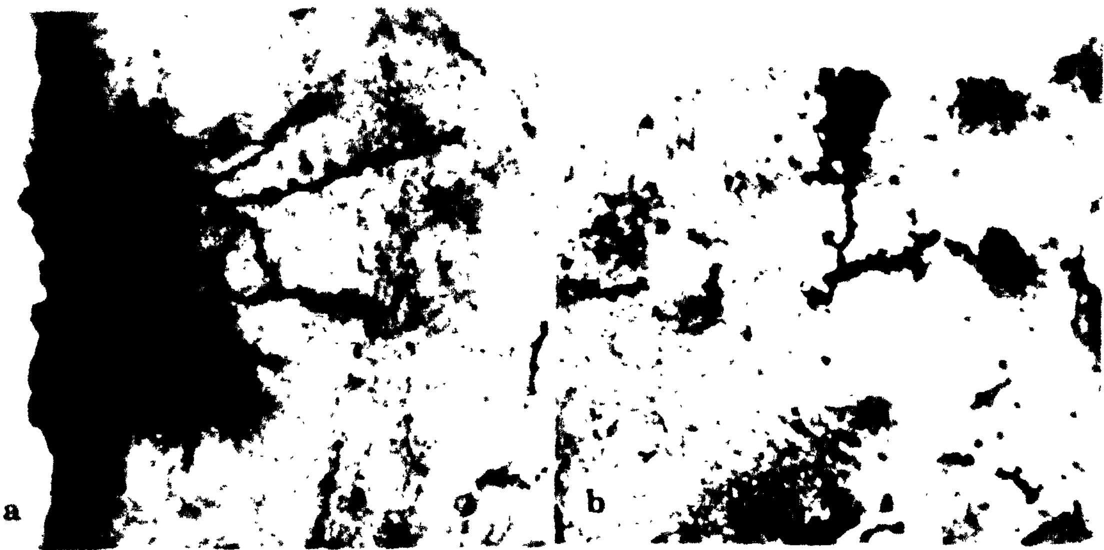
a, A cluster of ependymal cells within the body of the lateral ventricle. Delicate undivided basal processes emanate from the somata and course perpendicularly for only a short distance subependymally. X 700.

b, A solitary ependymal cell within the anterior horn of the lateral ventricle representative of a variety with basal processes that have an irregular knobby or spinous appearance. X 516.

c, The basal processes of some ependymal cells such as the one seen to originate from the cell on the extreme left, run parallel to and immediately beneath the ependymal lining sometimes for long distances. A part of this particular process shown as a shadow is stained but out of the plane of focus. Note that the remaining cells in the field have no processes at all. X 465.

d, Ependymal cells within the medial wall of the anterior horn near its floor with multiple processes extending for considerable distance into the subjacent neuropil. This population of cells is structurally similar to the tanycyte ependymal cells of the third ventricle. X 250.

e, As in fig. d above, A horizontal process extends immediately beneath the ependymal layer while vertically directed processes arborize with a tangle of processes within the subjacent neuropil. X 283.


a
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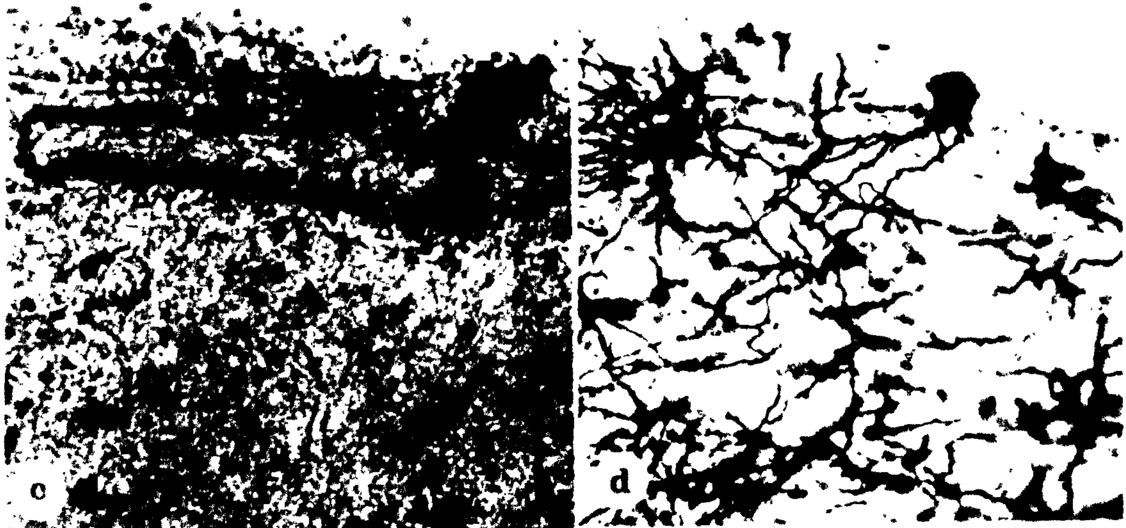
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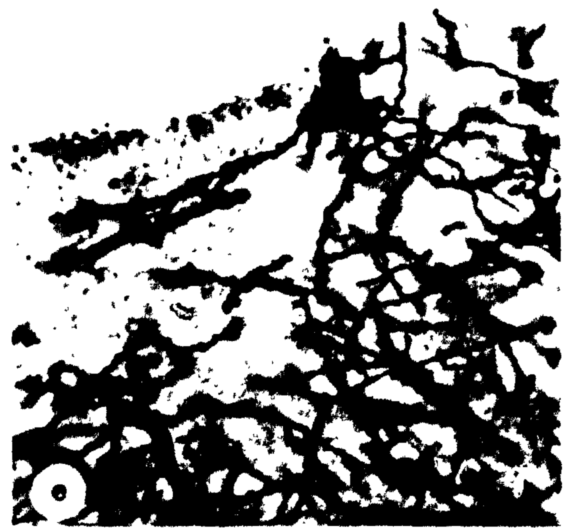
e

projected from their basal surfaces. Some basal prolongations were smoothly contoured while others possessed an irregular knobby or beaded appearance. Most either projected perpendicularly from the soma for a short distance only (Plate 6d, 7a, b) or extended for variable distances parallel to the luminal surface immediately beneath the ependyma (Plate 6c, 7c). These type II ependymal cells as a whole appear to be distinct from the tanycyte population of the third ventricle by virtue of the fact that frequently, though not invariably, (1) more than a single basal process is present, (2) the processes are comparatively short (<60 μm in length) and confined to the immediate hypendymal zone, and (3) they do not form intricate relationships within the neuropil. There is considerable disagreement in the literature as to whether ciliated cuboidal ependymal cells within the mammalian lateral ventricles possess basal processes. In the feline lateral ventricle, for example, no ependymal cells were observed to send processes into the neuropil (Klinkerfuss, 1964), whereas in the human lateral ventricles Malinsky (1968) concluded that "we cannot with certainty decide whether the ependymal cells have one long process each as the typical tanycytes, or whether some cells have more than one process while others have none at all." In the floor of the rat fourth ventricle, Palay (1958) described cuboidal or short columnar ependymal cells whose bases are drawn into tapering processes that extend into the depths of the brain substance. Peters, Palay and Webster (1976) have recently stated that "they (ependymal cells) are cells with essentially epithelial characteristics, and their bases do not project into the periventricular neuropil."

The third type of ependymal cell found within the walls of the

rabbit lateral ventricles possessed one or more long undulating basal processes (>100 μm) that penetrated deeply into the subjacent periventricular neuropil interdigitating with a tangle of glial and neuronal processes (Plate 7d, e). Ependymal cells exhibiting this distinctive characteristic were localized in the rostral portions of the lateral ventricles but the number of such cells identified was very small. Structurally, these cells exhibited features which were more common to the ependymal tanycyte of the third ventricle (see below) and to the more obscure "ependymal astrocyte." With the exception of a few localized regions, however, ependymal cells with long basal processes (tanycytes) have rarely been described in the adult mammalian ventricular system, outside of the third ventricle (Agduhr, 1932; Tennyson and Pappas, 1962, 1968a; Klinkerfuss, 1964; Schachenmayr, 1967; Bleier, 1971, 1972; Sarnat, Campa and Lloyd, 1975). Tennyson and Pappas (1962) found tanycytes among the ependyma of the rabbit midbrain aqueduct during early developmental stages but not in the adult form. Bleier (1971) likewise observed numerous ependymal processes in the rostral and ventral wall of the lateral ventricles, extending into the septum and caudate nuclei in neonatal and young animals but she has seen them only on rare occasions in mature animals. The ependymal cell variant, identified as an "ependymal astrocyte" has been distinguished within the subependyma of the feline lateral ventricles (Klinkerfuss, 1964). These cells with their thick processes penetrating deeply into the underlying neuropil presumably correspond to the "ependymal astrocytes" identified by Tennyson and Pappas (1962, 1968a) within the aqueductal ependyma of the fetal rabbit. The "ependymal astrocyte" as described by Horstmann (1954) in Selachians differs from

the tanycyte in that it possesses multiple twisted basal processes.

In the rabbit, a conspicuous accumulation of cells was distinguishable within a narrow zone immediately subjacent to ependyma of the lateral ventricles. A more or less continuous layer of subependymal cells is most prominent within the walls of the rostral portions of the ventricles (Plate 5b-e, 8e), especially within its lateral walls (Plate 5c-e; 8e). Rostrally, the subependymal layer of cells extends around the floor of the ventricle onto the medial walls (Plate 5b, c) while caudally it gradually disappears from the lateral walls of the ventricle in a ventral to dorsal gradient. Coextensive with the subependymal cellular layer, and closely related to the ependymal lining itself, is an extensive periventricular vascular network (Plate 5b-e; 8e). As noted in the lateral ventricles of the rat (Hirano and Zimmerman, 1967), ependymal cells often appear to directly abut these periventricular blood vessels.

The cellular composition and functional significance of the subependymal aggregations of cells is not fully known. It has been described by a number of investigators who believed it to represent a vestige of an actively mitotic lamina during embryonic life (Opalski, 1933; Kershman, 1938; Globus and Kuhlenbeck, 1944; Shimizu _et al._, 1957; Freide, 1961; Fleischhauer, 1961; Smart, 1961; Tennyson and Pappas, 1968a; Westergaard, 1970). From observations made in the rabbit astrocytes whose terminal expansions form the perivascular glia limitans by ensheathing the numerous blood vessels in the immediate vicinity make up a significant component of this lamina. There are few places in the central nervous system where astrocytes are so concentrated as they are subependymally within the periventricular stratum (Palay, 1958;

Klinkerfuss, 1964).

The presence of neuronal processes within the ependymal lining of the cerebral ventricles has been established in a variety of vertebrates (Chapter 4.3). In the rabbit cerebral ventricles (Plate 6a, lateral ventricle; Plate 16e, third ventricle) small multipolar neurons were occasionally found randomly distributed within the periventricular zone whose dendritic processes were directed toward the ventricle. Such dendrites commonly terminated immediately beneath the ependymal lining or followed a course parallel to but immediately subjacent to the bases of the ependymal cells. No evidence was found, however, to indicate that these processes actually extended beyond the ependyma into the lumen of the ventricle. Astrocytic processes have likewise been observed which take a similar course to the neuronal processes (Plate 16f). Other neuroglial cell types with processes that extend to the ventricular surface have been described both within the ependymal and subependymal regions of the ventricular walls. Processes of "spider cells" and microglial cells that extend to the ventricular surface and terminate beneath or within the ependyma have been described by Bleier (1971, 1972) and Cammermeyer (1965) respectively. It was suggested that on the basis of their relationships these cells may be engaged in transport between neurons, between the ependyma and the CSF, or between the CSF and blood. In the rabbit, the arcuate nuclei (Plate 12a, c, d, e) which lie within the periventricular zone frequently show condensations of their microglial-like cellular components which come to lie adjacent to the ventricular wall (Plate 12d, e).

Lining of the Third Ventricle

For the convenience of presentation, the third ventricle (with

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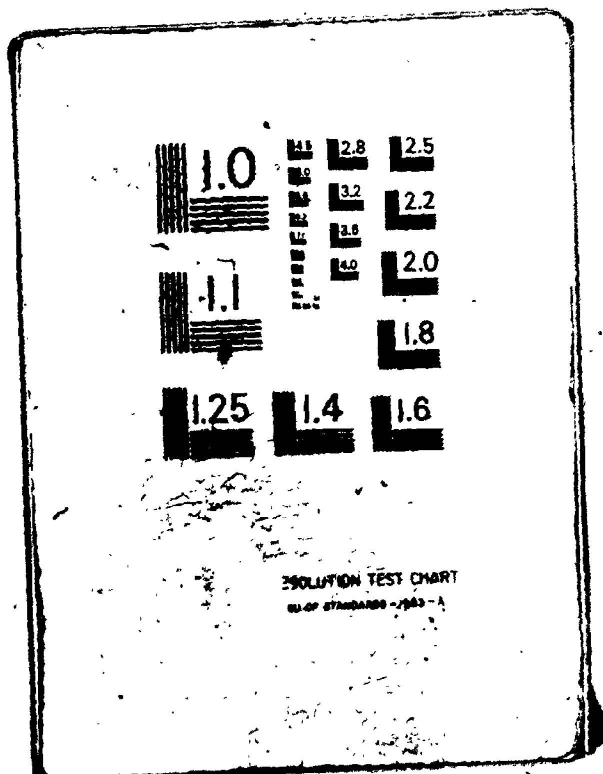


its ependymal lining) has been divided into three regions: (1) ventricle adjoining the rostral hypothalamus, (2) tuberal region, and (3) caudal hypothalamus. At the transverse level of the tuberal region, the third ventricle may be subdivided further into the dorsolateral walls on the one hand and the ventrolateral walls and floor on the other. The greater part of the lining of the third ventricle is comprised of ciliated cuboidal ependymal cells not unlike those already described throughout most of the lateral ventricles. In routine histological preparations, a single, uniformly organized layer of such cells was most commonly distinguished within the walls of the third ventricle bordering upon the rostral hypothalamus (Plate 8a, b), caudal hypothalamus and dorsal tuberal region (Plate 10b, c; 11c, d, e). As in the lateral ventricles, the ependyma of the third ventricle was also comprised of different kinds of cells which could be classified according to the structure of their basal poles. In Golgi-Cox preparations, the ventricular wall adjoining the rostral hypothalamus, dorsal tuberal region and caudal hypothalamus was distinguished by the presence of impregnated ependymal cell clusters (Plate 9c, 13b-d) bearing a striking resemblance to both the type I and II ependymal cells identified within the lateral ventricles (Plate 6b-d; 7a-c) of the rabbit. Although both ependymal cell variants conformed with the common mural type of ependymal cell in their distribution within the walls of the third ventricle, type II cells were more frequently encountered within the lateral walls of the supraoptic recess adjoining the rostral hypothalamus (figure 1, coronal levels 1-4) whereas type I cells predominated within the dorsolateral walls of the tuberal region (figure 1, coronal levels 5-10). The walls of the supraoptic recess at the level of the rostral

hypothalamus were further distinguished by the following additional features. In this location, a gradual transition (Plate 8b) may be seen from the typical cuboidal ependyma (Plate 8a) of the dorsolateral walls to a flattened layer of squamous cells stretching across the floor of the recess (Plate 8c). The flattened ependyma overlying the relatively acellular optic chiasma is similar morphologically to ependyma lining white matter elsewhere in the ventricles (Plate 5a). In contrast to the observations of Tennyson and Pappas (1968a), the lateral walls of the rabbit supraoptic recess were not found to consist of a flattened layer of ependymal cells except in the floor of the recess as noted above. Amid the ciliated cuboidal ependymal cells that cover most of the lateral walls of the supraoptic recess, the ependymal variant identified as the "tanycyte" was found. It was distinguishable from adjacent cells only by virtue of a basal process which extended into the subjacent neuropil (Plate 8a; 9a, b). In routine histological preparations, the faintly stained processes arose in this location from seemingly ciliated cells within and forming a part of the ependymal lining (Plate 8a) in this region of the ventricle. Some processes could be followed to their termination on blood vessels (Plate 8a) while others end indeterminately within the neuropil in close proximity to periventricular neurons and neuroglia (Plate 8a; 9a, b). In comparison to more caudal diencephalic levels, i.e., the tuberal region of the hypothalamus (see below), tanycytes were generally not found in large numbers within the walls of the ventricle adjoining the rostral hypothalamus (figure 1). Still fewer ependymal cells of this type were distinguished within either the dorsolateral walls of the tuberal region or in the caudal hypothalamus (figure 1).

Plate 8 a-e, Coronal sections taken through the rostral diencephalon at the level of the supraoptic nuclei. Selected regions of the ventricle wall represented in the photographs are illustrated in the diagram at the upper left. CAHP method. LV, lateral ventricle; CP, choroid plexus; CD, caudate nucleus; AC, anterior commissure; SO, supraoptic nucleus; MPO, medial preoptic nucleus; LPO, lateral preoptic nucleus; OCh, optic chiasma.

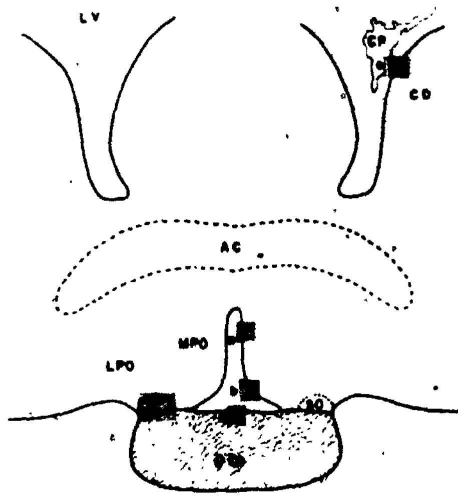
a, Within the lateral walls of the supraoptic recess, the scattered presence of tanycytes (arrows) can be distinguished among the predominantly ciliated cuboidal cells of the ependymal lining. The tanycytes have a process that extends peripherally from their base through a relatively cell-free zone into the adjacent preoptic periventricular neuropil. VIII, third ventricle. X 472.

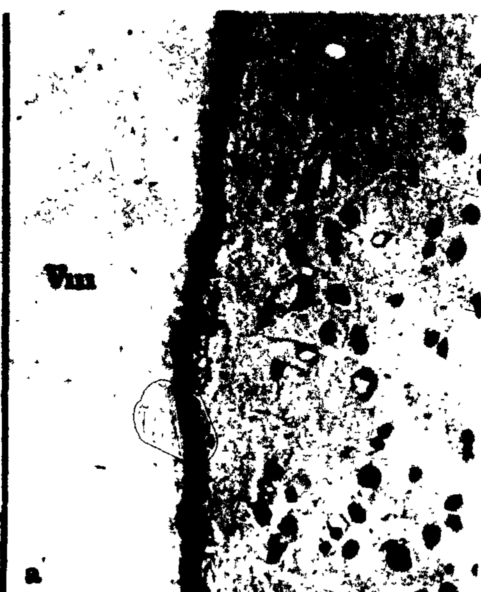
b, Within the ventrolateral walls of the recess, the ependyma gradually assumes the characteristics of the flattened cells which form its floor. Tanycyte ependymal cells were not seen within the ventral ependymal lining which is here closely applied to the diffusely organized cells of the periventricular and medial preoptic nuclei. X 464.

c, Attenuated ependymal lining of the optic chiasma (OCh) in the floor of the recess illustrating the laterally elongated cells with widely spaced nuclei characteristic of the lining in regions where the ventricles directly border white matter. X 468.

d, Large round or oval neurons of the magnocellular supraoptic nucleus (SO) along the dorsolateral border of the optic chiasma (OCh). The nucleus is richly vascularized with the neurosecretory cells clustered around the blood vessels. Some of these cells (arrowheads) contain granules, identified as secretory product which has reacted positively to the CAHP. X 282.

e, A single layer of ciliated cuboidal ependyma within the dorsolateral wall of the anterior horn of the lateral ventricle as in Plate 5e. Beneath the ependymal lining is an accumulation of subependymal cells characteristically seen within the lateral walls of the ventricle. At this rostrocaudal location, the subependymal cell accumulation is especially conspicuous. LV, lateral ventricle; CP, choroid plexus. X 480.


LV
CP
CD
AC
MPO
LPO
SO


a

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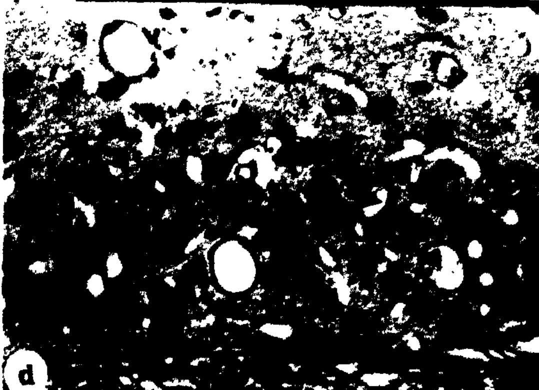
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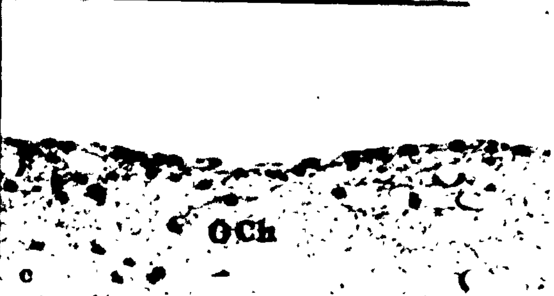
OCh
c

rabbit. Above the level of the infundibular recess, tanycyte processes generally extended horizontally, penetrating the hypothalamic neuropil to varying depths (Plate 11b, 12c, 13a) they were most commonly found to end by attaching to vascular walls (Plate 10a, 16a), in a tangle involving processes of neurons and glial cells (Plate 12c, 13a, 14, 15, 16b-d) or simply diffusely within the neuropil without an identifiable termination. No ependymal tanycyte processes could actually be traced from the ventricular surface to the pial surface in this region although a distinct population of tanycytes identified by Sharp (1972) as "dorsal tanycytes" (type I) in an equivalent region of the quail ventricle were presumed to terminate there. Tanycyte processes which extended from cells within the lateral walls of the infundibular recess assumed a ventrally directed curved course through the arcuate neuropil and tuber cinereum (Plate $10a_3$, 12). These processes appear similar to the fine non-varicose fibers of "type II tanycytes" described by Sharp (1972) in the quail. A number of these processes appeared to converge upon the external glial membrane at the lateral surface of the diencephalon while others were closely apposed to blood vessels at the tubero-emential interface (Plate $10a_3$, 12b). Whether or not direct contact is established between tanycyte terminals and cells of the pars tuberaliscould not be determined in this study although such an arrangement has been reported by Anand Kumar and Knowles (1967) and Knowles and Anand Kumar (1969) in the Rhesus monkey. From the discontinuous looking ependymal lining of the floor of the IR, most tanycyte processes project vertically through the ME. Most, if not all, processes appear to converge in bundles which subsequently split within the external zone of the ME into the typical "horsetail-like" terminal divisions (Plate

a

b

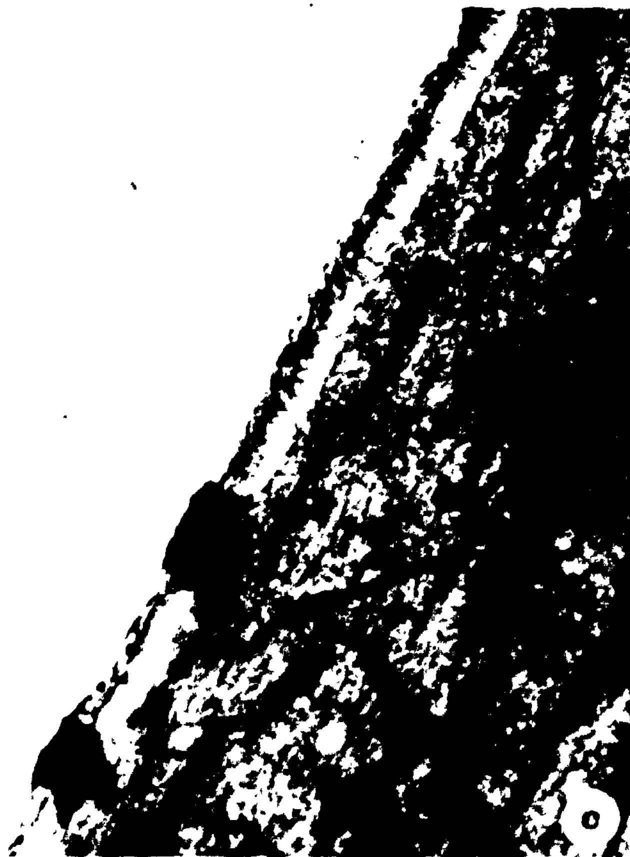
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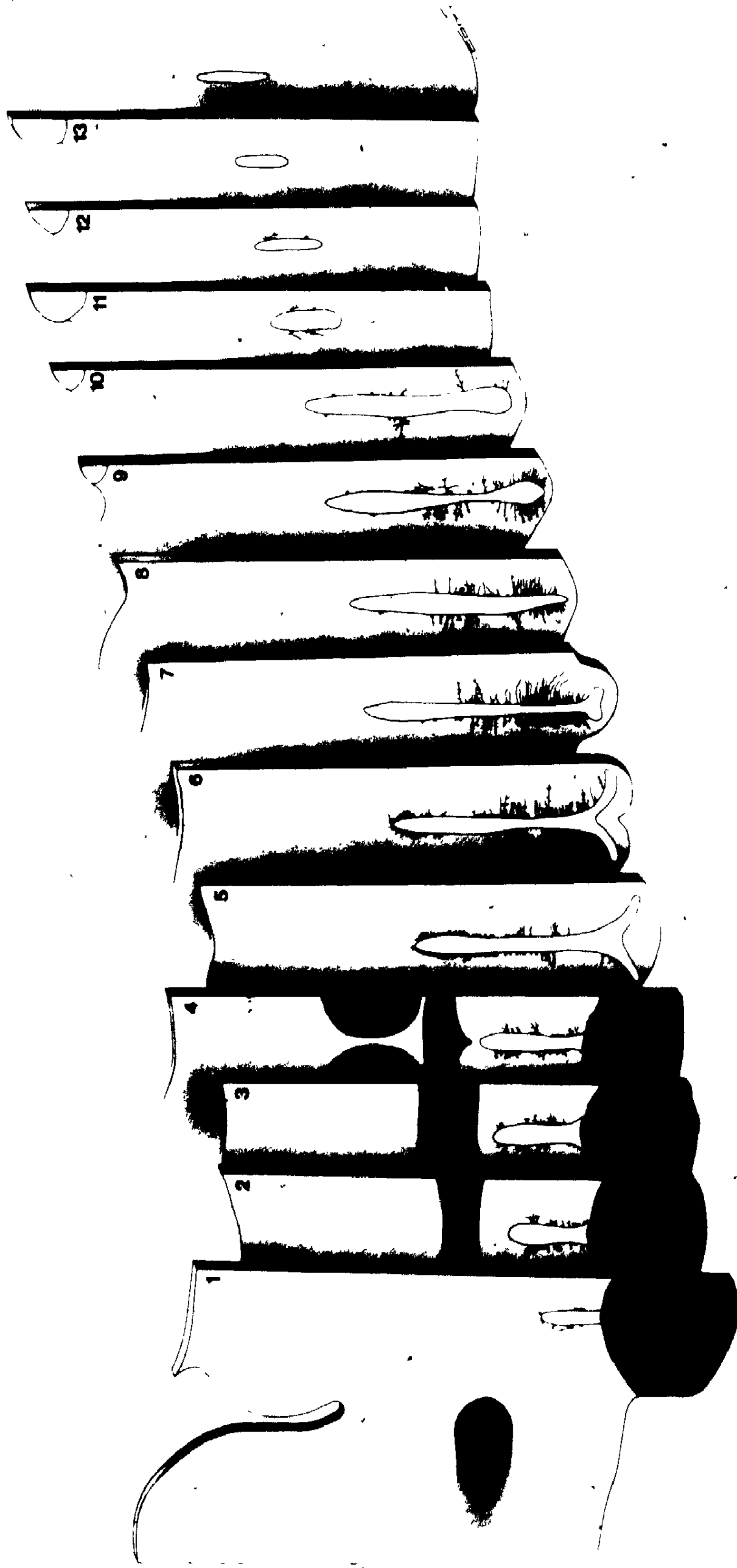
Figure 1

Composite diagram reproduced from a series of camera lucida drawings of coronal sections taken serially through the rabbit diencephalon. The diagram illustrates the number and distribution of impregnated ependymal cells within the walls and floor of the third ventricle as revealed in a typical Golgi-Cox preparation. Each coronal level (1 through 14) represents 3 serial sections each of 100 um thickness. The following regional histological variation may be noted: 1) the concentration of ependymal tanycytes along the ventrolateral wall and floor of the ventricle throughout the tuberal region of the hypothalamus (sections 5 through 10), 2) the conspicuous absence of tanycytes in regions of the ventricle adjoining the caudal hypothalamus (sections 11 through 14), 3) the presence of comparatively few tanycytes within the lateral walls of the ventricle in the preoptic and supraoptic regions (sections 1 through 4) and 4) the preferential localization of Type I and II ependymal cells within the rostral, dorsal tuberal and caudal hypothalamus.


1
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14

At the transverse level of the tuberal hypothalamus, morphological differences in the structure of the hypothalamic ependyma were most evident (figure 1). The ependymal lining of the ventrolateral walls and floor of the third ventricle possessed an appearance which differed markedly from the conventional ependymal lining of the dorsal hypothalamic wall. Dorsally, the ventricular wall was formed by a single, uniformly organized layer of ciliated cuboidal cells with prominent round or oval nuclei which appeared centrally placed within the cell body (Plate 10b, c; 11c, d). In contrast, the ependymal lining corresponding to approximately the ventral one-third of the wall was largely though not entirely non-ciliated, compressed, and in regions irregularly stratified (Plate 10a-a3; 11a, b). It was composed of ependymal cells containing irregularly-shaped nuclei. The cells were unevenly spaced and organized into tightly packed clumps or clusters which gave the lining itself an overall irregular appearance (Plate 10; 11a, b1). The most distinctive feature of ventrolateral walls at this level is the prominence with which tanycytes are found. Basal processes extend from almost all ependymal cells in regions of the ventricular wall which overlie the ventromedial and arcuate nuclei (Plates 10a-a3, 11a-b1; 12, 13a). They are distributed with approximately the same density throughout the rostrocaudal extent of the entire tuberal region of the hypothalamus (figure 1). Tanycytes in the rabbit, therefore, were not restricted to the anterior tuber cinereum as appears to be the case in the Rhesus monkey (Anand Kumar and Knowles, 1967; Anand Kumar, 1968a; Knowles and Anand Kumar, 1969). Neither was a uniformly double layer of tanycytes separated by a distinct space, as described by these authors in the monkey, seen in the

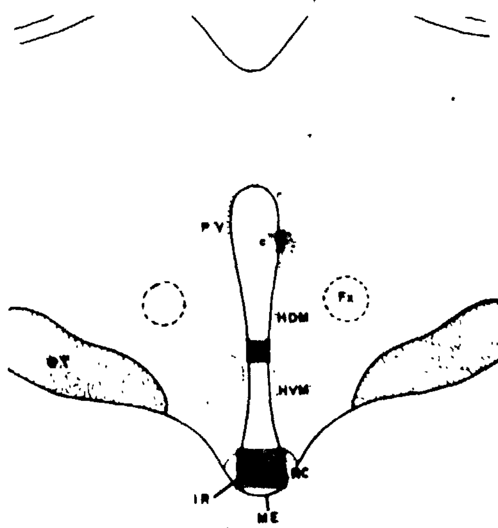
Plate 10 a-c Coronal sections taken through the tuberal region of the rabbit hypothalamus. Selected regions of the ventricle wall represented in the photographs are shown in the accompanying diagram. CAHP method. PV, paraventricular nucleus; Fx, fornix; OT, optic tract; HDM, dorsomedial hypothalamic nucleus; HVM, ventromedial hypothalamic nucleus; ARC, arcuate (infundibular) nucleus; IR, infundibular recess; ME, median eminence.

a, A survey photomicrograph of the ependymal lining of the ventrolateral walls and floor of the medial tuberal region of the third ventricle. The greatest density of tanycytes as may be seen by the large number of radially directed processes are situated within the ependymal lining bordering upon the ventral most part of the ventricle, infundibular recess and median eminence. Adjacent walls of the ventricle have been apposed in this photograph by removing a strip from the ventricle. X 132.

a_1, Representative segment of the ventrolateral wall of the third ventricle as in figure a above. Numerous faintly stained basal processes of ependymal tanycytes (arrowheads) extend radially ending on blood vessels in close proximity to the ependymal layer. Note that the morphology of the ependymal lining is more irregular than elsewhere in the ventricular system, with ovoid, elongate and lobate nuclei closely interdigitated. X 546.

a_2, Tanycytes of the ventral wall as in figure a above with basal processes extending radially for variable distances into the arcuate neuropil. Most processes cannot be traced to an exact termination although a number appear to encroach upon neurons and glia. Gomori-positive material (arrowheads) is present within the cytoplasm of cells scattered diffusely throughout the neuropil. Note the irregular organization of ependyma in clusters giving the lining an almost discontinuous appearance. X 342.

a_3, Tanycyte processes within the mediobasal tuber cinereum. Some processes (arrows) extend from the cell body at the ventricular surface to the pars tuberalis. In their course through the neuropil they appear to run together in bundles which split distally into 'horsetail-like' terminal divisions. Other processes (arrowhead) take a more abbreviated course terminating in contact with blood vessels within the arcuate neuropil. X 340.


PV
HDM
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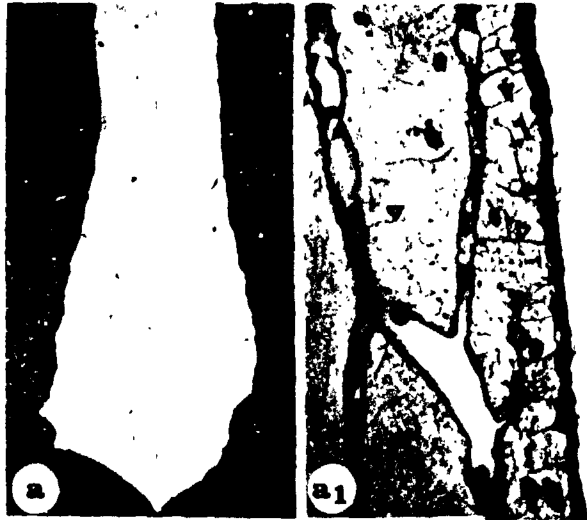
IR
ME


a
a1

a2

a3

Plate 10 b, A survey photomicrograph of a segment of the dorsolateral walls of the third ventricle at the medial tuberal level. Notice that tanycyte ependymal cells are not present at this level of the ventricle as evidenced by the obvious absence of their radially directed basal processes. X 126. Inset, shows the regular line of ependymal cell nuclei characteristic of cuboidal cells of the dorsolateral wall. Note the hypendymal cells provided with expansional attachments to blood vessels situated beneath the ependymal lining of the dorsal wall. The flattened elongate nuclei immediately subjacent to the ependymal lining are likely those of microglial cells. X 326.

c, Paraventricular nucleus (PV) and adjoining ependymal lining of the ventricle. As in the supraoptic nucleus (Plate 8d), the densely organized neurons of the PV nucleus contrast sharply with the surrounding neuropil by virtue of their distinctive staining properties. X 132. Inset, shows a single layer of regularly ordered cuboidal ependymal cells characteristic of the dorsal ventricular walls. Compare with figure $10a_1$, a_2, a_3. X 351.

b

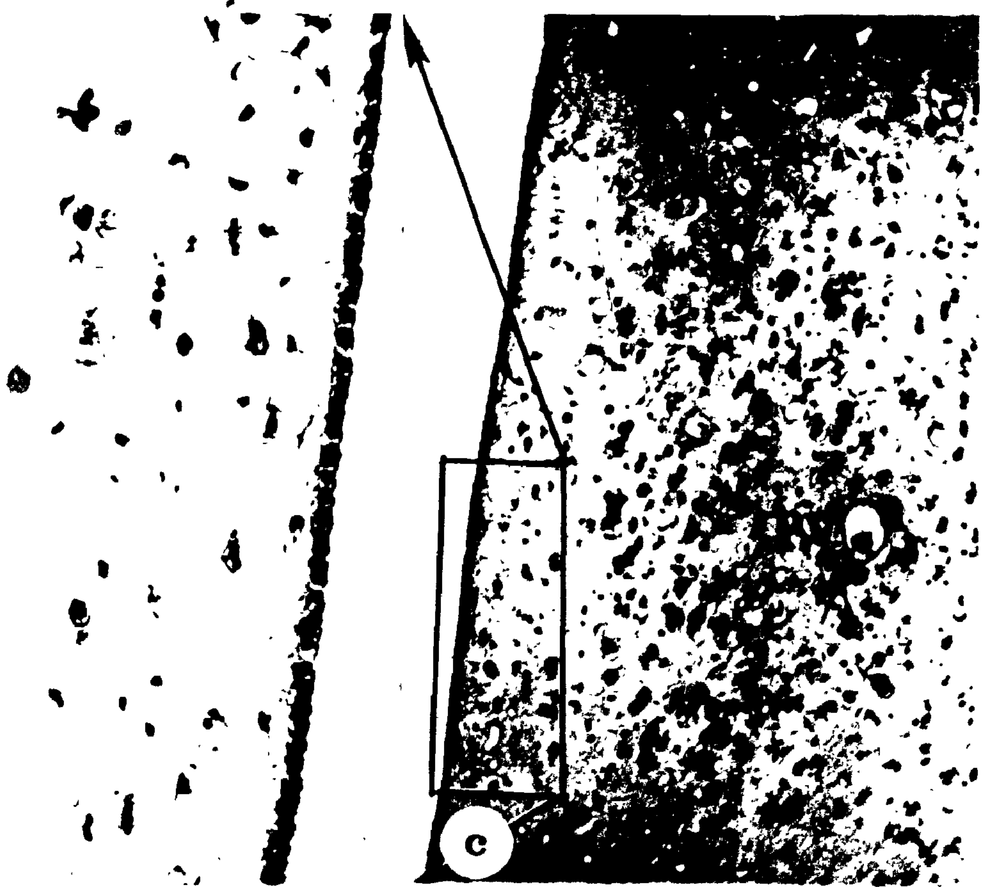
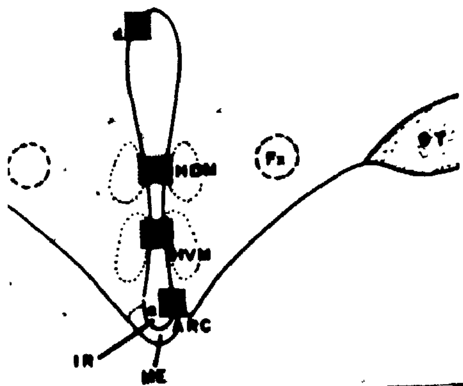

c

Plate 11 a-e, Coronal sections taken through the tuberal region of the rabbit hypothalamus. The shaded areas in the diagram indicate the region of the ventricular wall represented in the micrographs. Labels are as in Plate 10. CAHP method.

a, Neurons of the arcuate nucleus at the base of the ventricle border directly upon the ependymal lining. Tanycyte processes are seen extending into this nuclear group. X 150. a_1, as in fig. a but a few sections displaced showing a distinct but isolated accumulation of subependymal cells reminiscent of the subependymal cell plate in the lateral ventricles. X 135.

b, A survey photomicrograph at the level of the HVM nucleus. X 135. b_1, enlargement of an area corresponding to level of the arrow in fig. b showing numerous horizontally directed tanycyte processes. X 170. b_2, enlargement of an area corresponding to the level of the arrowheads in fig. b showing a pocket of subependymal cell concentration. X 350.



Fx
ARC
IR
ME

a

a1

b

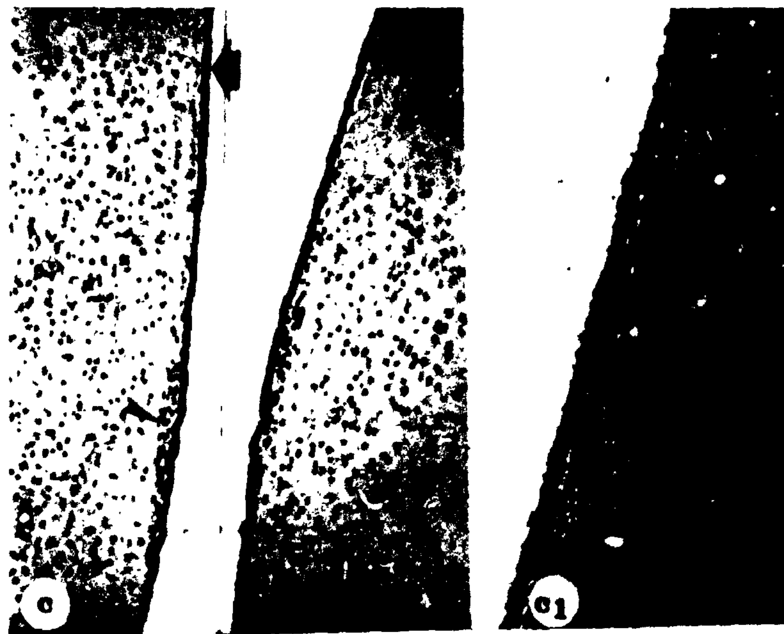
b1

b2

Plate 11 c, Tanycyte ependymal cells are lacking along the dorsal walls of the ventricle as evidenced by the absence of radially directed basal processes. Arrowhead, isolated concentration of subependymal cells. X 130. c_1, enlargement of an area similar to that illustrated in fig. c (arrow). Note the regular line of cuboidal cells and the hypendymal astrocytes. X 210.

d, Roof of the third ventricle showing the absence of tanycytes and a well defined zone of subependymal cells. X 125. d_1, enlargement of an area equivalent to that shown in fig. d (arrow). X 210.

e, A regular lining of ciliated cuboidal ependymal is found at the thalamic level of the ventricle. X 190. e_1, as in fig. e showing Gomori-positive granules (arrowheads) within hypendymal glial cell. X 350.


c
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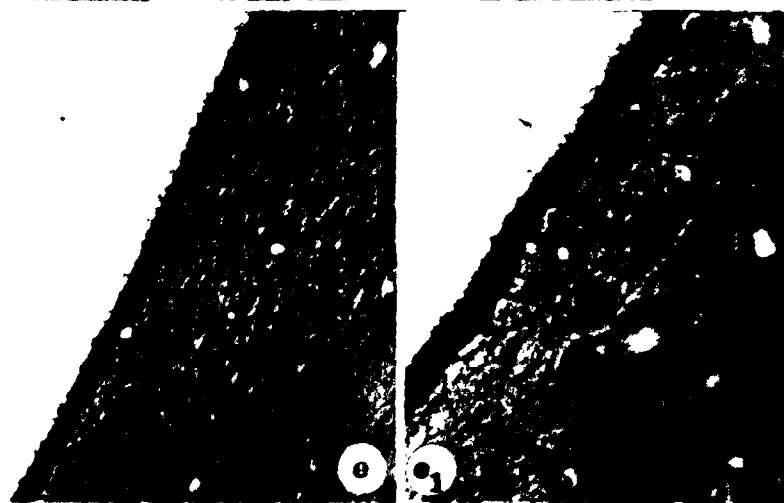

e
e1

rabbit. Above the level of the infundibular recess, tanycyte processes generally extended horizontally, penetrating the hypothalamic neuropil to varying depths (Plate 11b, 12c, 13a) they were most commonly found to end by attaching to vascular walls (Plate 10a, 16a), in a tangle involving processes of neurons and glial cells (Plate 12c, 13a, 14, 15, 16b-d) or simply diffusely within the neuropil without an identifiable termination. No ependymal tanycyte processes could actually be traced from the ventricular surface to the pial surface in this region although a distinct population of tanycytes identified by Sharp (1972) as "dorsal tanycytes" (type I) in an equivalent region of the quail ventricle were presumed to terminate there. Tanycyte processes which extended from cells within the lateral walls of the infundibular recess assumed a ventrally directed curved course through the arcuate neuropil and tuber cinereum (Plate $10a_3$, 12). These processes appear similar to the fine non-varicose fibers of "type II tanycytes" described by Sharp (1972) in the quail. A number of these processes appeared to converge upon the external glial membrane at the lateral surface of the diencephalon while others were closely apposed to blood vessels at the tubero-emential interface (Plate $10a_3$, 12b). Whether or not direct contact is established between tanycyte terminals and cells of the pars tuberaliscould not be determined in this study although such an arrangement has been reported by Anand Kumar and Knowles (1967) and Knowles and Anand Kumar (1969) in the Rhesus monkey. From the discontinuous looking ependymal lining of the floor of the IR, most tanycyte processes project vertically through the ME. Most, if not all, processes appear to converge in bundles which subsequently split within the external zone of the ME into the typical "horsetail-like" terminal divisions (Plate

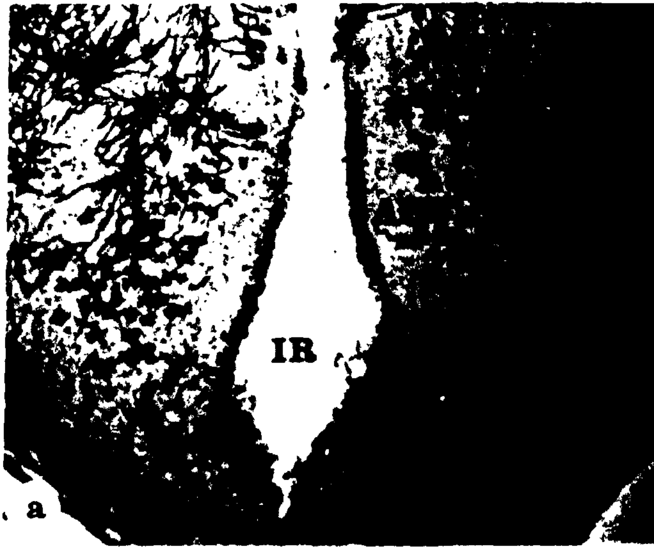
Plate 12 a, Coronal section through the rabbit infundibular recess (IR) at the caudal level of the arcuate nucleus (ARC) showing tanycyte ependymal cells within the lateral walls and floor of the IR. Their basal processes project almost vertically through the median eminence. Golgi-Cox. X 68.

b, A solitary tanycyte process (arrow) links the lateral wall of the ventricle near the floor of the infundibular recess (IR) with the pial surface of the brain. In its course through the tuber cinereum the process is undivided except at its termination where it splits into several short terminal branches. Golgi-Cox. X 177.

c, In Golgi-Cox preparations hypothalamic nuclear groups generally blend imperceptibly with each other. The arcuate nucleus (ARC) as illustrated in this figure is an exception. Its peculiar microglial-like cellular components facilitate its identification. Note that tanycyte processes extend horizontally into the VMN whereas inferiorly they assume a ventrally directed curved course through the arcuate neuropil. Golgi-Cox. X 91.

d, At higher magnification, the microglial-like components of the arcuate nucleus (arrows) may be seen insinuated among the tanycyte processes. Their form consists of elongated cell bodies with short tortuous processes arising from either pole. X 180.

e, Ventral walls of the third ventricle adjacent to the arcuate nucleus (as in fig. c) at higher magnification. Note that the majority of delicate processes appear to be undivided. They originate singly from their respective somata and penetrate the nucleus to varying depths. Golgi-Cox. X 178.


IR
a

IR
b

d

c

e

Plate 13 a, Tanycyte ependymal cells at the level of transition from the ventromedial to dorsomedial hypothalamic nuclei. Basal processes extend from tanycyte ependymal cells into the ventromedial nuclei (lower one third of photomicrograph) less frequently into the dorsomedial nuclei (upper two thirds of photomicrograph) and not at all at more dorsal levels of the ventricle wall (see figure 1). Golgi-Cox. X 80.

b - d, Type I (fig. c and d) and II (fig. b) ependymal cells from the dorsal wall of the third ventricle at the level of the tuberal region of the hypothalamus. Compare with Plate 6b, c, d; 7a, c; 9c. Golgi-Cox. b, X 651; c, X 760; d, X 715.

e - g, Photomicrographs of a few of the many tanycyte forms distinguished within the ventral walls and floor of the third ventricle.

e, A variety of tanycytes whose main process is comparatively smooth and undivided except distally at its termination. Note that the secondary branches end in terminal enlargements apposed to tangentially oriented processes of indeterminate origin. X 830.

f, Tanycyte ependymal cells at the arcuate-infundibular level of the third ventricle with coarse stout basal processes. Note their irregular knobby or spinous outlines. X 453.

g, A tanycyte ependymal cell at the level of the ventromedial hypothalamic nucleus with one coarse stout radially directed process from which emerges numerous spines and a second tangentially directed process which is comparatively thin and smoothly contoured. X 257.

$10a_3$) described by several investigators (Lofgren, 1959, 1960, 1961; Kobayashi *et al.*, 1970; Sharp, 1972). The divergent terminal processes end at the basal surface of the palisade layer of the ME upon the portal vasculature. Scott *et al.* (1972a, b) estimate that in this region tanycyte terminals occupy nearly half the total surface area of the portal bed. Kobayashi (1975) and Oota *et al.* (1974), however, reported that the number of ependymal endings abutting directly on the capillary walls of the ME was somewhat more variable and frequently species dependent. The tanycyte population of the rabbit ME was usually poorly impregnated in Golgi-Cox preparations (Plate 12a, b). A similar observation made in the quail ME using the Gallyas method of staining was interpreted by Sharp (1972) as a criterion for distinguishing what he considered to be the morphologically "protoplasmic" variety of tanycytes (Type 4) of the ME from the more "fibrous" categories of tanycytes (Types 1, 2 and 3) within the ventrolateral and dorsolateral walls. In the present study, the basal processes of a number of tanycytes distinguished in the ME, divided at their termination into several short terminal branches similar to that illustrated in Plate 12b. In this respect they resemble tanycytes whose appearance is believed by Bleier (1971, 1972) to be characteristic of the ME and which we think to be equivalent to the terminal "horsetail-like" configuration seen in routine histological preparations (Plate $10a_3$).

Although tanycytes along the ventrolateral wall and floor of the rabbit third ventricle were similar enough to be recognized as a distinct population, their processes frequently assumed a variety of forms, lengths, diameters and branching patterns (Plates 12, 13e-g, 14, 15). Specific structural variations among tanycytes however could not be

Plate 14 Photomicrographs of tanycyte ependymal cells encountered at various loci within the ventrolateral walls of the third ventricle. The ependymal lining lies horizontally along the top edge of each photomicrograph. Golgi-Cox.

a, The elongated tanycyte soma measuring approximately 11 - 15 um borders on the ventricular lumen. The main process is approximately 160 um long. It has a very irregular knobby outline. Note the lateral branches which emerge from the main process. X 526.

b, Two radially directed processes each approximately 130 - 150 um long appear to emerge from the one soma which is of the order of 8 - 15 um. Both processes show varicosities along their length and appear unbranched as they span the cell free periventricular zone. P, protoplasmic A. X 538.

c, This photomicrograph was taken at the level of the arcuate nucleus. It shows multiple vertically oriented processes emerging from the basal pole of what appears to be a single tanycyte soma. Notice also the horizontally directed process. Compare with Plate 7d, e. X 500.

d, Tanycyte within the ependymal lining bordering the ventromedial nucleus. Note that its thick process tapers distally along its length. X 800.

e, Tanycyte within the ependymal lining bordering the ventromedial nucleus. Note the presence of numerous short clustered spines (arrowheads) distributed along the length of its process, as well as what appear to be collateral branches (arrow). X 553.

Plate 15 Photomicrographs of tanycyte ependymal cells seen within the lateral walls of the third ventricle at the level of the IR. The ependymal lining lies horizontally along the top edge of each photograph.

a, Collateral branches (arrowheads) spring perpendicularly from the main process shortly after it emerges from the soma. The main process, approximately 70 um in length, is otherwise undivided except distally (arrows) as it encroaches upon a cluster of microglial-like cells in the arcuate nucleus. Golgi-Cox. X 632.

b, Collateral branches (arrowheads) emerge more distally along the tanycyte process which in this case is approximately 185 um in total length. Note that the vertical process on the left appears to emerge from a cell body which lies beneath the ependymal lining (a displaced tanycyte). Golgi-Cox. X 594.

c, Tanycyte process approximately 70 um long with many lateral branches (arrowheads). Golgi-Cox. X 611.

Plate 16 a, Tanycyte ependymal cell processes in contact with blood vessels within the periventricular neuropil. Golgi-Cox. X 410.

b & c, Illustrated is the relationship established by ependymal tanycytes and neuroglia in the form of a complex tangle of processes. The silhouetted glial images in these photomicrographs are assumed to be fibrous astrocytes. Contact between the two cells is established (fig. c, arrowhead). Golgi-Cox. b, X 252; c, X 250.

d, Microglial-like component of the arcuate nucleus receiving a single undivided tanycyte process. This process measures approximately 150 μm in length. Golgi-Cox. X 530.

e, Small pyramidal neuron (arrowhead) within the periventricular neuropil with dendrites that extend to the ventricle where they course immediately beneath the ependymal lining. Golgi-Cox. X 627.

f, A neuroglial cell assumed to be a fibrous astrocyte with expansions in contact with the base of the ependymal lining. Golgi-Cox. X 627.

a

b

c

d

e

f

related regionally to any particular hypothalamic location and in general they were not dissimilar enough in their morphology to permit separate classification. These findings are in substantial agreement with observations made by Bleier (1971, 1972) in rabbits, rats, mice and cats. They differ, however, from observations made by Millhouse (1971, 1975) in rats and mice. He recognized that most tanycytes along the third ventricle could be divided into three distinct parts: (1) a cell body next to the ventricle, (2) a neck portion which emerged from the cell body, was confined to the periventricular zone and usually contacted a vessel wall, and (3) a thin smooth tail portion distal to the neck. Dorsal tanycytes and those in the ME, however, were found by Millhouse to be sufficiently exceptional morphologically to be distinguishable as separate populations. Dorsal tanycytes lacked neck processes, consisting of only cell body and tail portion, whereas tanycytes in the ME lacked tail portions. It was not possible, in the present study to recognize such subtle distinctions in tanycyte morphology in the rabbit.

With few exceptions (Plate 11a_1, b, b_2, c) a subependymal cell layer, as well defined as that found within regions of the lateral cerebral ventricles, was generally not observed within the third ventricle. Hypendymal cells, however, commonly occurred individually or in groups at irregular intervals subjacent to the ependyma (Plate 10b, 11d, e_1). They were most conspicuous within the dorsolateral walls and roof of the third ventricle throughout its rostrocaudal extent, and in this respect appeared to be related inversely to the pattern of tanycyte distribution. Cammermeyer (1965), identified similar cells, which were found a short distance beneath the ependyma in the gray

matter of the rabbit third ventricle, as tanycytes. Tennyson and Pappas (1968a) have even suggested that the ependymal tanycyte of the rabbit may be a "migratory" cell, since the subependymal region contains cells with a similar morphology.

It is well known that Gomori's chrome alum hematoxylin phloxine and paraldehyde fuchsin methods selectively stain hypothalamic neurosecretory material classically associated with the magnocellular hypothalamo-neurohypophysial system. With the first method, the main mass of both the supraoptic (Plate 8d) and paraventricular (Plate 10c) nuclei contrast sharply with the surrounding neuropil by virtue of the distinctive staining properties of their neurosecretory cells. In addition to these neurosecretory nuclei, CAHP-positive material was also observed within neuronal and glial cells scattered throughout the tuber cinereum (Plate 10a_2, a_3) and subjacent to the ependyma within the thalamic portion of the ventricle walls (Plate 11e_1). A survey of the literature revealed that Gomori-positive granules have been demonstrated in cells identified as both neuroglial and microglial, situated periventricularly within the hypothalamus of a variety of species Cammermeyer, 1965; Polak and Azcoaga, 1969). In rodents, Smith (1951) noted a small number of Gomori-positive neuroglial cells in the subependymal zone of the floor of the infundibulum. Kroon and Goossens (1974) have localized their presence to glial cells of the arcuate nucleus and adjoining tuberal region of the hypothalamus while Wislocki and Leduc (1952, 1954) called attention to their scattered occurrence throughout the central grey substance around the third ventricle and cerebral aqueduct. Accumulations of such cells in some circumventricular organs (notably the SFO, AP and SCO) and surrounding regions have

also been reported (Wislocki and Leduc, 1954; Teichmann, 1967; Koritsánszky, 1969).

The location of Gomori-positive glial cells within the subependymal zone of the third ventricle and in close association with areas of neurosecretion within the hypothalamus is considered by some to be evidence that glial cells may simply have phagocytosed their complement of granules (Wislocki and Leduc, 1952, 1954; Ford and Kantounis, 1957; Teichmann, 1967). This view, however, is not shared by all investigators. For example, the belief has also been expressed that these Gomori-positive glial cells may be related to the neurosecretory system, to the transfer of neurosecretory material or that they may represent modified glial cells with the capacity to secrete Gomori-positive material (Koritsánszky, 1969; Polak and Ascoaga, 1969; Kroon and Goossens, 1974).

In the present investigation, Gomori-positive material was not observed in typical mural ependymal cells or tanycyte ependymal cells and their processes. This is in contrast to demonstrations of selective Gomori-positive staining of ependyma within circumventricular organs (Wislocki and Leduc, 1954; Vigh et al., 1962; Diederen, 1969; Vigh, 1969; Vigh and Vigh-Teichmann, 1969) and within distinct but less circumscribed regions of the general ventricular lining (Vigh et al., 1962; Vigh et al., 1963; Vigh, 1964). In the Rhesus monkey, tanycytes situated in the antero- and latero-ventral walls of the anterior hypothalamus were reported by Anand Kumar and Knowles (1967) and Anand Kumar (1968b) to contain coarse granules which stained selectively with the CAHP method. In the rat hypothalamus, Kroon and Goossens (1974) observed granules stainable with CAHP within ependymal

cells of the infundibular recess, though never in the processes of tanycytes or anywhere in the rest of the ependymal lining. This selective staining contrasts with the non-specific CAHP-positive reaction of ependymal cells in all parts of the rodent lateral ventricles which prompted Westergaard (1970) to conclude that lipofuscin rather than a specific ependymal product was responsible for the staining.

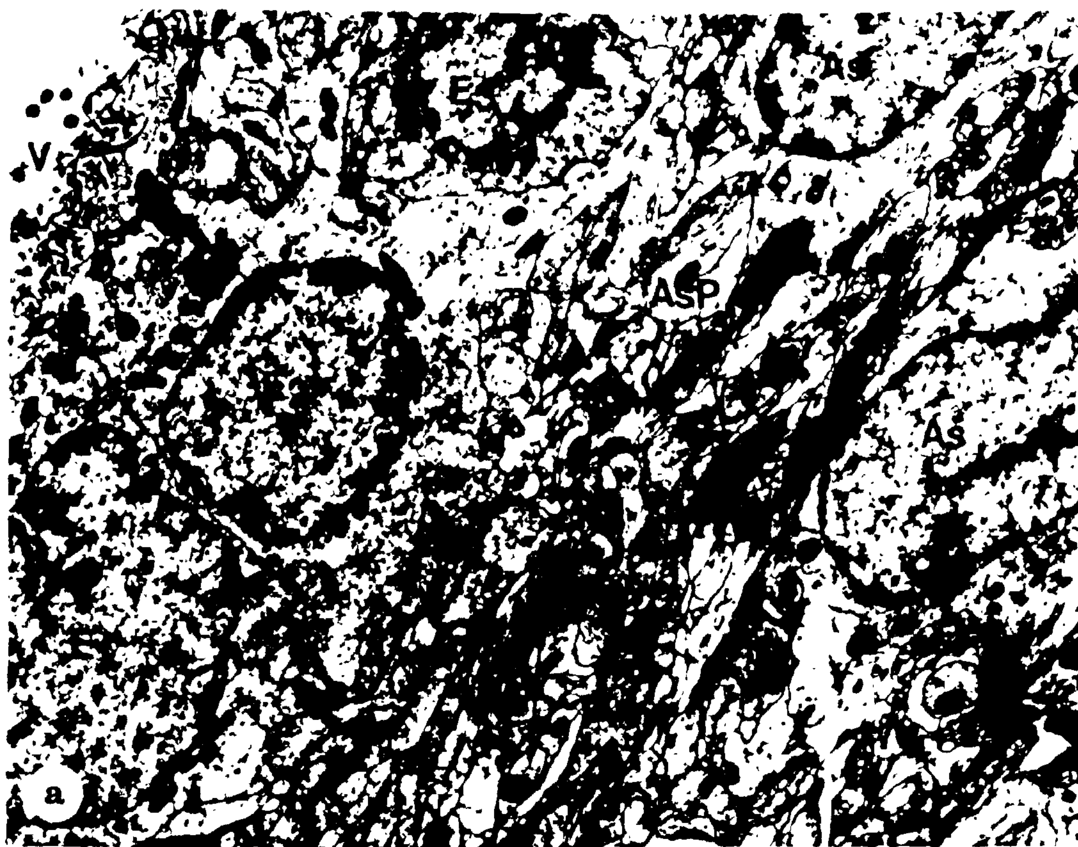
ii) Transmission Electron Microscopic Observations

A TEM study of the lateral walls and floor of the third ventricle of the rabbit confirms, as predicted from LM observations, the presence of at least two morphologically distinct types of ependymal cell: 1) ciliated cuboidal ependymal cells, and 2) non-ciliated ependymal tanycytes.

The principal cell type of the third ventricle was the cuboidal ependymal cell. Its morphology was, with minor exceptions, fundamentally similar to ultrastructural descriptions of common mural ependyma at various loci within the cerebral ventricles of a number of other mammalian species (Tennyson and Pappas, 1962, 1968a; Brightman and Palay, 1963; Klinkerfuss, 1964; Malinsky, 1968; Knowles and Anand Kumar, 1969; Westergarrd, 1970; Anand Kumar, 1972; Knowles, 1972; Millhouse, 1972, 1975; Sharp, 1972). One of the more conspicuous features of the common mural cells was the ciliated nature of their apical surfaces (Plate 17a, 18b, 19, 22a). Cilia were of indeterminate length, approximately 0.26 μm in diameter and were composed of the usual distinctive "9+2" axial filament complex. The basal bodies of the cilia were a regular feature of the apical cytoplasm of the cells

Plate 17 a, Cuboidal ependymal cells (E1, E2, E3) lining the dorsolateral wall of the rabbit third ventricle in the region designated 5 in Plate 2. Large spherical or ovoid nuclei are eccentrically placed and occupy a substantial portion of the cell volume. The ventricular surface (V) is provided with many cilia and microvilli. The lateral surfaces of adjacent cells may be relatively straight (arrows) or elaborately interdigitated (arrowhead). Fibrous astrocytes (As) are found underlying the ependyma in a subependymal glial layer. AsP, astrocytic processes; f, glial filaments. X 7,750.

b, A single layer of cuboidal ependymal cells (E1, E2) from a region of the dorsolateral wall of the rabbit third ventricle which we have arbitrarily designated 5 in Plate 2. Just below the ventricular border zonulae adhaerentes (za) are present. The lateral cell membranes (arrowheads) in this section are gently convoluted. A prominent Golgi complex (G) is present in a supranuclear position. Note that the basal surface of the ependymal cells is indented by numerous cytoplasmic processes (arrowheads) that exhibit the same cytological features as the soma and therefore are assumed to originate from the base of these cells. Identifiable within the subependymal zone are pale processes of fibrous astrocytes containing bundles of filaments and small neuronal processes (n). N, neuron. X 11,550.


V
E
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As
a

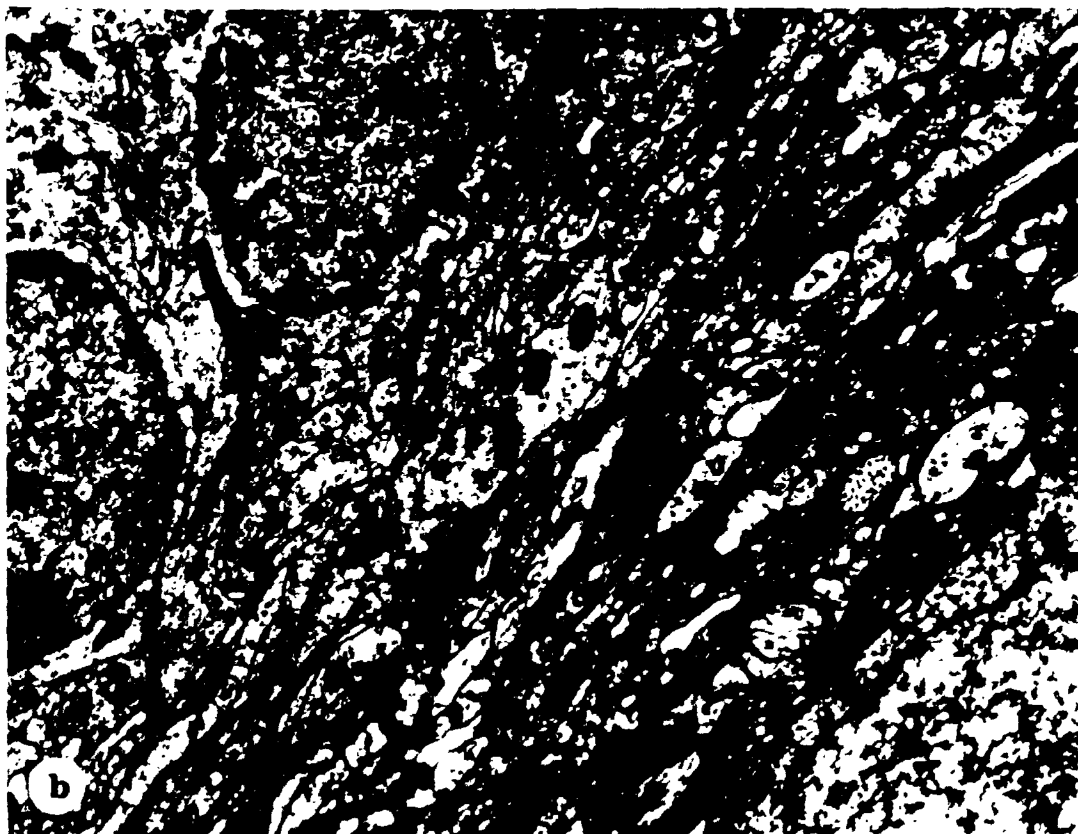
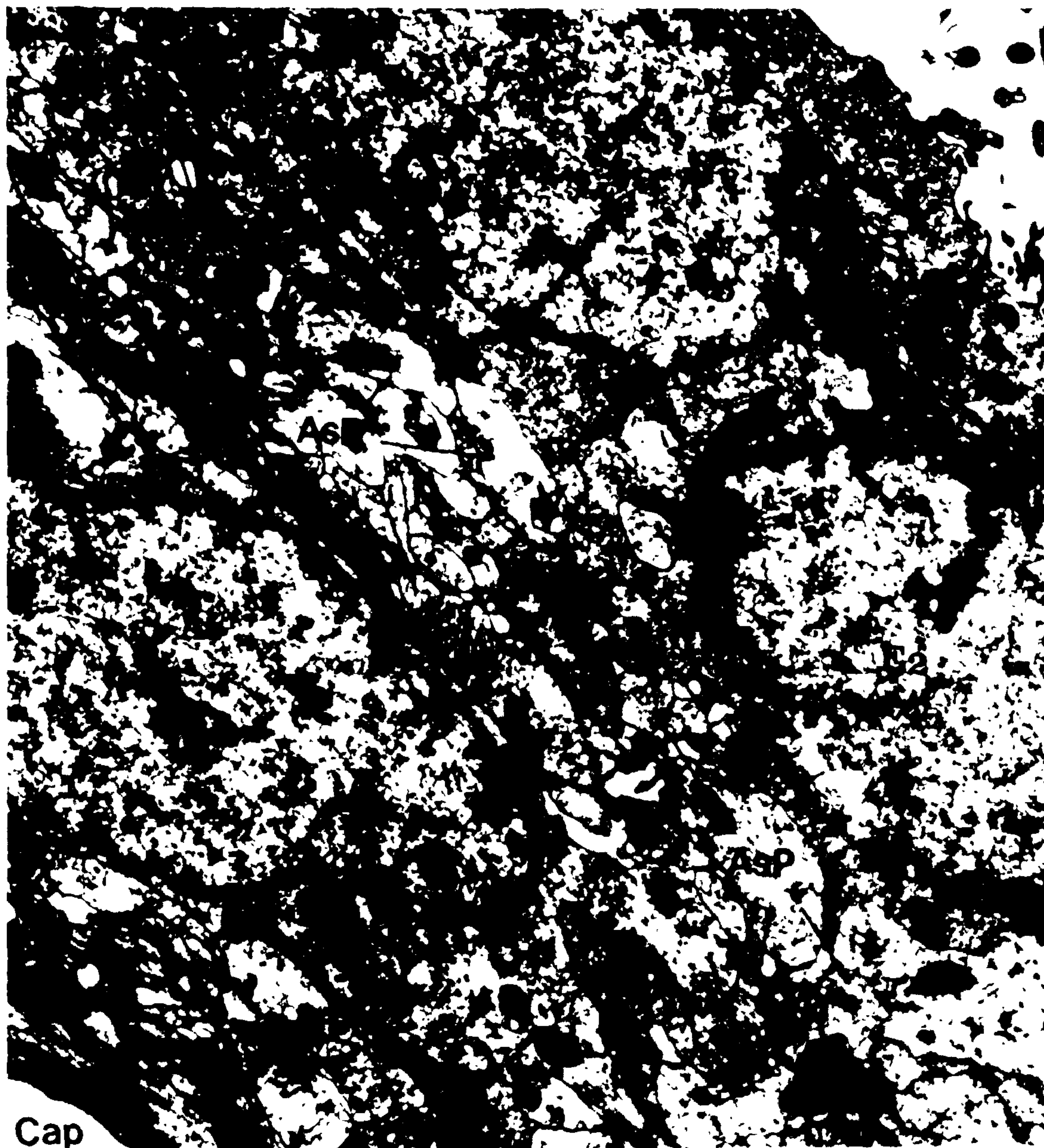

b

Plate 18 a, Ependymal cells (E_1, E_2, E_3) lining the dorsolateral wall of the rabbit third ventricle in the region designated 6 in Plate 2. Notice the extensive array of closely interdigitated processes (Ep) at their basal poles. mv, microvilli. X 7400.

b, Ependymal cell from the region of the ventricle designated 5 in Plate 2. Notice the presence of cilia (cil), microvilli and surface blebs at its apical pole. At its basal pole it is intimately associated with a hypendymal microglial cell (M) and a fibrous astrocyte (AsP). G, Golgi apparatus; f, cytoplasmic filaments. X 11,400.

Plate 19 Two adjacent ciliated cuboidal ependymal cells (E1, E2) from the dorsolateral wall of the rabbit third ventricle (sample area 5, Plate 2), each displaying a large oval nucleus. The lateral membranes (solid arrowheads) in this section are relatively straight. Mitochondria are conspicuous apically in association with the zonula adhaerens (clear arrowhead) and throughout the cytoplasm. Golgi complexes (G) are prominent in the apical cytoplasm above the nuclei. Elements of the endoplasmic reticulum, lysosomes (Ly) and clusters of ribosomes are evident in the cytoplasm. A fibrous astrocyte (As) is identifiable at the capillary (Cap) level beneath the ependymal lining. Surrounding the capillary is a terminal process of a fibrous astrocyte (*) forming the perivascular glia limitans. i, island of ependymal cytoplasm encompassed by zonula adhaerens; AsP, astrocytic processes; Ep, ependymal processes. X 13,330.


As
Cap

(Plate 20). In addition to cilia, the apical surfaces usually exhibited variable numbers of microvilli measuring approximately 0.02 - 0.08 μm in diameter (Plate 17a, 18a, 21a, 22a). Brightman and Palay (1963) considered such projections were too irregular in their dimensions to be appropriately termed microvilli. The microvilli of the ependyma in the rabbit were irregular filliform extensions of apical cytoplasm which frequently broadened at their bases. They usually lacked a well defined internal structure, although occasionally fine filaments were evident within the inner core of some microvilli. Other modifications of the free ependymal surface included irregular bulbous protrusions of the apical cytoplasm which extend into the ventricle (Plate 18b). Their appearance suggests that they are a cytologically unspecialized variety of cellular extension.

The cuboidal ependymal cells contained a large regularly spherical or oval vesicular nucleus which was often placed eccentrically and which occupied much of the cell volume. The karyoplasm was coarsely granular and a single prominent nucleolus was occasionally present in the plane of section. The nuclear membrane was distinct with condensed chromatin masses lying against its inner margin. The cytoplasm surrounding the nucleus had a granular appearance of moderate electron density. Within the cytoplasmic matrix, the normal complement of organelles could be distinguished. The mitochondria were numerous and comparatively small measuring approximately 0.2 μm in diameter. They frequently appeared as spherical, oval or elongated rod-shaped profiles in sections and were concentrated in the apical cytoplasm of the cell and perinuclear region. The Golgi apparatus was also generally supranuclear in position and often displaced toward the lateral cell membrane

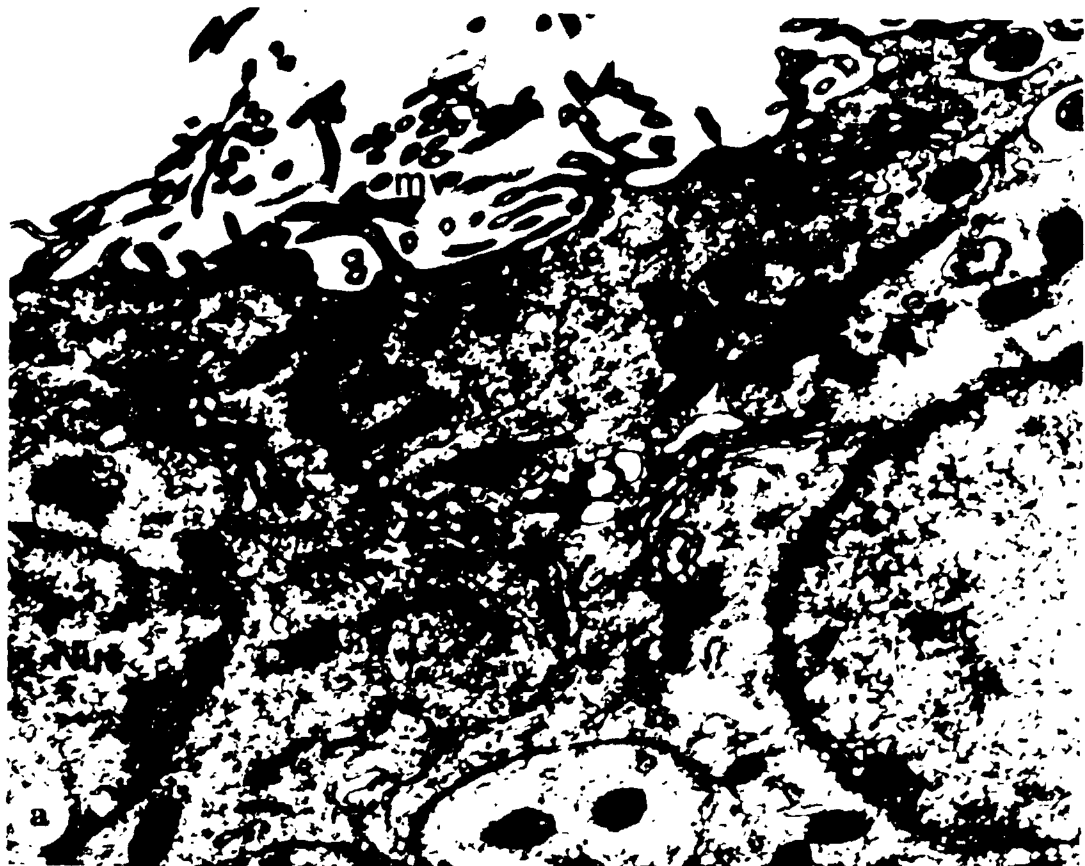
Plate 20 Ciliated cuboidal ependymal cell from the dorsolateral wall of the third ventricle. Its reniform nucleus (Nuc) is eccentrically placed in the cell. The cytoplasm contains ribosomes (r), basal bodies (bb) and numerous mitochondria. A few microtubules as well as scattered segments of granular reticulum can also be seen. Two gap junctions (arrowheads) are found along the lateral membrane at some distance from the apical surface. Note also the neuronal profiles (n) inserted among the ependymal processes. mvb, multivesicular body (0.3 μ diam.). X 30,700.

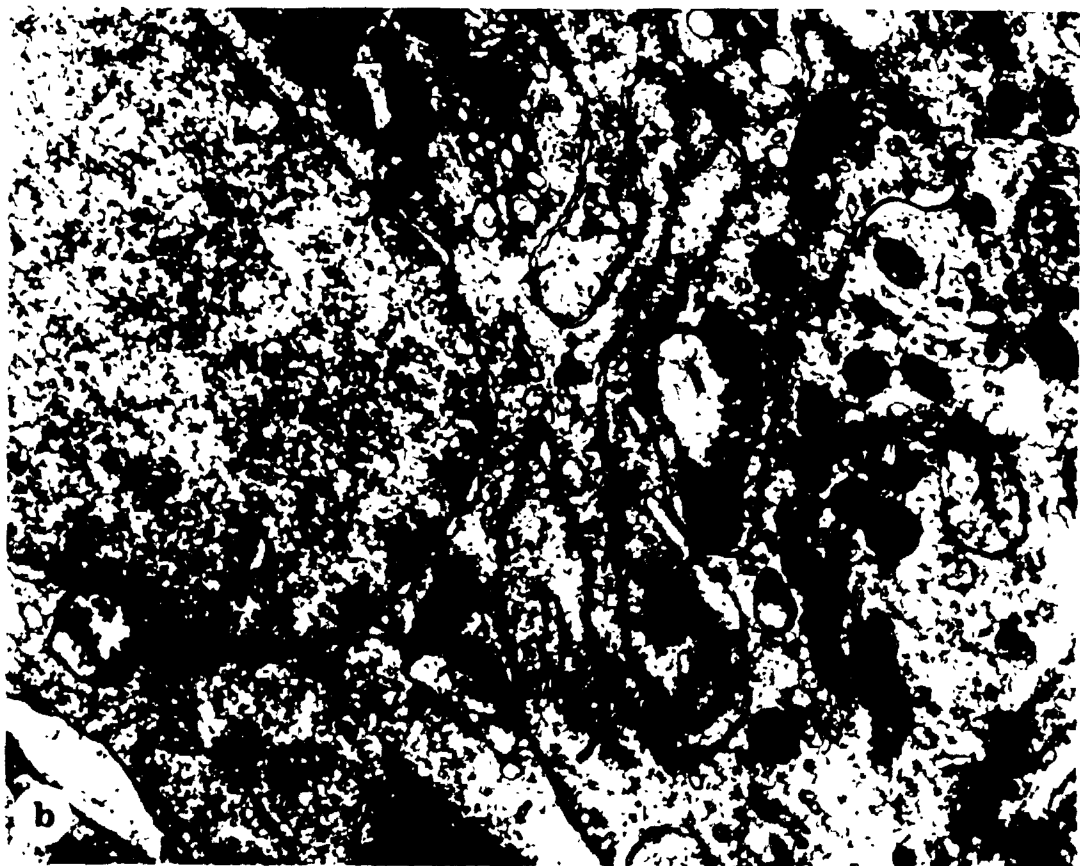
(Plate 17b, 18b, 19, 20, 21a). It consisted of the usual laminar stacks of flattened cisternae with associated clusters of vesicles. Irregular vesicular and short cisternal profiles of the smooth endoplasmic reticulum were abundant and dispersed, apparently randomly, throughout the cytoplasm, whereas cisternae of granular endoplasmic reticulum were sparse. Free ribosomes, however, were widely dispersed throughout the cytoplasm in small clusters or rosettes. Randomly dispersed cytoplasmic filaments and a small number of microtubules were commonly observed, but the dense perinuclear aggregates of filaments found in the rat (Brightman and Palay, 1963; Millhouse, 1972) were not observed in this study. This is in agreement with Klinkerfuss' (1964) observations of ependyma in the lateral ventricle of the cat. Membrane bound multivesicular bodies and lysosomes were also a feature of the cytoplasm of the ependymal cell. In general, the common mural ependymal cell is rather unremarkable in terms of its complement of organelles and inclusions.

The lateral cell membranes of adjacent cuboidal ependymal cells are comparatively simple and usually without elaborate folds or extensive interdigitations (Plate 17a, 19, 20, 21a). Occasionally, however, the lateral borders were rather tortuous and interdigitations were seen between adjacent lateral membranes in the apical region of some cells (Plate 17). Detailed reports of the complex intercellular junctions between contiguous ependymal cells have been provided by Brightman and Palay (1963), Brightman and Reese (1969; 1975) and Brightman <u>et al</u>. (1975). Our observations in the rabbit are largely in agreement with these findings. Zonula (or puncta) adherens, gap junctions and/or zonula occludens were usually seen apically as well as at irregular intervals along the lateral interface (Plate 17b, 19, 20, 21, 22a). It was

Plate 21 a, Supranuclear cytoplasm of typical mural ependymal cells illustrating the abundance of juxtaventricular organelles. The apical membranes adjoining the ventricle are organized into numerous microvillus-like projections (mv) and cilia. The lateral surface of contiguous cells are joined by intermediate (za) and occluding (arrowhead) junctions. G, Golgi complex; mit, mitochondria; Nuc, ependymal nuclei; ER, granular endoplasmic reticulum; arrows, microtubules; solid arrowheads, filaments. X 31,100.

b, Adjoining ciliated cuboidal ependymal cells from the rabbit third ventricle illustrating the cytoplasmic complement of organelles. The section was taken from the sample region designated 6 in Plate 2. The large granular nuclei (Nuc) are surrounded by cytoplasm containing numerous mitochondria (mit), dispersed clusters of ribosomes (r) and a few lysosomes (Ly). The granular endoplasmic reticulum (ER) is sparse, however vesicular and cisternal elements of the agranular reticulum are widely dispersed in the cytoplasm. In addition, the cytoplasm contains a tracery of randomly dispersed fine filaments accompanied by occasional microtubules (arrows). The lateral walls of the neighbouring cells are joined by zonulae adhaerentes (za) and gap junctions (arrowsheads). i. islands of ependymal cytoplasm within the cells. X 29,550.


mv
g
a

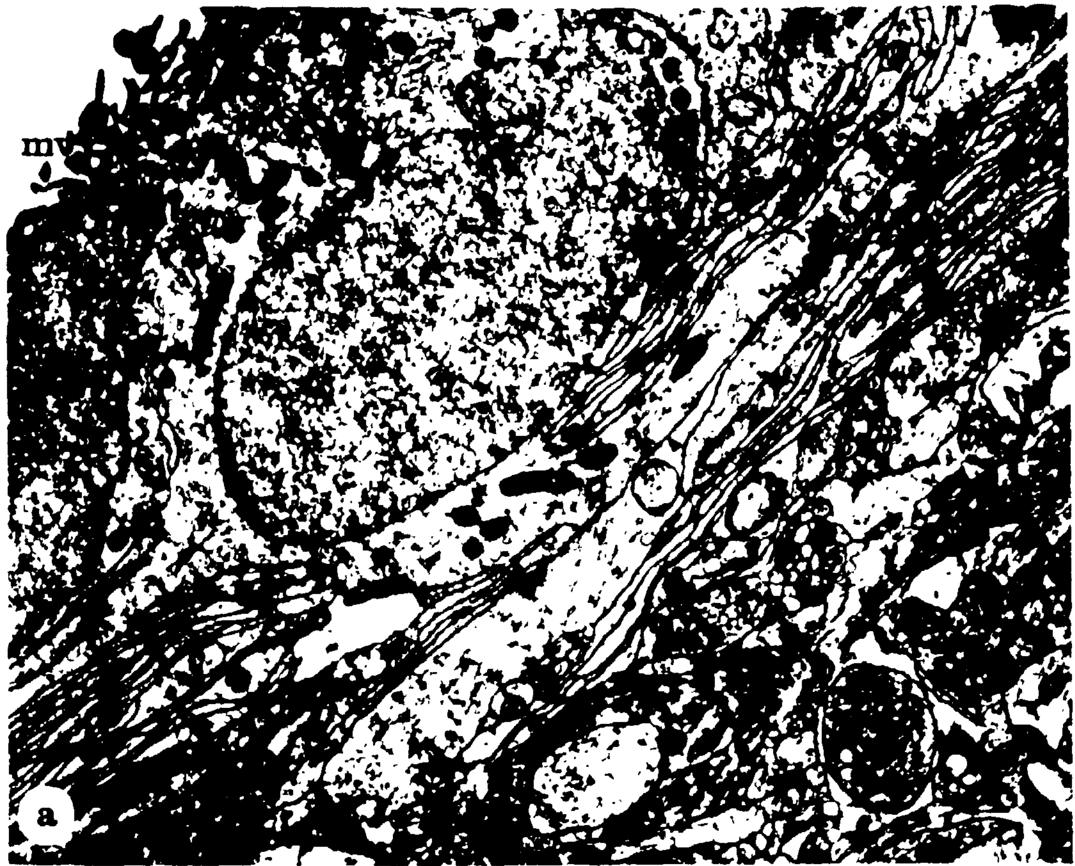

b

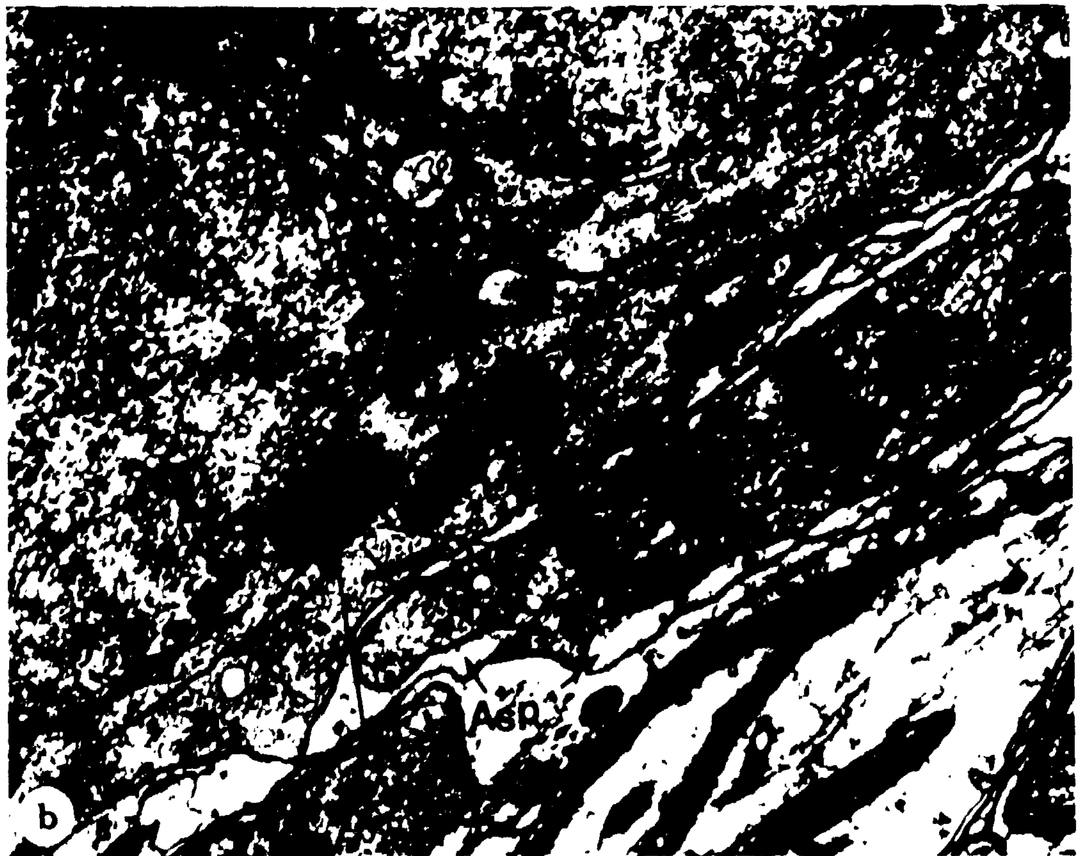
often not possible however to distinguish between occluding and gap junctions with certainty.

The ependymal lining of the dorsolateral wall of the third ventricle was separated from the underlying neuropil by a narrow zone of glia often referred to as the "internal glial layer" or "inner limiting glial membrane". As is clearly shown in Plates 17, 19 and 22, astrocytes and their processes were among the principal contributors to the formation of the subependymal cell and fiber layers respectively. Additional contributors to the subependymal fiber layer were the ependymal cells themselves (i.e., type II cells) whose basal surfaces were drawn out into numerous cytoplasmic processes (Plate 17, 19, 22). Rather than penetrating radially into the neuropil these basal cytoplasmic processes appeared to extend tangential to the surface of the ventricle. Consequently in coronal sections, they invariably appeared as irregular oblique or cross-sectional profiles that were closely interdigitated in a jig-saw like fashion (Plate 17, 18, 19, 22). They exhibited the same cytological features as the ependymal soma which, depending upon the size of the profile, included one or more of the following: a granular cytoplasm of moderate electron density, clusters of free ribosomes, mitochondria, fragments of granular and agranular reticulum and cytoplasmic filaments. These basal ependymal processes were observed to be closely interwoven with oval, circular and sheet-like profiles of astroglial cytoplasm. Astrocytic processes had a lucent cytoplasmic matrix that was less electron dense than that of ependymal processes. Many astrocytic processes were filled with filaments; while non-filamentous areas frequently appeared empty. In addition, a number of the larger processes often contained mitochondria. Insinuated among the myriad of ependymal and astrocytic processes of the hypendymal

Plate 22 a, A single layer of ependymal cells from the rabbit third ventricle. The section is taken from sample area 4 shown in Plate 2. The low cuboidal cell occupying the center of the field has an elongated vesicular nucleus with indentations. Cilia and thin microvilli (MV) project into the lumen of the ventricle. Apically a number of zonulae adhaerentes (arrowheads) are situated between cells. Notice that the thin cytoplasmic process (solid arrowheads) running horizontally immediately beneath the surface ependymal cell clearly originates from the base of the cell. Compare with the cells shown in Plates 6c, 7c and 9c. Arrows, complex interdigitation of basal cytoplasmic processes of adjacent ependymal cells. X 12,700.

b, Basal poles of neighbouring ciliated cuboidal ependymal cells (E1, E2). The section is taken from a region of the dorsolateral wall of the rabbit third ventricle that is designated 6 in Plate 2. A number of cytoplasmic processes (Ep) assumed to be of ependymal origin are interposed between the ependymal lining and the subjacent neuropil. Electron-lucent laminae of astrocytic cytoplasm (AsP) are interwoven with the ependymal processes. Note that no basement membrane exists between the ependymal lining and the underlying neuropil. X 31,250.


mv
a


AsD
b

glial zone were usually a number of typical axonal and dendritic profiles assumed to be extensions of the subjacent neuropil. The transition from subependymal glial membrane to neuropil was often irregular and indistinct; it was characterized, however, by a gradual increase in the number of visible neuronal profiles and a corresponding decrease in the number of glial profiles.

As stated previously neither the ependyma nor the subependyma were morphologically homogeneous throughout the ventricular system. Nowhere, however, were structural changes more obvious than along the ventrolateral wall and floor of the third ventricle where typical ependymal elements were completely displaced by tanycyte ependymal cells (Plate 23, 24). Tanycytes at this level of the rabbit ventricle exhibited convex apical surfaces which appeared to lie partially free in the ventricle. They are provided with very few cilia; rather their apical surfaces were furnished with microvilli and large numbers of irregular bulbous extensions of apical cytoplasm which projected into the lumen of the ventricle (Plates 23, 24 and 25). These cytoplasmic blebs did not show any unusual ultrastructural specialization; they consisted of fragments of tanycyte cytoplasm which contained ribosomes and small vesicular profiles. With the exception of mitochondria which were occasionally encountered in the broader processes, other organelles were usually excluded from the bleb cytoplasm. Their organization suggests that they represent an inconstant, and transient variety of cell extension. The apical blebs on the luminal-tanycyte surface have been considered by some investigators as a morphological expression of an apocrine release of some unknown secretory product (Scott et al., 1972a; Kozlowski et al., 1973; Mestres et al., 1974).

Plate 23 A reconstruction of a portion of the ventrolateral wall of the rabbit third ventricle (equivalent to the region designated 2 in Plate 2). The montage provides a survey of the relationship of tanycyte ependymal cells (EpT) to a broad region of the underlying neuropil. Notice the irregular shape of the tanycyte somata. Their free surface exhibits numerous cytoplasmic projections (arrowheads). Note also the basal processes of tanycytes (Tp) directed toward the subjacent capillary (Cap). Immediately subjacent to the tanycyte somata and wedged between basal processes is cross-sectional profiles of neuronal and glial elements of the neuropil. N, neuronal perikarya; P, pericytes. X 2,750.

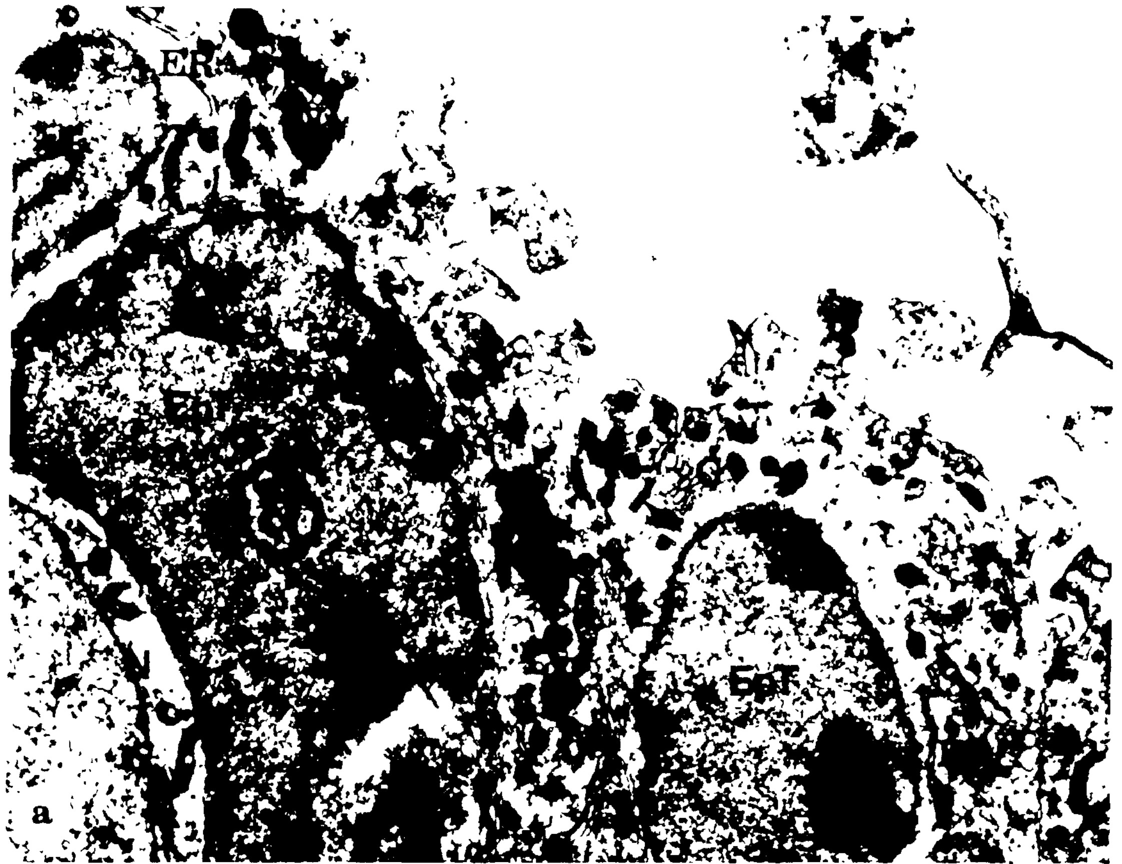
Cap
P
P
P
C

Plate 24 The field shown in this montage of photomicrographs is as illustrated in Plate 23 but displaced serially. Tanycyte ependymal cells (EpT) and some of their basal prolongations (Tp) at a level of the hypothalamus designated 2 in Plate 2 are shown at higher magnification in this figure. Note the convex apical surfaces of tanycyte somata (arrowheads) which appear as a series of well defined bulges into the ventricle and the bulbous projections (arrows) of the ventricular surface membrane. Tanycyte nuclei are clustered closely together, are elongated and often deeply indented. Note also the spatial relationship between tanycyte terminal processes (Tp), perivascular space (arrows) and a capillary (Cap). Within the feltwork of intermingled neuronal processes of the subjacent neuropil, are a few myelinated axons and several axonal profiles containing electron dense granules. N, neuronal perikarya; P, pericytes. X 4,650.

P
P
Cap

Plate 25 a, Apical surface of tanycyte ependymal cells (EpT) along the ventrolateral wall of the rabbit third ventricle (taken from the region designated 2 in Plate 2). Notice the irregular cytoplasmic projections of the order of 0.5-1.0 μm. A neuron (N) is inserted next to the tanycyte without evidence of intervening glial sheath. ER, granular reticulum; G, Golgi apparatus; Ly, lysomes; arrows, lucent vesicles; arrowheads, zonulae adhaerentes. X 12,200.

b, As in fig. a above showing organelles contained within the rim of cytoplasm between adjoining tanycyte ependymal cells. X 19,600.


ER
EpT
a

b

In comparison with the ciliated-cuboidal ependymal cells, tanycytes are more irregular in shape (Plate 23, 24, 30, 31, 39). Their irregular nuclei contrasted markedly with the regular ovoid or spherical nuclei of the cuboidal cells. Tanycyte nuclei were generally elongated along an axis perpendicular to the ventricle and often deeply indented (Plate 23, 24, 30, 31). Frequently they appeared to be clustered closely together, because of only a narrow rim of cytoplasm along their lateral margins.

The cytoplasm of tanycytes tended to be more electron dense than that of cuboidal ependymal cells. Millhouse (1972) attributed this density to the presence of a condensed flocculent material that could barely be resolved, whereas this same material in common ependymal cells was more dispersed. Contained within the cytoplasm were the normal array of organelles which resembled those of the ciliated cuboidal cells. Dispersed within the cytoplasm were clusters of ribosomes and only a few isolated profiles of a poorly developed granular endoplasmic reticulum. Vesicles and cisternae of the smooth endoplasmic reticulum were abundant and widely scattered throughout the cytoplasm. Mitochondria were numerous particularly in the apical and basal cytoplasm and throughout tanycyte processes. The Golgi complex was prominent and situated either supra- or infranuclearly. Lysosomes were a common feature of tanycyte somata as were small numbers of randomly dispersed cytoplasmic filaments. Microtubules, however, were numerous and found throughout the cytoplasmic matrix.

In reviewing the literature we have noted some inconsistency among investigations concerning the cytological features which serve to distinguish tanycytes from the banal variety of ependymal cell.

Millhouse (1972, 1975) cited nuclear shape, matrix density, the absence of bands of cytoplasmic filaments and the abundance of microtubules as the principal criteria for distinguishing tanycytes in the rat. On the other hand, Tennyson and Pappas (1962) found the ultrastructure of rabbit ependymal cells to be so similar that differentiation of cell types in the ependyma could only be determined by the morphology of their basal portions.

Doubtless the most recognizable feature distinguishing the tanycyte from the common ependymal population of cells is its basal prolongation. The tanycyte process emerged from the basal pole of the cell, projected into the subjacent neuropil sometimes for considerable distances. It was frequently directed toward capillaries (Plate 23, 24, 40). These processes exhibited a number of identifying cytological features: 1) an electron dense cytoplasmic matrix equal to that of the soma, 2) a complement of numerous paraxially oriented mitochondria, and 3) numerous longitudinally oriented microtubules. Dilated cisternae of the endoplasmic reticulum vesicles and clusters of ribosomes were also present; they were particularly conspicuous at the origin of the processes from the base of the cell (Plate 26, 27).

In the postnatal ribbit Tennyson and Pappas (1962) reported that the number of organelles diminished distally in tanycyte process of the cerebral aqueduct. In the rabbit, the basal processes of tanycytes did not repeatedly branch to form a syncytium as described by Knowles and Anand Kumar (1969) in the monkey. The tanycyte process in its course through the neuropil had an irregular or knobby outline (Plate 27a, 28b) and appeared to be largely unbranched except at its termination where it often divided into several slender branches (Plate 29, 40). Distally

Plate 26 a, Tanycyte ependymal cells located along the ventro-lateral wall of the ventricle in the region designated 1 in Plate 2. Notice that the organelles of the basal cytoplasm continue into the proximal process. mit, mitochondria; nuc, tanycyte nuclei; G, Golgi apparatus; r, ribosomes; ER, granular reticulum; arrowhead, agranular reticulum; n, profiles of subjacent neuronal processes. X 18,700.

b, As in fig. a above showing the somata of tanycyte ependymal cells. Labels are as in fig. a. arrowhead, gap junction between contiguous cells. X 30,900.

Plate 27 a, Basal surface of ependymal tanycytes (EpT) from a region of the ventrolateral wall of the third ventricle designated 1 in Plate 2. A process (Tp) arises from the basal pole of the cell and extends into the subependymal region where other tanycyte processes (Tp) are seen. Mitochondria (Mit) are numerous in the basal cytoplasm and extend into the proximal process. The Golgi complexes (G) are situated infranuclearly. The tanycyte process has an irregular outline (arrowheads) and its surface is surrounded by cross-sections of neuronal processes. X 14,550.

b, Proximal portion of a tanycyte ependymal cell process in the subependymal region of the ventricle wall designated 1 in Plate 2. The basal process measures approximately 0.6 - 0.9 um in diameter and borders partially on neighbouring tanycyte processes. Mitochondria (Mit) and microtubules (arrows) are longitudinally aligned in the process. Smooth endoplasmic reticulum is represented by a number of short tubules and vesicular profiles (arrowheads). X 34,615.

Plate 28 a, Isolated profiles of preterminal tanycyte processes (Tp) in longitudinal section illustrating their relationship to neuronal elements of the arcuate neuropil. Tanycyte processes measure approximately 1-1.2 μm in diameter and frequently adjoin one another. Notice the sheets of astrocytic cytoplasm (arrows) enveloping their external surface. D, dendrites; N, arcuate neuron. X 11,700.

b, As in fig. a above (but several sections removed), tanycyte processes (Tp) in longitudinal section (1-1.6 μm diameter). Note their electron dense cytoplasmic matrix. Their complement of mitochondria and microtubules parallel the axis of the processes. Tanycyte processes exhibit irregular knobby outlines (arrowheads) and are ensheathed by thin sheets of astrocytic cytoplasm (arrows). D, dendrites; N, arcute neuron. X 12,500.

tanycyte processes frequently converge but never anastomose with one another (Plate 23, 24). A number of pericapillary tanycyte terminals abutting directly on the basement membrane of a capillary perivascular space is shown in Plate 29. The relationship of tanycyte terminals to blood vessels is very much like the terminal expansions or end-feet formed at the surface of capillaries by perivascular astrocytes elsewhere in the neuropil. The functional significance of this anatomical relationship as far as tanycytes are concerned is still unknown.

The similarity of astrocytes and ependymal cells have been noted by a few investigators (Hild, Takenaka and Walker, 1965; Hirano and Zimmerman, 1967; Peters, Palay and Webster, 1976). Sarnat et al. (1975) presumed an inverse relationship to exist between the relative development of ependymal cells, glial cells and the blood supply. While ependymal cells predominate as the principal glial form in non-mammalian vertebrates, with increased vascularity as occurs in higher mammals, astrocytes become the predominant glial form. Leonhardt (1966) considered the tanycyte as an intermediate form between the ependymal cell and the astrocyte.

Isolated terminal processes of tanycytes are often times difficult to differentiate from other processes in the neuropil despite descriptions of their cytological features and their relationship with other tissue components. Brawer (1972) for example, reported that in the rat mitochondria of tanycytes were less than one-half the size of those of glial and neuronal mitochondria and suggested that this was a reliable means of identifying isolated tanycyte profiles. We have found this to be an unreliable diagnostic criterion for the

Plate 29 a, Micrograph showing spatial relationship of several terminal tanycyte processes (Tp) to the basal lamina of a capillary (Cap) perivascular space in the hypothalamic neuropil. Compare with Plate 40. The tanycyte pericapillary processes contain vesicles, tubules of ER, circular and elongated mitochondrial profiles and accumulations of microtubules. N, neuronal soma. X 12,000.

b, Portion of the capillary wall enveloped largely by tanycyte processes (Tp) as outlined in fig. a above. Notice their relationship to the comparatively pallid profiles of neuroglial components of the pericapillary sheath (AsP). Processes of glial cells are crowded with glial filaments that appear in transverse section as dots of uniform size evenly distributed throughout the cross-section. Arrows, basal lamina of the perivascular space. X 15,000.

Plate 30 a, Tanycyte ependymal cells along the ventrolateral wall of the rabbit third ventricle in the region designated 2 in Plate 2. A tanycyte process (arrow) can be seen which establishes intimate contact with a neuron (N) and possibly one of its dendrites (D) in its course through the arcuate neuropil. M, microglial cell; P, pericyte. X 4800.

b, This micrograph (taken from the region designated 2 in Plate 2) illustrates the close relationship frequently seen between tanycytes and neurons of the arcuate neuropil. X 12,200.

Plate 31 a, Ependymal cells lining the ventrolateral wall of the third ventricle in the region designated 2 in Plate 2. Note that a subependymal glial layer is lacking and that the tanycytes rest directly upon the subjacent neuropil. N, arcuate neuron; M, microglial cells. X 4700.

b, Enlargement of part of the field shown in fig. a. The basal ependymal surface is here moulded to accommodate three large clusters of axonal and dendritic (D) processes. M, microglial cells. X 7600.

The field shown in these two micrographs is as illustrated in Plate 30a but several sections removed.

identification of tanycyte processes in the rabbit neuropil.

In contrast to the dorsolateral wall of the ventricle the ependymal lining of the ventrolateral wall and floor rests directly upon the neuropil (Plates 23, 24, 30, 31). A subependymal "internal glial layer" was lacking—an observation that has been made by a number of other investigators (Christ, 1951; Polak and Azcoaga, 1969). The basal membrane of ependymal tanycytes can be seen to accommodate the neuronal elements of the subjacent neuropil. Frequently, clustered circular profiles of unmyelinated axons, dendrites and even neuronal perikarya were found adjoining the basal ependymal surface or were insinuated between the basolateral surfaces of adjacent tanycyte somata (Plate 25a, 30, 31). Little insight in terms of the possible functional significance of this relationship can be provided.

4.2 Comparative Morphology of the Ependymal Lining of the Mammalian Third Ventricle: A Scanning Electron Microscopic Investigation

A diagrammatic representation of the rabbit diencephalon showing the entire third ventricle in mid-sagittal section precisely as viewed with the SEM is provided in figure 2. The third ventricle can be conveniently divided into three regions on the basis of observed variation in the morphology of the ependymal surface. These divisions and their position relative to the underlying hypothalamic nuclei are illustrated schematically in a coronal plane through the mid-tuberal level of the hypothalamus in the same figure. Although modeled on data obtained in the rabbit, the other mammalian species examined in this study were sufficiently similar so that the diagram is applicable to them as well. In the rabbit (Plate 32), as in the mouse (Plate 35a), rat (Plate 32c)

Figure 2

An excised diencephalic block showing diagrammatically the entire rabbit third ventricle in mid-sagittal section as viewed 'en face' with the SEM. The corresponding coronal section taken through the mid-tuberal level of the hypothalamus illustrates the divisions of the ventricular surface and their position relative to the underlying hypothalamic nuclei. AC, anterior commissure; MTN, midline thalamic nuclei; OCh, optic chiasma; MM, mammillary body; PC, posterior commissure; IR, infundibular recess; ARC, arcuate nucleus; HDM, dorsomedial nucleus; HVM, ventromedial nucleus; VIII, third ventricle; MR, mammillary recess.

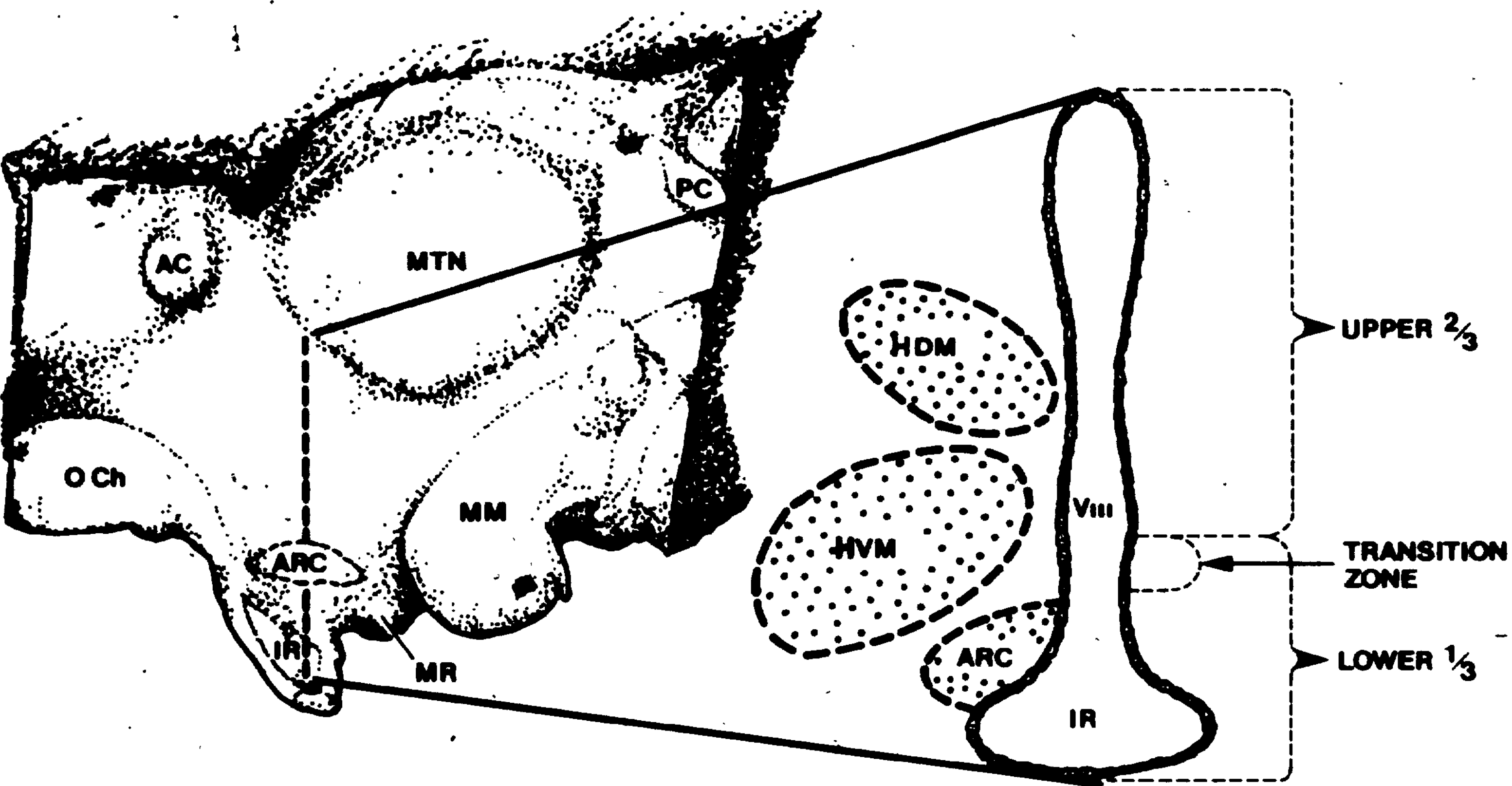

AC
MTN
PC
O Ch
MM
ARC
IR
MR
HDM
HVM
ARC
IR
VIII
UPPER 2/3
TRANSITION ZONE
LOWER 1/3

Plate 32 a, Scanning electron micrograph of a representative portion of the dorsal two-thirds of the ependymal surface of the rabbit third ventricle. A profusion of cilia extend into the lumen of the ventricle; only a few non-ciliated patches (arrow) are observed. X 2700.

b, Representative sample of the ependymal surface taken from the rabbit cerebral aqueduct. Cilia project from the surface of ependymal cells in clusters which appear orientated in parallel longitudinal rows, quite unlike their non-specific orientation in the third ventricle. This may bear some relationship to the axial orientation exhibited by the ependymal cells themselves in the cerebral aqueduct and interventricular foramen. X 1100.

c, The luminal surface of the dorsal two-thirds of the rat third ventricle is similar to that of the rabbit (fig. a, above). Note the presence of a feltwork of microvilli between the cilia (arrows). X 5500.

(Camphene sublimation method)

and rat kangaroo the major expanse of the wall of the third ventricle was lined by ependymal cells densely covered with long cilia which extended into the lumen of the ventricle without specific orientation. Small patches of non-ciliated ependymal surface were only infrequently encountered. This type of surface topography was found throughout the rostrocaudal extent of the dorsal two-thirds of the wall (figure 2) continuing uninterrupted into the interventricular foramen and the cerebral aqueduct (Plate 32b). In general, it was not possible to determine any significant variation in either the number or distribution of cilia, or in the morphology of the apical ependymal membrane itself because individual cell outlines were invariably obscured by the cilia. Interspersed among the cilia and ordinarily hidden from view by them was a feltwork of microvilli (Plate 32a, c; 35a) which was more easily distinguished ventrally as the ependymal surface gradually became less ciliated (Plate 34a, 35b). In certain regions of the ventricular system, notably the cerebral aqueduct and the interventricular foramen, cilia occasionally appeared oriented in parallel longitudinal rows as if they were fixed in synchronous wave-like patterns of activity (Plate 32b). This may bear some relationship to the distinctive axial orientation of the ependymal cells themselves in these two regions (Bruni et al., 1973).

A gradual transition in the morphology of the ependymal surface occurred consistently along the ventral one-third of the rabbit ventricular wall, at the location of the underlying ventromedial nucleus (figure 2). Here cilia were invariably less frequent; non-ciliated ependymal cells predominated (Plate 33). The non-ciliated cells appeared rounded or polygonal in shape although their outlines often were

Plate 33 Ventral one-third of the rabbit ventricular wall. A transition in the morphology of the ependymal surface can be seen. Ciliated ependymal cells occur less frequently. Non-ciliated cells predominate. They are rounded or polygonal in shape and their apices bulge only slightly into the ventricular lumen, forming a rather flattened and smoothly contoured surface. X 3520. Inset, a portion of the surface of the rabbit transitional zone viewed under higher magnification. Note the presence of knob- or bleb-like protrusions of the ependymal plasma membrane. X 12,000.

(Camphene sublimation method)

indistinct. Although the apical surface of a number of the non-ciliated ependymal cells bulged into the lumen of the ventricle, nevertheless the surface of the entire transitional zone retained an overall smoothly contoured appearance. A limited covering of microvilli and numerous stout knob- or bleb-like protrusions were commonly seen to interrupt the apical membrane of the non-ciliated cells. This transition in the morphology of the ependymal surface was seen consistently in the third ventricle of the rat kangaroo, rat (Plate 34a) and of the mouse (Plate 34b, 35b) as well. Unique to the transitional zone of the mouse and rat, however, was the abundance of readily discernible microvilli covering the surface of the non-ciliated cells (Plate 34a, 35b).

Immediately ventral to the transitional zone, the ventrolateral wall and floor (infundibular and mammillary recesses) of the third ventricle of the rabbit, is almost devoid of ciliated ependymal cells. Only infrequent clusters of cilia project from the surface of isolated cells (Plate 36a). It was not uncommon to find clusters of 25 or more cilia originating from the apical surface of a single cell (Plate 36b). The cilia were not uniform in diameter along their length but were noticeably thinner distally, and terminated in a bulbous enlargement (Plate 36b). In addition, their surface was rough and irregular with almost a segmented appearance (Plate 36b, inset). Often only a single cilium projected from the surface of isolated cells into the lumen of the ventricle (Plate 37a). The topography of the ventrolateral wall and floor is transformed from the flattened and smoothly-contoured appearance of the transitional zone to a more irregularly contoured surface of variable complexity (Plate 37, 38). The irregular contour of the region is attributable to the fact that most of the non-ciliated

Plate 34 a, Scanning micrograph of the transitional zone in the rat third ventricle. The luminal ependymal surface of non-ciliated cells is characterized by numerous microvilli. Smooth patches denuded of such surface specializations are seen infrequently. X 6500.

b, Low magnification micrograph of the ventrolateral wall of the mouse third ventricle illustrating the transition that occurs from a densely ciliated surface to a largely non-ciliated surface in this region of the ventricle. The free supraependymally-lying cells (arrows) are presumed to be macrophages. X 1440.

(Camphene sublimation method).

ciliated ependymal cells abound within most of the third ventricle, the interventricular foramen and the cerebral aqueduct not unlike that which we have reported in the other species examined (Plate 41a). From our preliminary observations on selected regions of the lateral cerebral ventricles, it seems very likely that this organization persists throughout the entire ventricular system in the human. This is in contrast to erroneous descriptions which may still be found in recent textbooks. Intervening non-ciliated patches which periodically interrupt the ciliated surface exhibit a feltwork of microvilli. The human third ventricle exhibited essentially the same regional variations in surface morphology as did the other species we have studied but they are not as sharply defined. A gradual and almost inperceptible transition to a sparsely ciliated surface occurs along the middle one-third of the ventricle wall (Plate 41b). This observation has recently been confirmed by Gito (1975) in his study of the human ventricular system. Compared with either the rabbit, rat or mouse, this means that non-ciliated ependymal surface occupies a larger expanse of the lateral wall in the human. Human fetal preparations of 13 weeks, however, exhibit sharply defined and abrupt transitions in the organization of the ventricular wall comparable to that of other mammalian species (Scott et al., 1972b, 1973, 1974b). We can only speculate on the possible reason for such differences. Worthington and Cathcart (1963) have suggested that more cilia may be present in fresh preparations of human adult brain than are encountered after routine fixation. In addition to post-mortem autolytic changes, age related changes, disease processes and possibly cyclic renewal and degeneration are a few to be considered. Cilia diminish progressively

Plate 36 a, Ventral to the transitional zone in the region overlying the arcuate nucleus in the rabbit, the ependyma is largely devoid of cilia except for the infrequent cluster which projects from the surface of isolated cells. X 2680.

b, About 25 cilia extend in a cluster from the surface of a single ependymal cell. The diameter of the ciliary shaft tapers toward the tip, terminating in a bulbous enlargement (arrows). X 7200. Inset, reveals that the ciliary surface is irregular (arrowheads) and almost segmental in appearance. X 14,600. Enlargement of a portion of the field shown in fig. a above.

(Camphene sublimation method)

cells now bulge conspicuously into the lumen of the ventricle, their lateral borders demarcated by deep furrows (Plate 37c, 38b, 39). It was not uncommon to find that individual ependymal cells differed in the morphological appearance of their apical surface which suggests a certain plasticity in the cells under normal conditions. Numerous irregular blebs or knob-like protrusions (ranging from 0.1 - 1.0 μm diam) extended from the surface of most cells (Plate 37a, b; 38a, b). They represented the most common form of surface specialization encountered. Microvilli, although present, were generally not uniformly distributed or found in very large numbers in the rabbit. Some isolated cells projected only a feltwork of microvilli from their luminal surface (Plate 38b) but it was more common to find cells that exhibited a combination of surface specializations. The surface of many nonciliated cells was frequently denuded in whole or in part of any form of specializations thus appearing comparatively smooth and structureless (Plate 37c, 38a). The equivalent region of the ventricle in both the mouse and the rat was similar except that microvilli were usually more prevalent in both of these species.

It was not possible to identify tanycyte ependymal cells or to distinguish them with any certainty from the ordinary mural variants on the basis of their surface morphology alone. It was possible, however, to identify tanycytes by examining the exposed fracture-face of cleavage planes through the ventrolateral wall of the ventricle (Plate 39, 40).

Very little information is available regarding the morphology of the ventricular ependyma in the human, particularly from an SEM perspective. In the cerebral ventricles of the human adult densely

Plate 37 a, Along the lateral walls of the rabbit infundibular recess only occasional solitary cilia rather than ciliary bundles are encountered. The characteristic feature of this region is the rather expansive non-ciliated surface. E_1, E_2, E_3, individual ependymal cells. X 5300.

b, High magnification micrograph of the ventricular surface as shown in fig. a above. Pleomorphic blebs or knob-like protrusions are the most common form of surface specialization encountered. X 12,600.

c, Paired stereomicrographs representing a region along the ventrolateral wall near the floor of the rabbit third ventricle. The flattened and smoothly contoured surface of the transitional zone is gradually transformed to a more irregular surface of variable complexity. X 1880.

(Camphene sublimation method)

Plate 38 a, High magnification paired stereomicrographs as in Plate 37c. In addition to the presence of numerous knob- or bleb-like protrusions, microvilli emerge from the juxtaventricular ependymal surface. X 4700

b, Ventrolateral wall near the floor of the rabbit third ventricle comparable to that illustrated in Plate 37c. Most ependymal cells in this preparation bulge conspicuously into the ventricle. Their lateral borders are clearly demarcated by deep furrows. Note the predominance of microvilli on the surface of two isolated ependymal cells in the field (arrowheads). X 1850

c, Higher magnification of a region of the rabbit ventricle similar to that shown in Plates 37c and 38a, b. X 2975

(Camphene sublimation method)

ciliated ependymal cells abound within most of the third ventricle, the interventricular foramen and the cerebral aqueduct not unlike that which we have reported in the other species examined (Plate 41a). From our preliminary observations on selected regions of the lateral cerebral ventricles, it seems very likely that this organization persists throughout the entire ventricular system in the human. This is in contrast to erroneous descriptions which may still be found in recent textbooks. Intervening non-ciliated patches which periodically interrupt the ciliated surface exhibit a feltwork of microvilli. The human third ventricle exhibited essentially the same regional variations in surface morphology as did the other species we have studied but they are not as sharply defined. A gradual and almost inperceptible transition to a sparsely ciliated surface occurs along the middle one-third of the ventricle wall (Plate 41b). This observation has recently been confirmed by Gito (1975) in his study of the human ventricular system. Compared with either the rabbit, rat or mouse, this means that non-ciliated ependymal surface occupies a larger expanse of the lateral wall in the human. Human fetal preparations of 13 weeks, however, exhibit sharply defined and abrupt transitions in the organization of the ventricular wall comparable to that of other mammalian species (Scott _et al._, 1972b, 1973, 1974b). We can only speculate on the possible reason for such differences. Worthington and Cathcart (1963) have suggested that more cilia may be present in fresh preparations of human adult brain than are encountered after routine fixation. In addition to post-mortem autolytic changes, age related changes, disease processes and possibly cyclic renewal and degeneration are a few to be considered. Cilia diminish progressively

Plate 39 Scanning micrographs arranged as a montage showing a small segment of the ventrolateral wall of the rabbit third ventricle. Note the flask-shaped or ellipsoidal form of the ependymal tanycyte somata (EpT) elongated in an axis perpendicular to the ventricular lumen. Their non-ciliated apical convexities (arrows) project into the ventricle while a single thick process (arrowheads) commonly extends from the basal pole. The transition from cell body to basal process is clearly defined. X 6800

(Camphene sublimation method)

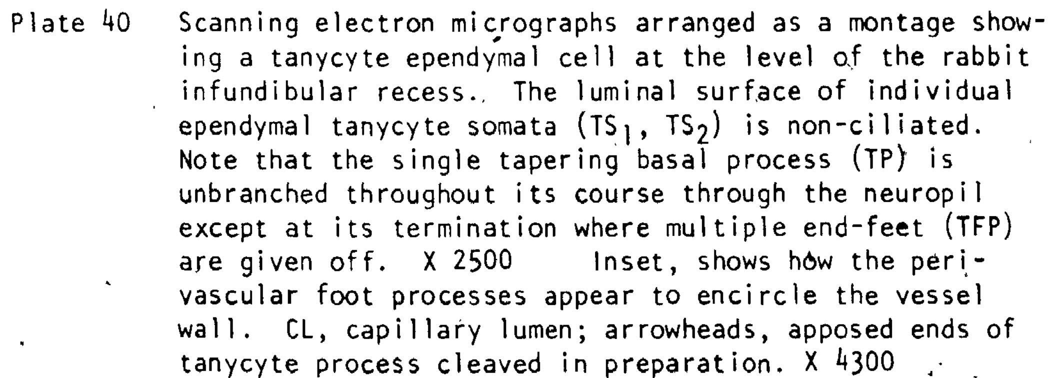


Plate 40 Scanning electron micrographs arranged as a montage showing a tanycyte ependymal cell at the level of the rabbit infundibular recess. The luminal surface of individual ependymal tanycyte somata (TS_1, TS_2) is non-ciliated. Note that the single tapering basal process (TP) is unbranched throughout its course through the neuropil except at its termination where multiple end-feet (TFP) are given off. X 2500 Inset, shows how the perivascular foot processes appear to encircle the vessel wall. CL, capillary lumen; arrowheads, apposed ends of tanycyte process cleaved in preparation. X 4300

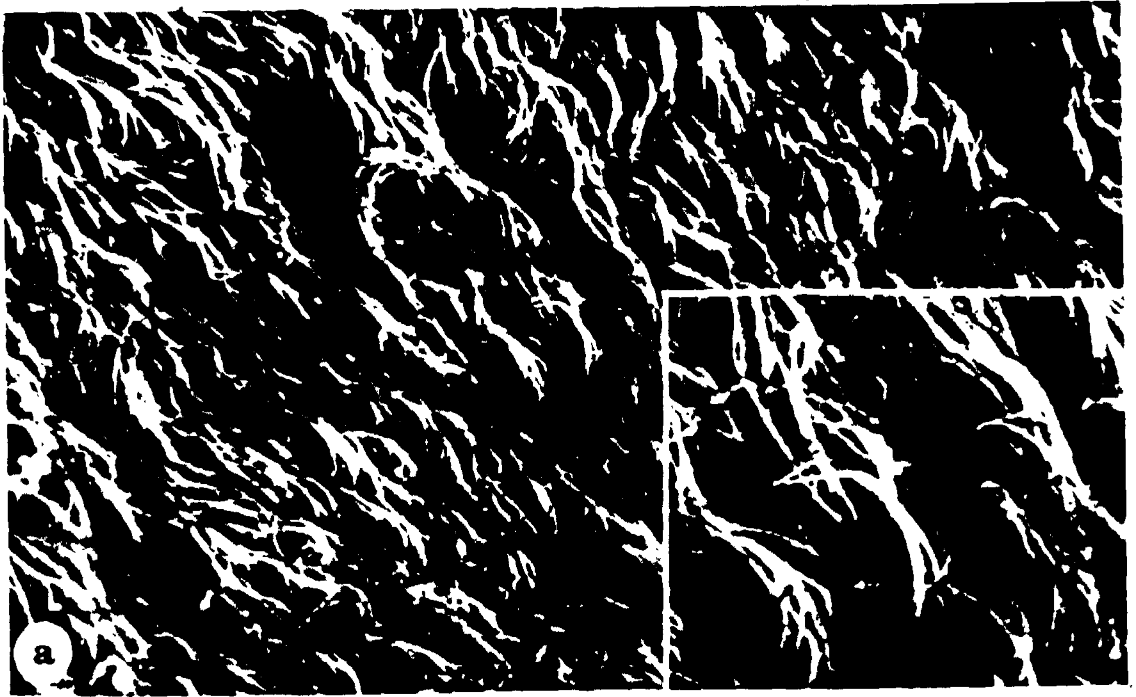
(Camphene sublimation method)

Plate 41 a, Scanning electron micrograph of the dorsolateral wall of the human third ventricle. The ependymal surface, not unlike the other species examined, is characterized by a thick mat of cilia with intervening non-ciliated patches of variable size. X 1000. Inset, Higher magnification of a portion of the dorsal wall as shown in fig. a illustrating the presence of a felt-work of microvilli between the cilia. X 2000.

b, A broad portion of the transitional zone in the human third ventricle is shown in this scanning micrograph. Cilia become progressively fewer on the lateral walls of the ventricle along a dorsal to ventral gradient. X 900.

c, The luminal surface of non-ciliated ependymal cells along the ventrolateral wall near the floor of the ventricle is shown in this micrograph. Surface blebs may be seen in this field. X 2700.

(Camphene sublimation method)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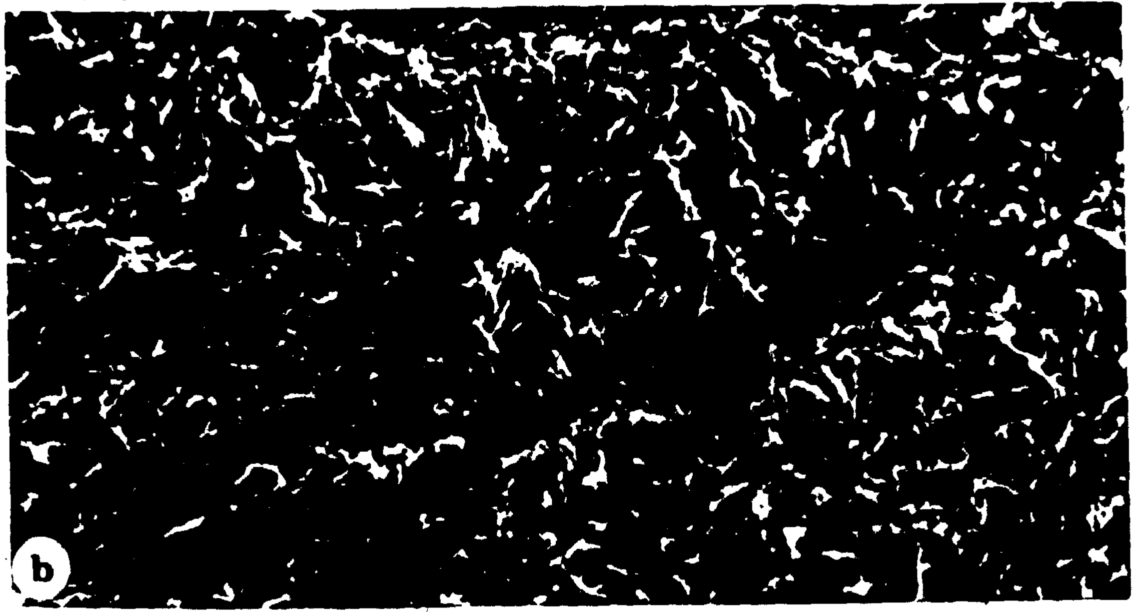
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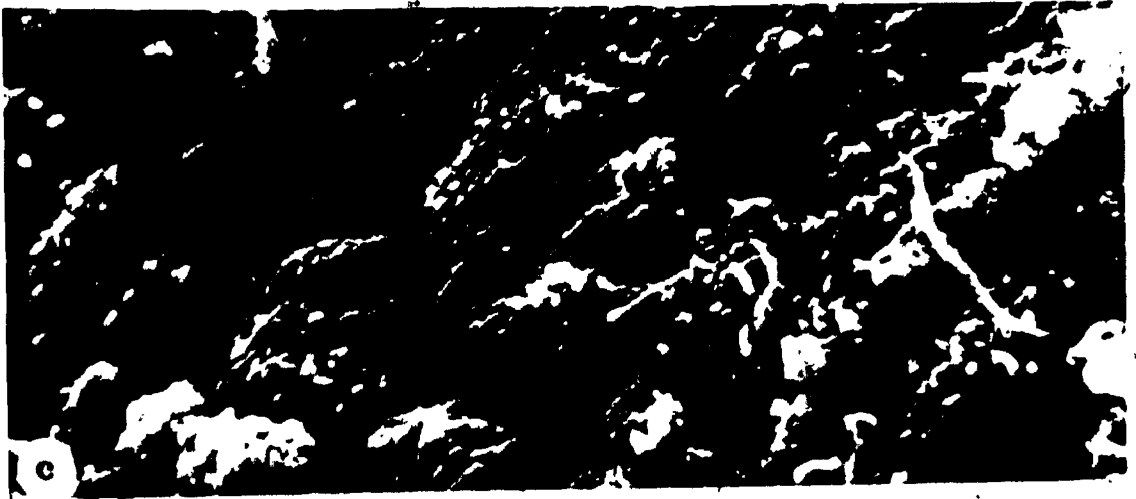
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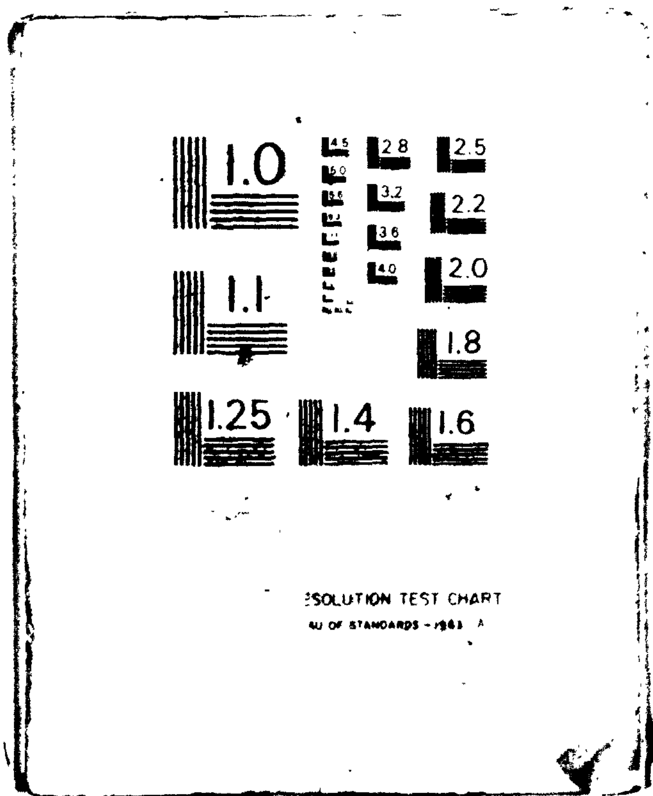

in a dorsal to ventral gradient and are entirely lacking in the depths of the ventricle along the ventrolateral wall and floor (Plate 41c). It was generally difficult to determine with certainty the nature of the surface specializations exhibited by the non-ciliated ependymal cells in this region since preparations were frequently of less than optimum quality. Nevertheless there were indications that surface blebs and microvilli like those of the other animal species studied were present.

Sulcated areas were seen frequently in some regions of the human third ventricles that were examined (Plate 44). This observation has been made previously in the human particularly the third ventricle Friede, 1961; Scott et al., 1972b) and in the lateral ventricles of both fetal and adult sheep (Kozlowski et al., 1972; Scott et al., 1974b). Such areas are therefore presumed to be normal and consistent features of the ventricular walls.

The observations of this comparative study substantiate suggestions that the ependymal lining of the cerebral ventricles is almost completely ciliated and that the conspicuous absence of cilia in circumscribed regions of the ventricular system is exceptional. The dorsolateral wall of the third ventricle in all species examined has a relatively constant appearance. The ependymal surface is characterized by a dense mat of cilia which extends into the lumen of the ventricle totally obscuring the underlying surfaces. This structural organization is found in several mammalian species including the rat, cat, mink, sheep, monkey and man (Clementi and Marini, 1972; Scott et al., 1972b, 1973, 1974b; Kozlowski et al., 1973; Mestres et al., 1974). The ependymal cilia of third ventricle are generally uniform and

homogeneous, however, species variation in density and possibly size is suggested. They are similar in structure to those found within the other brain ventricles. Generally, tufts of 25 or more cilia emanate from the central apical part of the luminal cell surface projecting into the ventricle in different directions. Unlike those of the cat (Clementi and Marini, 1972) or of the rat lateral ventricle (Peters, 1974), cilia particularly in the rabbit are not of a constant thickness from base to tip; rather they taper distally ending in terminal bulbous enlargements which have not been described previously. Such terminal dilatations at the apex of each cilium (the diameter of which exceeds that of the shaft), have since been seen in the brain ventricles of sheep (Kozlowski et al., 1973), monkey (Coates, 1973a, b) and the mink (Scott et al., 1973). While no special significance can be attached to these terminal dilatations analagous surface specializations (designated ciliary crowns) have recently been demonstrated in the cilia from all parts of mouse oviduct with both the scanning and transmission electron microscopes (Dirksen and Satir, 1972). These authors described terminal dilatations as an extracellular surface specialization which ultrastructurally consisted of a crown of fine hairs 60 Å thick, 225 Å long and which appear to insert into the cell membrane.

The ciliary surface in the rabbit, unlike that of either the mink (Scott et al., 1973) or the cat (Clementi and Marini, 1972) which possess smooth membrane profiles without particular substructure, is irregular with small thickenings and an almost segmental appearance. Such morphology may reflect a means of extending the surface area for purposes of ciliary pinocytosis (Brightman, 1965). Occasionally, the

ependymal cell membrane could be observed to invaginate around the cilium forming a pericilliary moat surrounding the base of the shaft (Kohno and Usui, 1966; Brightman and Palay, 1963). The function of cilia within the ventricular system is somewhat conjectural. While gradients of cerebrospinal fluid production and absorption are generally regarded as important determinants for the direction of CSF flow within the brain ventricles, studies do indicate that ependymal cilia are motile and capable of influencing the direction of current flow and the movement of particulate matter (Hild, 1957; Cathcart and Worthington, 1964; Worthington and Cathcart, 1963, 1966; Dalen, Schlapfer and Mamoon, 1971). Kohno and Usui (1966), however, concluded that ependymal cilia are not sufficiently polarized to maintain the directional circulation of CSF within the ventricular system. Speculation as to their physiological significance ranges from suggestions that they may subserve a sensory or receptive function to suggestions that they are simply non-functional.

In contrast to the rest of the ventricular lining, the ventrolateral wall and floor of the third ventricle is consistently devoid of cilia. The luminal surface of these ependymal cells is characterized by numerous irregular membrane specializations, some of which resemble microvilli. This distinctive morphology is constant among most other mammalian species and no doubt reflects a unique functional capacity. Of equal significance, is the close correspondence of this region with the large numbers of tanycytes distinguished in the area by other means of investigation (Chapter 4.1). It is not possible, however, to apply this topographical-structural correspondence too rigidly since 1) it is possible that not all ependymal cells in this

region of the ventricle are tanycytes, 2) tanycytes are not confined only to this region of the third ventricle, and 3) it is not possible to distinguish with certainty tanycytes from common mural ependymal cells on the basis of their surface morphology alone.

Elaborations of the cell surface are generally considered to be structural adaptations for specialized cell functions. From a functional standpoint, microvilli such as those of intestinal and renal epithelia are known to be associated with an absorptive function, although their presence cannot always be equated with such a function (Fawcett, 1962). A limited covering of microvilli has also been demonstrated on certain epithelial cells—notably, the mucous and parietal cells of the gastric mucosa as well as the goblet cells of the intestinal epithelium whose principal function is secretion (Fawcett, 1962, 1965). On the other hand, it has been proposed that microvilli of the serosal surfaces of major organs and tissues function in protecting the underlying mesothelium from abrasive or frictional damage (Andrews and Porter, 1973). Notwithstanding the fact that even cells whose surface have a similar appearance may actually be diverse in their functions, on none of the non-ciliated ependyma in the ventrolateral wall were microvilli found to be as numerous, as uniform, or as orderly in their arrangement as they are in the cells and tissues characterized above. The predominant features of these cells as determined by SEM observation are the large numbers of pleomorphic apical blebs. Ultrastructurally, Matsui and Kobayashi (1968) and Kobayashi and Matsui (1969) ascribed three basic types of ependymal membrane profiles to an equivalent area of the third ventricle in the rat and the white-crowned sparrow—bleb-like microvilli or bulbous protrusions, finger-like

microvilli and surface folds. The apical blebs commonly seen on the luminal surface in this region of the ventricle have been suggested to be a morphological expression of an apocrine release of some unknown secretory material into the ventricle (Scott et al., 1972a; Kozlowski et al., 1973; Mestres et al., 1974). Some authors alluded to the morphological similarities between ependyma of the ventrolateral wall of the third ventricle, of the choroid plexus and of the circumventricular organs (structures either known or suspected to have a secretory function), in supporting the general notion that the ependyma is an active secretory epithelium and possibly also an extrachoroidal source of CSF (Weindl and Joynt, 1972a, b; Kozlowski et al., 1973; Scott et al., 1973, 1974b).

Large lucent excrescences or spheroids described as emanating from the ependymal cells or lying freely upon the floor of the third ventricle (Knigge and Scott, 1970; Clementi and Marini, 1972; Scott et al., 1972a, b, 1973; Mestres et al., 1974) have been frequently encountered in our studies, however, it seems doubtful that they represent either a single population or a unique feature of the ventricle wall. Similarly the presence of smooth and granular-surfaced rounded structures among ciliated ependymal cells similar to those described by Clementi and Marini (1972), Mestres et al. (1974) and Scott et al. (1974b) has in our studies been invariably associated with contamination of the ventricular surface by formed elements of the blood. It is difficult, therefore, to accept the suggestion that they represent true *in vivo* morphological features of the ventricular surface. Two unusual structures, however, which we have encountered in the course of our investigations are shown in Plate 42. Their identity could not

(1-3 μm diam.) that extended for some distance over the ependymal surface. Smaller collateral branches could be followed for variable distances and at points appeared to pierce the ependyma, as if to extend into the underlying layers of the ventricle wall (Plate 43b). Occasionally adjacent processes were interwoven at what appeared to be their terminus in a tangled arborization (Plate 43c). While the neuronal nature of these profiles remains to be established, they may be analogous to the neuronal-like structures described by Noack et al. (1972) in the cat lateral ventricle and the unmyelinated nerve fibers identified on the surface of the rat lateral ventricle (Westergaard, 1972). These structures were described by Westergaard as containing neurotubules (200-300 Å diam.), rod shaped mitochondria with longitudinally arranged cristae and vesicles of diverse size, shape and content. Richards, Lorez and Tranzer (1973) recently confirmed and extended these observations demonstrating that supraependymal nerve terminals within the ventricles of the rat brain selectively accumulate 5-hydroxydopamine and have been specifically characterized as monoaminergic. Although nothing of the origin, distribution or function of these nerve fibers has been determined, investigators have speculated extensively on their function. It has been suggested that they may be capable of regulating the function of ependymal cells, that they may be involved in neuroendocrine regulating mechanisms, that they may act as receptors or that they secrete directly into the CSF.

While these structures may resemble neural processes and may correspond to SEM images described in other species, their infrequent occurrence, unusual morphology and proximity to the cut surface of the

Plate 42 a, An unusual crater-like formation protruding from the lateral wall of the rabbit supraoptic recess. X 126. a_1, Higher magnification of an identical formation both in structure and location observed in a second rabbit specimen. Note that the ridges appear to consist of ciliated ependymal cells continuous with those of the adjacent wall of the ventricle. X 992.

b, An unusual structure assumed to be a blood vessel emanating from the ventricular surface of the rabbit infundibular recess. It has an outer sheath of cells (fibroblasts, meningeal cells) continuous with ependymal cells lining the recess. X 410. b_1, Higher magnification of the same structure illustrating the fusiform or spindle shaped outer collar of cells. X 1600. b_2, The cut end of the same structure in cross-section showing what could conceivably be construed as a lumen. X 3700.

(Camphene sublimation method)

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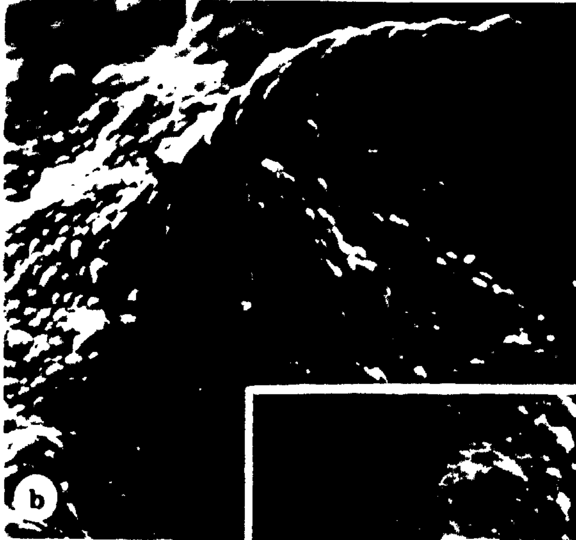
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b2

vesicles and distinctive small mitochondria were encountered at the free ependymal surface and closely interdigitated with ependymal cells in the rat (Brightman and Palay, 1963). Matsui and Kobayashi (1968), however, reported that such unmyelinated axonal endings protruding into the third ventricle were not a frequent finding in this species. In the third and fourth ventricles of the rabbit and cat brain, a great number of bulb-like processes of unmyelinated nerve fibers containing both the small synaptic type and the larger dense cored vesicles, as well as large mitochondria, have also been described (Leonhardt and Lindner, 1967; Leonhardt, 1968; Leonhardt and Prien, 1968; Leonhardt and Backhus-Roth, 1969). Between the bulb and the apical plasmalemma of the ependyma, desmosomes and synapse-like contacts were found. The significance of these intraventricular neuronal processes and their relevance to the present discussion is based on the fact that they are considered by some investigators to represent a potential mode of entry into the CSF for biologically active principles. This supposition is not without precedent since the presence of small concentrations of a number of such principles in the CSF has been documented. They have included thyroxine and triiodothyronine (Siersbaek-Nielsen and Mølholm Hansen, 1970; Hagen and Solberg, 1974), insulin (Margolis and Altzuler, 1967), cortisone and hydrocortisone (Baron and Abelson, 1954), progesterone (Lurie and Weiss, 1967), estrogen (Anand Kumar and Thomas, 1968; Anand Kumar, 1973), HCG (Bagshawe, Hilary Orr and Rushworth, 1968), GH (Linfoot _et al._, 1970), TSH (Bakke, 1963) and ACTH (Kendall, McGilura and Lamorena, 1973). Feldberg and Myers (1966) described the presence of serotonin, catecholamines and a hydroxy acid lipid, later presumed to be prostaglandin, in the CSF of

the cat. The presence of a peptide with ADH activity has also been demonstrated in the ventricular CSF (Heller, Hasan and Saifi, 1968; Vorherr et al., 1968; Heller, 1969; Rodriguez and Heller, 1970; Pavel and Coculescu, 1972). As we have already stated elsewhere, it is speculated that the specialized ependyma of the lateroventral wall and floor of the third ventricle may be responsive to the presence of circulating hormonal principles within the CSF. Using the SEM, Clementi and Marini (1972) identified small round formations on the floor of the cat third ventricle which they have likened to the club-like nerve endings described above. In contrast, we have not been able to identify the presence of such distinct interependymal neuronal projections within the ventricle in our studies. It is conceivable that their presence may, however, be obscured by the knob or bulb-like protrusions which populate the ependymal surface in this region of the ventricle.

i) Supraependymal Neuronal Processes

In addition to the presence of neuronal processes which traverse the wall of the ventricle and come to lie within the lumen, a variety of intraluminal structures lying freely on the ependyma have been described within the mammalian cerebral ventricles (Schwanitz, 1969). In the third and fourth ventricles of the rabbit and of the cat, glial cells have been reported to lie freely within the lumen (Leonhardt and Lindner, 1967); in the rabbit, cat, rat and mouse third ventricle microglial-like cells were reported to lie on the ependymal surface (Bleier, 1971, 1972). The existence of epiplexus (Kolmer) cells in most if not all vertebrate species is well documented (Carpenter et al., 1970). More recently, their structure has been characterized with

the SEM (Hosoya and Fujita, 1973; Chamberlain, 1974; Peters, 1974).

The presence of a curious population of multiprocessed supra-ependymal cells of various sizes and configurations does not appear to be an isolated phenomenon since they have recently been demonstrated by SEM in the cat (Clementi and Marini, 1972), squirrel monkey and rabbit third ventricle (Weindl and Joynt, 1972a, b). Similar intra-luminal cells have also been observed along the floor and in the IR of the monkey (Coates, 1972, 1973a, b, c), mink (Scott et al., 1973) and human (Scott et al., 1972b), third ventricle as well as within the lateral ventricles of the rat (Peters, 1974) and cat (Noack et al., 1972). It has been proposed that these structures represent intra-luminal glial and/or neuronal elements whose precise nature and function is as yet undetermined. While this investigation has failed to document the presence of significant numbers of such structures within the third ventricle, supraependymal cell-like structures with many long branching processes that extend over the underlying ependymal surface were observed in a few preparations. Their morphology and disposition are in accord with descriptions provided by Noack et al. (1972) and Peters (1974) of similar structures within the cat and rat lateral ventricles respectively and within the cat, mink and human third ventricles (Clementi and Marini, 1972; Scott et al., 1972b, 1973, 1974b). These intraluminal structures are shown in Plate 43. They were situated along the non-ciliated wall of the infundibular recess and were clearly distinct from the subjacent luminal ependymal surface. Large oval or spherical shaped bodies (8 μm diam.) with a relatively smooth surface were particularly conspicuous (Plate 43d). Arising from these swellings were long cylindrical processes

(1-3 μm diam.) that extended for some distance over the ependymal surface. Smaller collateral branches could be followed for variable distances and at points appeared to pierce the ependyma, as if to extend into the underlying layers of the ventricle wall (Plate 43b). Occasionally adjacent processes were interwoven at what appeared to be their terminus in a tangled arborization (Plate 43c). While the neuronal nature of these profiles remains to be established, they may be analogous to the neuronal-like structures described by Noack et al. (1972) in the cat lateral ventricle and the unmyelinated nerve fibers identified on the surface of the rat lateral ventricle (Westergaard, 1972). These structures were described by Westergaard as containing neurotubules (200-300 Å diam.), rod shaped mitochondria with longitudinally arranged cristae and vesicles of diverse size, shape and content. Richards, Lorez and Tranzer (1973) recently confirmed and extended these observations demonstrating that supraependymal nerve terminals within the ventricles of the rat brain selectively accumulate 5-hydroxydopamine and have been specifically characterized as monoaminergic. Although nothing of the origin, distribution or function of these nerve fibers has been determined, investigators have speculated extensively on their function. It has been suggested that they may be capable of regulating the function of ependymal cells, that they may be involved in neuroendocrine regulating mechanisms, that they may act as receptors or that they secrete directly into the CSF.

While these structures may resemble neural processes and may correspond to SEM images described in other species, their infrequent occurrence, unusual morphology and proximity to the cut surface of the

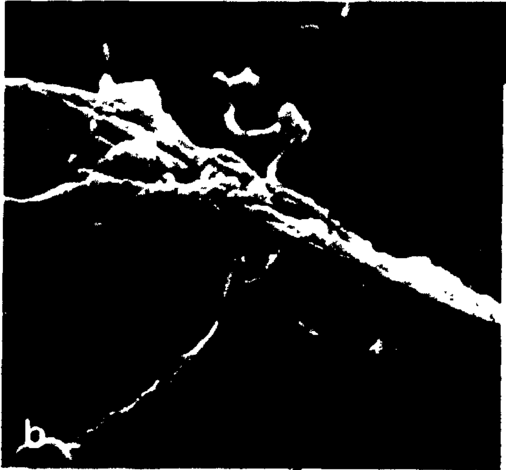
Plate 43 a, Scanning electron micrograph of the anterolateral wall of the rabbit IR. Occasionally, supraependymal structures with long branching processes were seen lying on the non-ciliated ependymal surface of the recess. X 700.

b, Branches which appear to extend into the underlying ependymal surface (arrowheads). X 1600.

c, Occasionally processes appeared to be interwoven at their termination in a tangled arborization. X 1760.

d, Most processes were observed to originate from oval or spherical bodies having a relatively smooth texture. X 1800.

(Camphene sublimation method)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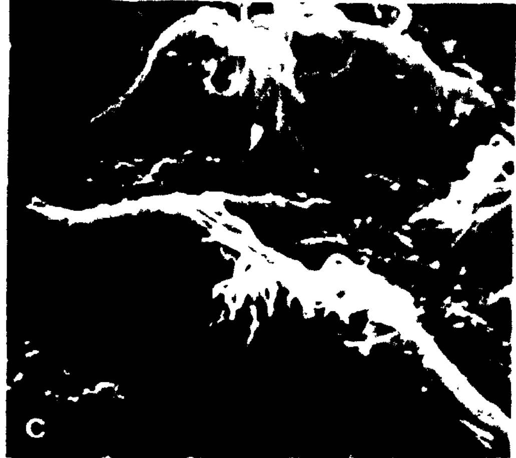
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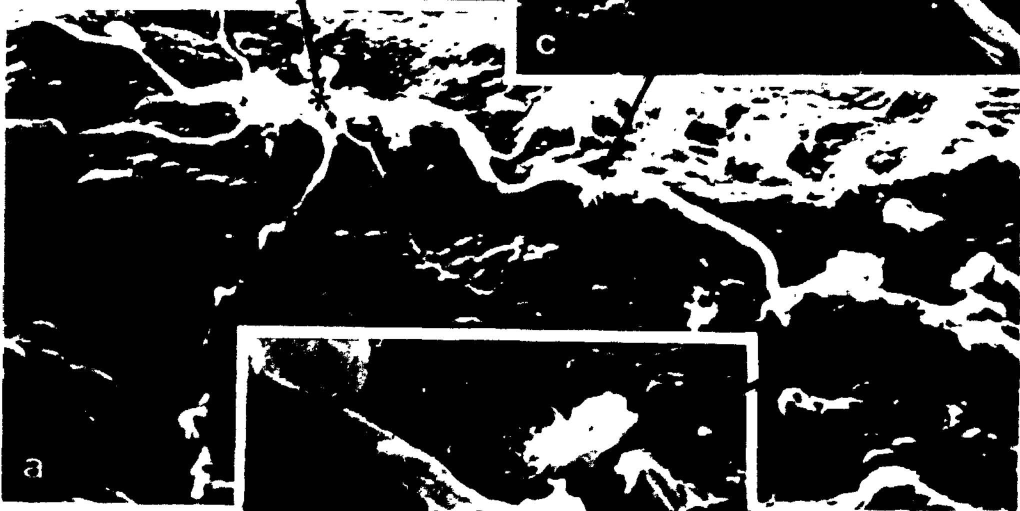
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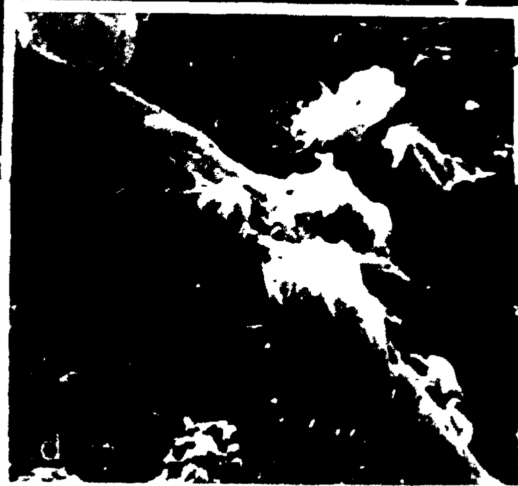
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tissue does not preclude the possibility of preparational artifact and therefore warrants cautious interpretation.

ii) Anomalous Course of the Dorsal Supraoptic Decussation

The supraoptic decussations or commissures of the third cerebral ventricle are poorly understood fiber bundles that course rostrally through the hypothalamus, descend abruptly rostral to the fornix and cross the midline in the chiasmal ridge dorsal to and behind the decussating optic tract.

Vonderahe (1937) noted an anomalous course of the dorsal supraoptic decussation in 2% of a sample of human brains examined. Instead of descending and crossing in the region of the chiasma in the usual manner, the decussating fibers traversed the third ventricle at the level of the paraventricular nucleus. In such brains, some fibers of the dorsal supraoptic decussation were traced to the paraventricular nucleus of the opposite side indicating their probable termination. A scanning electron micrograph of a human male third ventricle viewed in mid-sagittal section is shown in Plate 44. The fiber bundle (0.3 mm diam.) emanating from the ventricular surface presented in this figure is assumed to be the anomalous commissure described by Vonderahe. Its surface is covered by a layer of ciliated ependymal cells continuous with the ciliated cells lining the wall in this region of the ventricle. Microscopically, the structure is said to be composed of finely myelinated and nonmyelinated fibers. It is interesting to note that in many such cases, this anomaly was associated with a neoplastic growth elsewhere in the body; this was also found to be true in the present study.

Plate 44 Scanning electron micrograph of a human male third ventricle as viewed in mid-sagittal section showing what is assumed to be the anomalous course of the dorsal supraoptic decussation (arrowhead) as it bridges the third ventricle at about the thalamic level. Note the sulcations (small arrows) in the thalamic (left) and retrochiasmatic (right) areas of the ventricle wall. X 25. VIII, third ventricle; large arrow, site of pituitary stalk attachment.

(Camphene sublimation method)

4.3 Morphology of the Ependymal Lining of the Rabbit Third Ventricle Following Ovariectomy

Ovariectomy is a commonly accepted procedure for studying endocrine relationships. In reflex ovulators such as the rabbit, detailed reports on the complex hormonal changes that occur following ovariectomy are not numerous. It is established, nevertheless, that interruption by ovariectomy of the ovarian steroid negative-feedback system which controls tonic LH secretion initiates the discharge of LH. In the rabbit, a modest but progressive increase in LH levels occurs following ovariectomy which stabilizes at about 1.5 times control values within the first few days post-operatively (Dufy-Barbe et al., 1972; Dufy-Barbe, Franchimont and Faure, 1973). Scaramuzzi et al. (1972) reported that serum LH values in female rabbits ovariectomized for 4-14 months were elevated 4-fold over those of estrous females and were not correlated with the period of castration. An early and progressive elevation in FSH levels is similarly seen in the female rabbit following ovariectomy. Dufy-Barbe et al. (1972, 1973) have reported a 3-fold elevation in FSH levels 2 days after ovariectomy, a 6-fold increase at the end of 1 week and by 90 days, values approaching 10 times basal levels. Against such a background of dynamically changing hormonal milieux, the interpretation of concomitant morphological changes, if any, resulting from ovariectomy is at the very least difficult.

In this investigation as in all subsequent studies to be reported, examination of the ependymal surface of the third ventricle with the scanning electron microscope revealed a pattern of regional variation in morphology entirely consistent with descriptions which have been previously reported (Chapter 4.2). On the basis of this variation in

surface morphology, the ventricle has been conveniently divided into three distinct regions: 1) the dorsolateral wall, 2) a transitional zone, and 3) the ventrolateral wall and floor (infundibular and mammillary recesses). The largest part of the ventricular surface, the dorsolateral walls, is distinguished by the presence of a more or less homogeneous population of densely-ciliated cuboidal cells. In contrast, the luminal surface of ependymal cells forming the ventrolateral wall and floor of the ventricle, which corresponds to the region of greatest tanycyte concentration (Chapter 4.1), exhibits an appearance quite distinct from the adjacent ventricular surfaces and is characteristically non-ciliated. The transitional zone, which is interposed between the ciliated dorsolateral wall and the non-ciliated ventrolateral wall exhibits surface features which are common to both regions as the one is gradually transformed into the other.

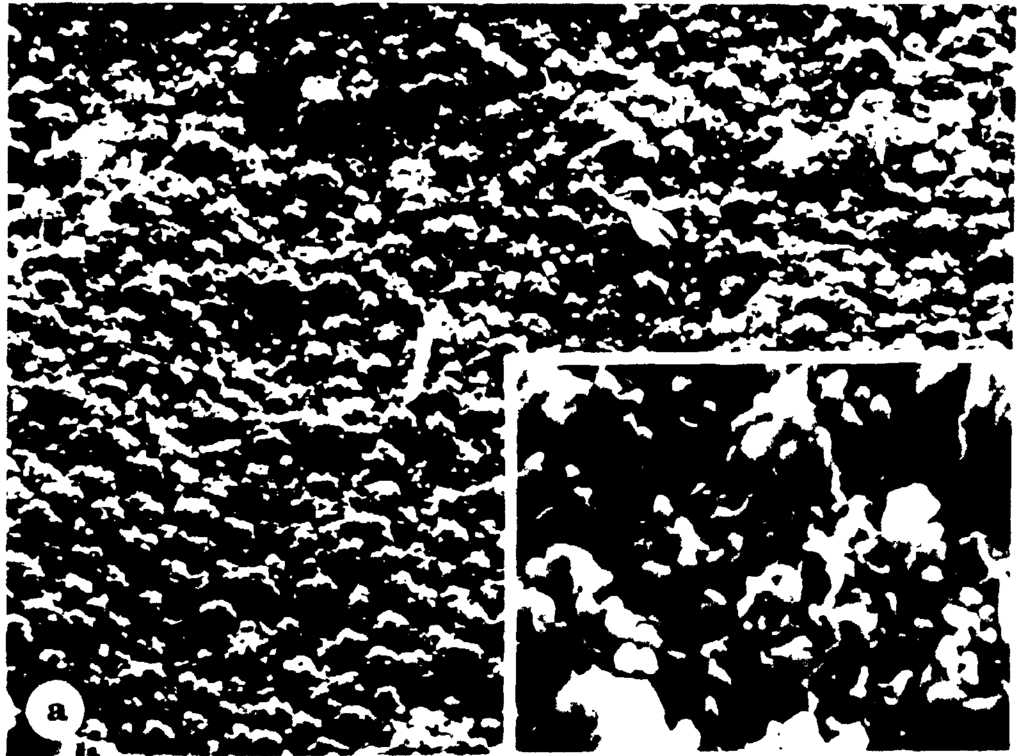
The dorsolateral wall and the transitional zone maintained the same constant appearance and was unchanged from animal to animal regardless of the experimental procedures employed in this and subsequent investigations. In most instances it was not possible because of the ciliated nature of the dorsolateral walls to directly visualize the luminal ependymal surface. For these reasons and to avoid unnecessary repetition, we have chosen not to illustrate these two regions and they have been excluded from further consideration. Henceforth attention will be focused on the ventrolateral wall and floor of the ventricle, for it is in this region that the ependymal cells appear to be uniquely organized for the translocation of substances from the CSF and are allegedly responsive to changes in the hormonal state of the animal. Care was taken to select equivalent areas of this small region in both

control and experimental animals for comparison, to ensure that any changes seen in the ependymal surface could validly be ascribed to castration and not to random variation in ependymal morphology. The accompanying micrographs (Plate 45, 46) therefore are representative of the middle one-third of the lateral wall and floor of the IR corresponding to the region designated b in Plate 57 (i.e., the adjoining parts of the regions designated 1 and 2 in Plate 2). The non-ciliated ependymal surface of this small region has at low magnification, a cobble-stone appearance. This topographical pattern was evident in both ovariectomized rabbits and laparotomized controls (Plate 45, 46). It was largely attributable to the fact that the apices of most of the ependymal cells bulge into the lumen of the ventricle. As a consequence, individual cells were circumscribed by furrowed borders that made them comparatively easy to distinguish from one another. Measurements made of a representative sample of these cellular profiles in both ovariectomized and laparotomized animals over the 34 week post-operative period are compared in figure 3. It is important to note that measurements taken from single rather than stereo-micrographs will most likely be inaccurate because the image of the surface is foreshortened due to the tilt of the specimen relative to the electron beam. Thus the measurements provided can be regarded as very approximate only and are intended to provide an indication of relative rather than absolute values. Two points may be noted in figure 3: 1) the overall similarity in the size distribution of cellular profiles in both controls and ovariectomized rabbits, and 2) the abrupt and permanent shift in the distribution of cell size that occurs at 1 and 3 weeks in control and experimental animals respectively.

Plate 45 a, Scanning electron micrograph showing the ependymal surface of the IR in a laparotomized rabbit sacrificed one week post-operatively. Note the cobble-stone appearance of the non-ciliated ependymal surface. X 1480. Inset, is an enlargement of a small part of the field shown in fig. a. Most of the bleb-like profiles in this narrow field are < 0.6 um diameter. X 7400.

b, Scanning electron micrograph of a region of the ventricle equivalent to that shown in fig. a above in an ovariectomized rabbit sacrificed one week post-operatively. No significant morphological changes were noted when compared with controls. X 1540. Inset, is an enlargement of a small part of the field shown in fig. b. The bleb-like and globular profiles in this field range from < 1 to 3 um diameter. X 7500.

(Camphene sublimation method)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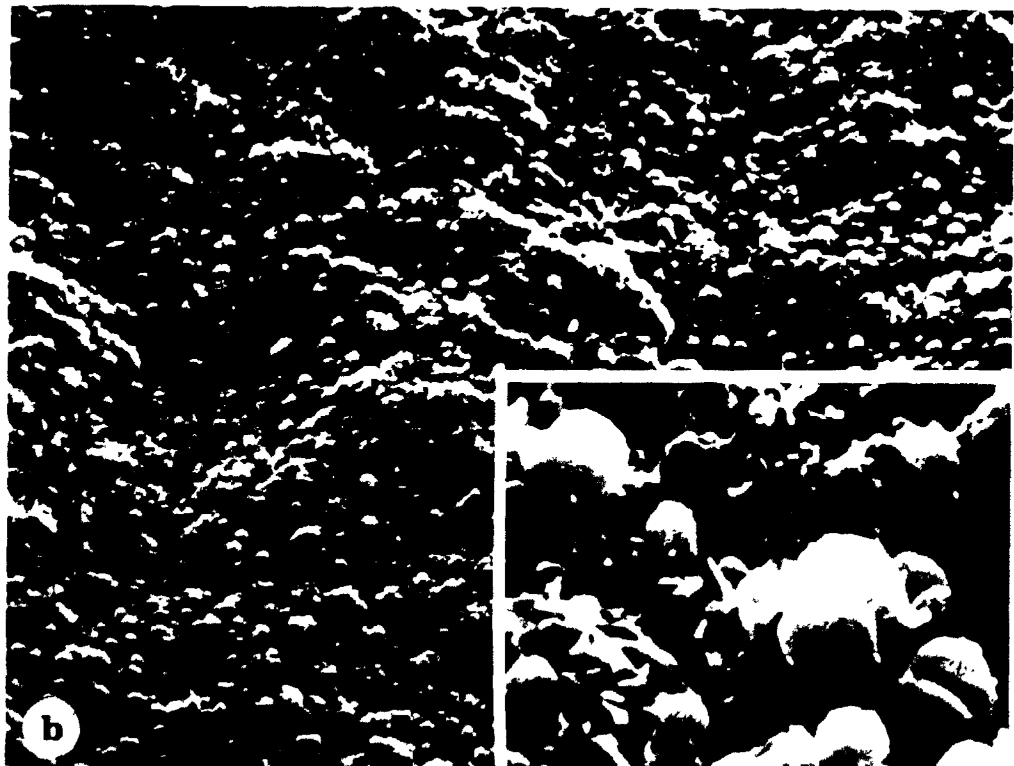
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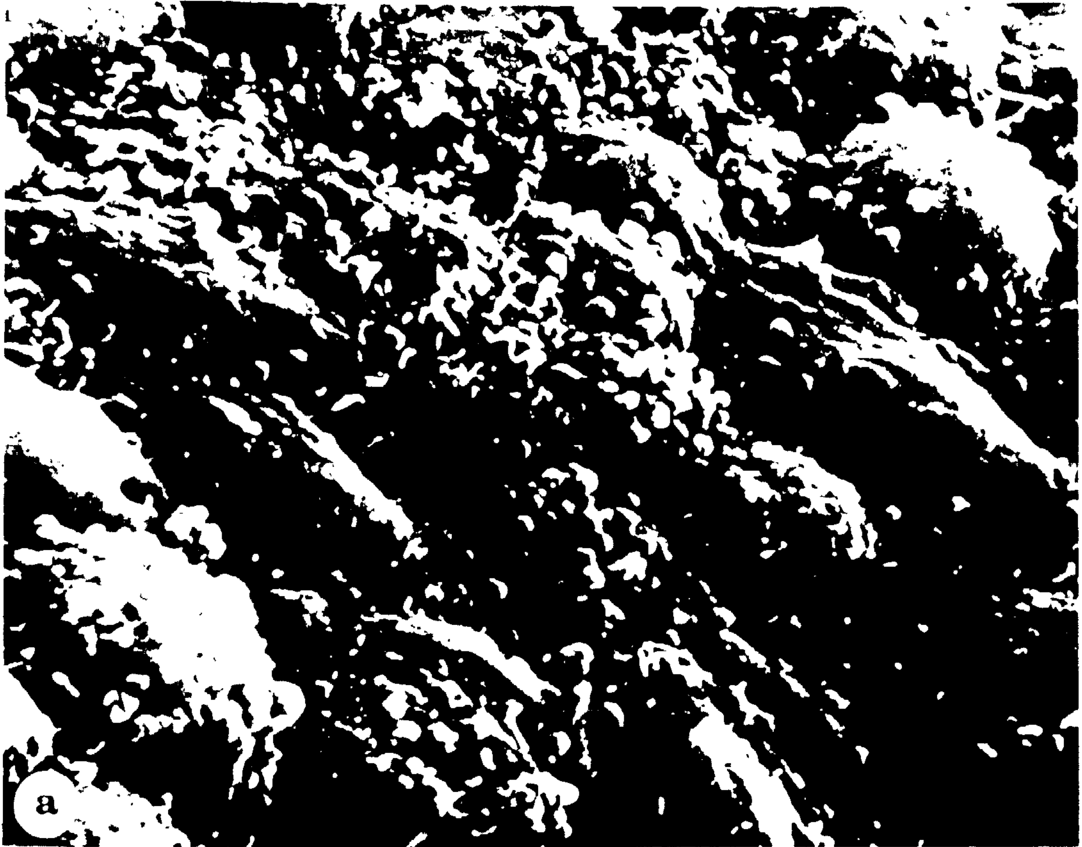
Plate 46 a, Scanning electron micrograph showing a small part of the ependymal surface of the IR in a laparotomized rabbit sacrificed 6 weeks post-operatively. The variable complexity seen in the morphology of the ependymal surface is shown in this figure. Open arrows, bleb-like profiles; solid arrows, microvilli. X 4150

b, Scanning electron micrograph of the ependymal surface of the IR in an ovariectomized rabbit sacrificed 6 weeks post-operatively. X 740. Inset, is an enlargement of a small part of the field shown in fig. b. X 3000.

(Camphene sublimation method)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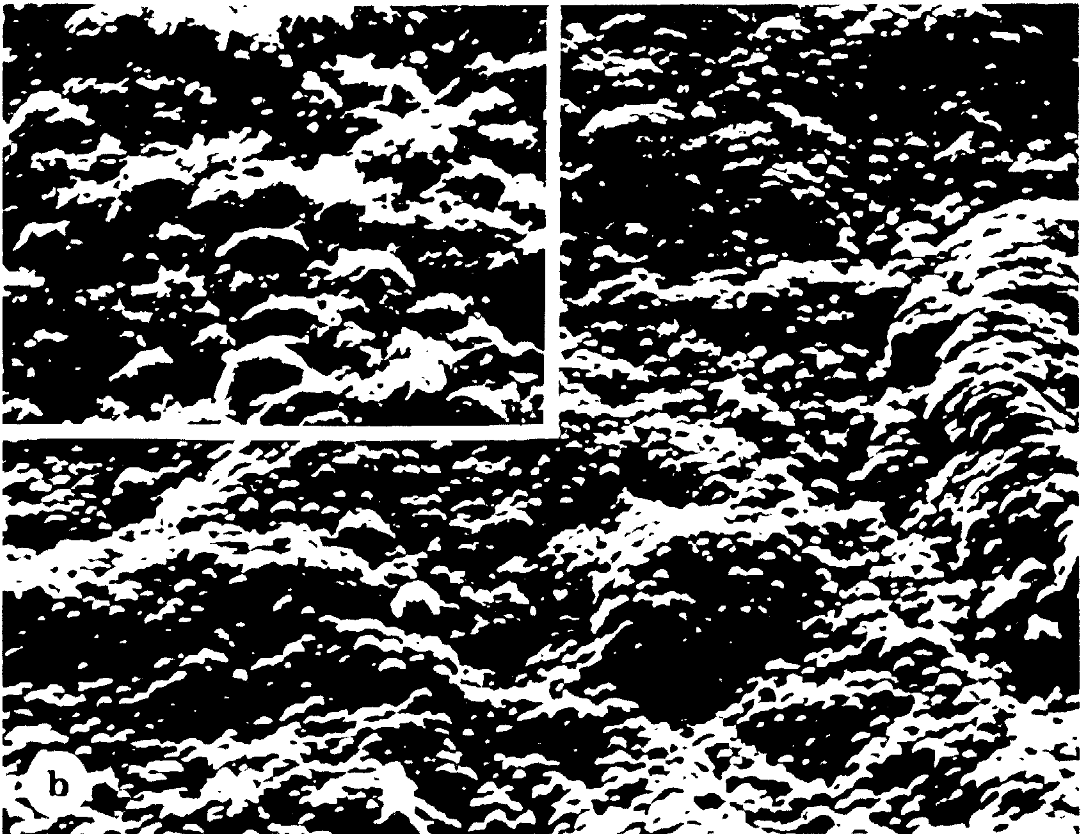
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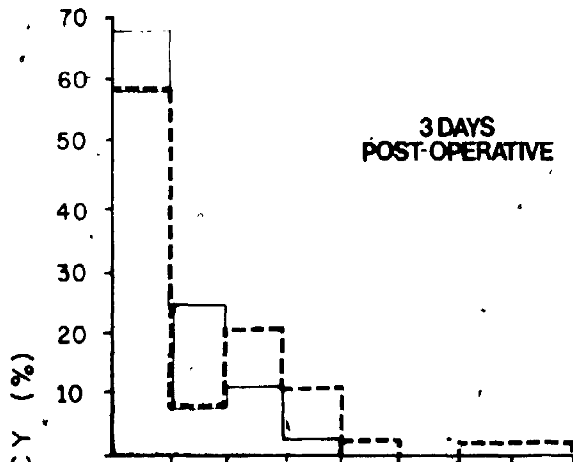
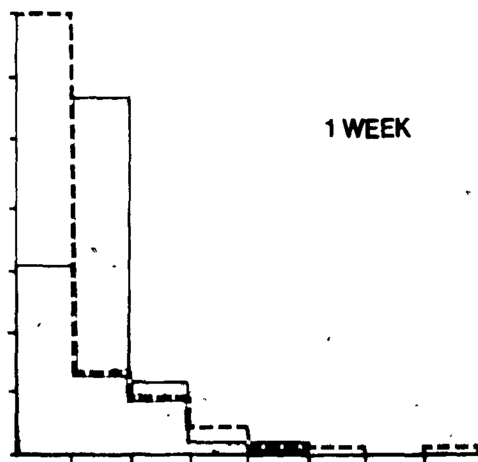
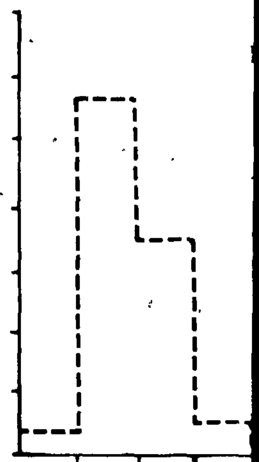
Figure 3

Histograms of the apical diameters of ependymal cells within the IR of laparotomized and ovariectomized rabbits from 3 days to 34 weeks post-operatively. Measurements of cellular dimensions were made directly from micrographs of constant field (18 cm^2) and magnification (X 1600). Each histogram is drawn from data derived from 2 micrographs (1 per rabbit) at each post-operative period. Approximately 150 cellular profiles were measured in each micrograph.







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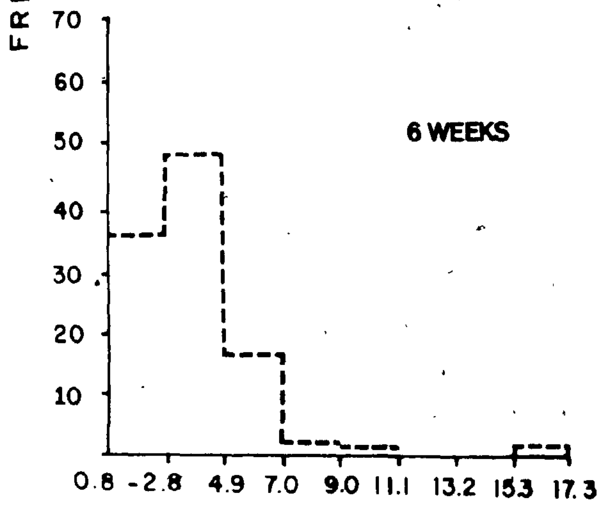
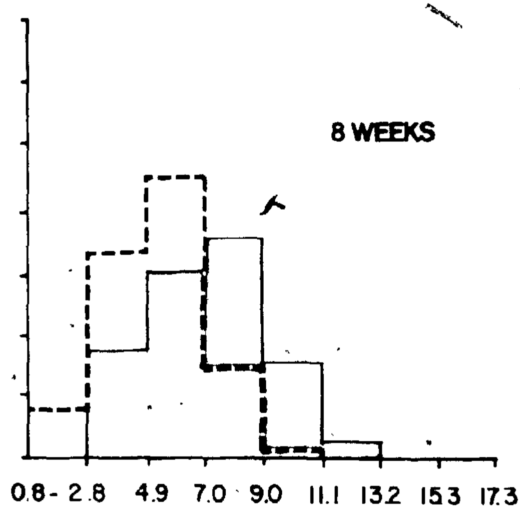
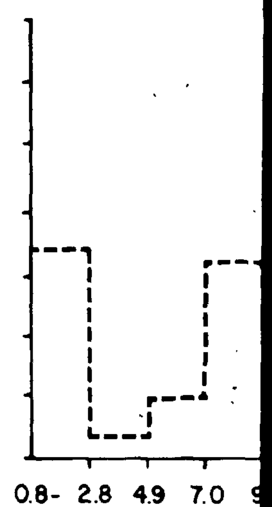


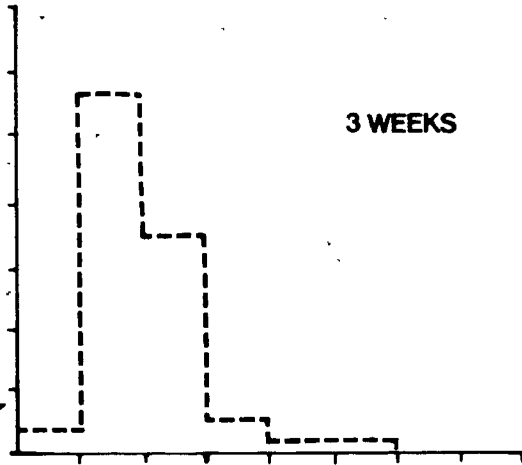




DIAMETER (µm)

1of 1

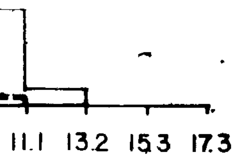
1 WEEK

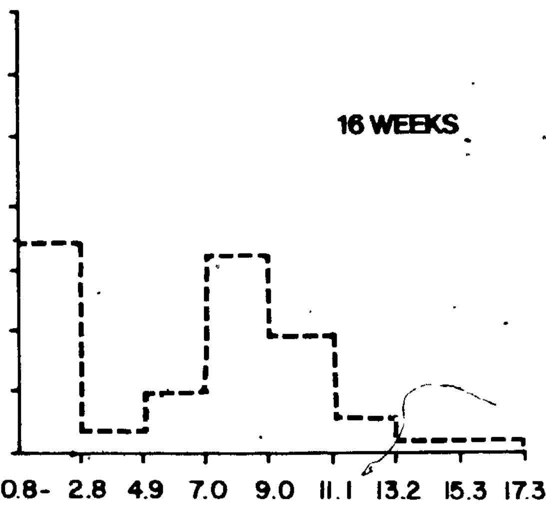


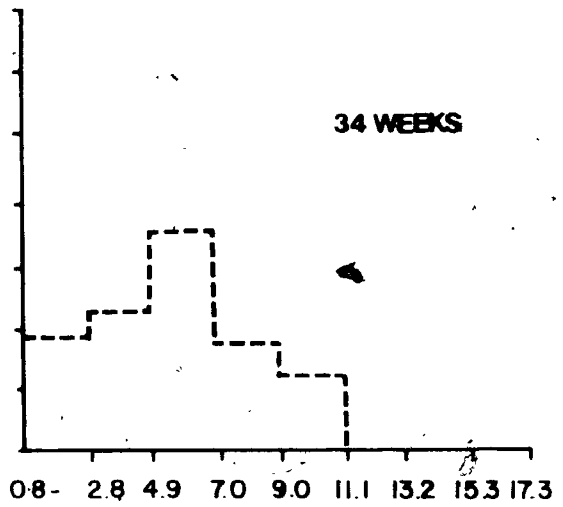
- - - - OVARIECTOMY

CONTROL LAPAROTOMY

8 WEEKS







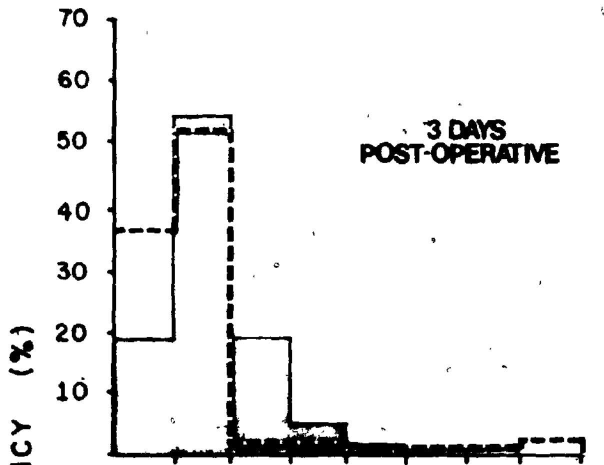
DIAMETER (µm)

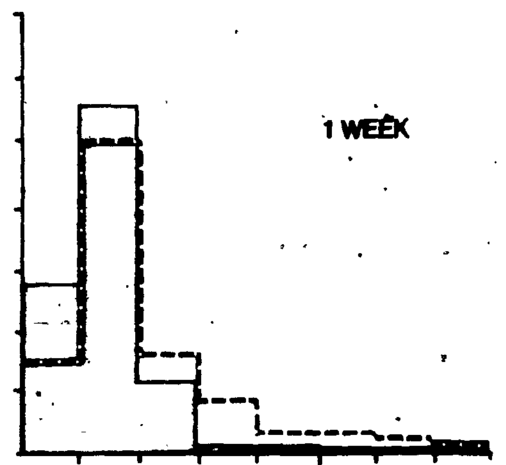
While the significance of this last observation is not immediately apparent, it is unlikely, however, to be related to ovariectomy since the same pattern was established in controls. The broadened distribution of cellular dimensions seen at 16 and 34 weeks may be explained by the fact that in one of the two animals at each of these post-operative periods broad regions of the IR were atypically flattened and largely devoid of surface eruptions. Brawer, Lin and Sonnenschein (1974) noted that the basal ventricular wall in the rat had a similar glabrous appearance with individual ependymal cells outlined by ridge-like elevations only during diestrus. They have characterized this as a quiescent stage of activity in ependyma which they observed to undergo cyclic changes coinciding with different phases of the estrous cycle. We are uncertain, however, of the extent to which these vagaries reflect true structure or are attributable to less than optimal preparation of the tissue.

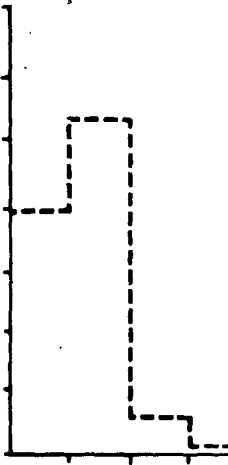
The specializations or eruptions of the ependymal surface which are peculiar to this region of the ventricle represent the second parameter that was considered. Measurements were made of a representative sample of these profiles in both ovariectomized and laparotomized animals over the experimental period. Figure 4 provides an indication of the variability encountered with respect to this parameter. The data is based on measurements that were made of only the knob- or bleb-like surface profiles. Two points may be noted in this figure: 1) the relative uniformity in the average size distribution of the knob- or bleb-like profiles in both controls and ovariectomized animals during the experimental period, and 2) that a broader range of profiles (i.e., 1-3.5 μm range were more prevalent during the 3 day to 6 weeks

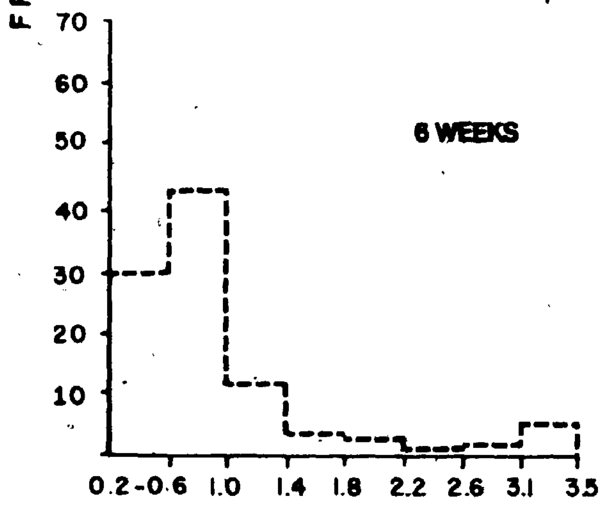
Figure 4

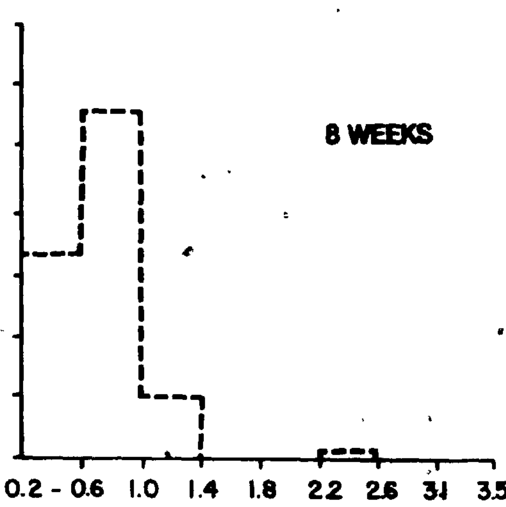
Histograms of the diameters of bleb-like profiles on the surface of ependymal cells within the IR of laparotomized and ovariectomized rabbits from 3 days to 34 weeks post-operatively. Measurements were made directly from micrographs of constant field (18 cm^2) and magnification (X 8000). Each histogram is drawn from measurements derived from 2 micrographs (1 per rabbit) at each post-operative period. Approximately 150 of the bleb-like profiles were measured in each micrograph.

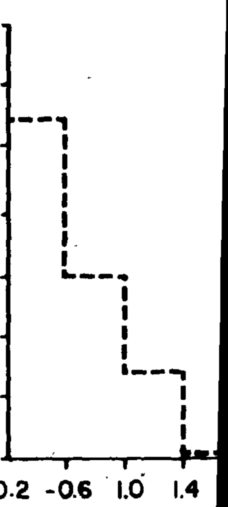






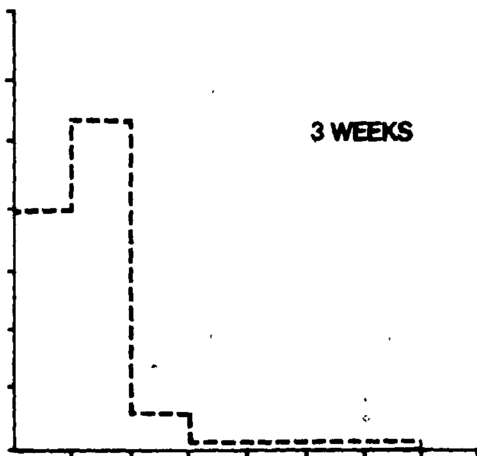






DIAMETER (μm)

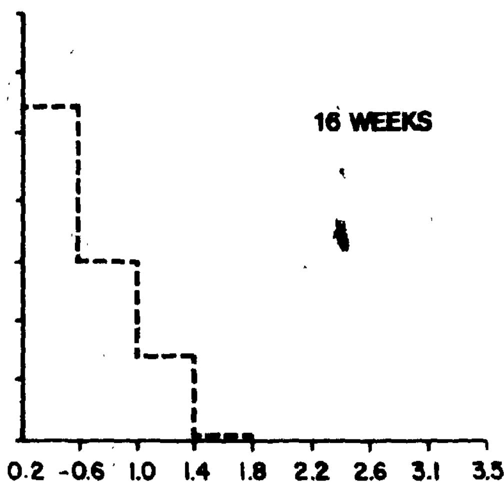
WEEK

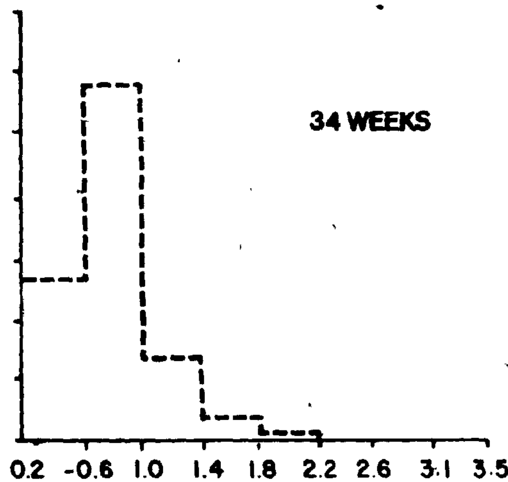


---- OVARIECTOMY

CONTROL LAPAROTOMY

8 WEEKS





DIAMETER (μm)

post-operative period (where profiles within this size range represented approximately 27-35% of the total population) than during the subsequent 8-34 week post-operative period (where they represented only 13-15% of the total population).

Few investigations describing the ependyma following ovariectomy have been undertaken using the scanning electron microscope. While the SEM appeared to be ideally suited for a study of this type by providing a total view of the structure of large areas of the ventricular surface, the results obtained however are at variance with images acquired from reconstruction of thin sections. Kobayashi and Matsui (1969) and Kobayashi, Matsui and Ishii (1970) have found that three weeks after ovariectomy most ependymal and hypendymal cells and their nuclei in the female rat ME are enlarged and cytoplasmic organelles are increased in number. A comparable response was obtained in the mouse by Oksche *et al.* (1972) who reported an increase in the nuclear volume of ependymal cells adjoining the ME and arcuate nucleus 6 days after ovariectomy; by 12 days, only the ependyma adjacent to the arcuate nucleus remained in the stimulated state.

With regard to surface specializations, in the monkey, both microvilli and rounded bulbous projections are normally distinguished on the ependymal ventricular border. Following ovariectomy (1-3 mos) however, the bleb-like projections vanish almost entirely (Knowles and Anand Kumar, 1969; Coates, 1974). In the rat, Kobayashi and Matsui (1969) and Kobayashi *et al.* (1970) found that both microvilli and bulbous protrusions are poorly developed on the apical surface of most ependyma 3 weeks after ovariectomy, whereas Oksche *et al.* (1972) noted only a marked decrease of microvilli on the apical plasmalemma 6-12 days after

ovariectomy in the mouse. Most investigators (Knowles and Anand Kumar, 1969; Kobayashi and Matsui, 1969; Kobayashi et al., 1970; Coates, 1974) agree however, that subsequent replacement treatment with estrogen restores, and even increases, the number of apical bleb-like and bulbous protrusions which disappeared after gonadectomy. In contrast to these reports, castration produced no changes in the ependyma of the rat (Brawer, 1971) or the Coturnix quail (Sharp, 1972). In addition, Sharp (1972) was unable to find any differences between castrated and estrogen or testosterone treated birds.

On the basis of our observations, in the rabbit we have arrived at the same conclusion. No significant changes in ependymal morphology occur that can be correlated with the increased gonadotropic hormone secretion associated with ovariectomy. From a functional point of view there is no evidence to indicate enhanced or diminished secretory or absorptive activity of ependyma triggered by ovariectomy. It is plausible to conclude therefore that ependymal cells lining the lateral wall and floor of the IR are not directly responsive to changes in adenohypophysial activity and therefore on this basis may not be implicated as a communicating link between the CSF and pituitary for feedback regulatory effects.

The normal variation commonly observed in the complexity of the luminal cell surfaces while unrelated to the hormonal changes occasioned by ovariectomy does suggest that ependyma in this region of the ventricle may have a highly dynamic surface which is morphologically plastic even under normal conditions. The ependymal surface may be reshaped in accordance with changes in CSF composition, production and/or pressure (Schechter and Weiner, 1972) or may simply reflect differences in the

functional state of cells at the time of fixation.

Finally, the following additional considerations merit attention: 1) our inability to correlate changes in the ependymal surface of the IR with ovariectomy may be due to the variable complexities of this specialized surface itself, a view advanced by Schechter and Weiner (1972) to explain their failure to observe changes in the ependymal surface of the rat lateral recess in response to catecholamines, 2) the response of a cell to hormonal changes may not be reflected as a surface phenomenon, and 3) the normal variations in the complexity of this specialized surface may explain why the present results are at variance with ultrastructural studies in that the sampling necessary in thinly sectioned material for TEM may miss regional variations in a given area. Chamberlain (1973) found this to be a very plausible explanation for the inconsistencies which he noted in the development of ependyma and choroid plexuses in prenatal rat brains.

4.4 Ovulation in the Rabbit Following Administration of Synthetic Luteinizing Hormone-Releasing Hormone (LH-RH)

i) Intravenous Administration of Synthetic LH-RH in Conscious and Urethane Anesthetized Rabbits

The minimum effective dose (MED) of synthetic LH-RH required to induce ovulation in 100% of conscious rabbits when administered intravenously was found to be 300 ng/kg body wt (Table 1). Two-thirds of this dose (200 ng/kg body wt) was effective in inducing ovulation in 60% of the animals. There was no evidence that synthetic LH-RH in relatively large doses (8x or 20x the MED_{100}) increased the number of ova released significantly above the number (6.7 ± 1.9) obtained with minimum

TABLE I

INDUCTION OF OVULATION IN CONSCIOUS RABBITS FOLLOWING

INTRAVENOUS INJECTION OF SYNTHETIC LH-RH

Dose of LH-RH (ng/kg body wt.)	Body Wt. (kg) ($\bar{x}$±S.D.)	No. of Rabbits Ovulated/N	Total No. of Ruptured Follicles	No. of Ruptured Follicles per Rabbit ($\bar{x}$±s.e.m.)
Saline Controls	3.7±0.6	0/3 (0%)	0	0
100	4.6±1.5	0/3 (0%)	0	0
200	5.2±0.4	3/5 (60%)	18	6.0±2.1
300	4.3±1.1	3/3 (100%)	20	6.7±1.9
2450	5.1	1/1 (100%)	7	7
5950	4.2	1/1 (100%)	8	8

effective dose levels (Table I). Rabbits that ovulated, did so 13-16 hours after LH-RH administration. The time at which ovulation occurred following injection was not related to the dose of synthetic decapeptide administered.

Parallel results obtained in urethane anesthetized rabbits injected intravenously with synthetic LH-RH are shown in Table II. Treatment with 250 and 300 ng/kg body wt of synthetic LH-RH adminstered as a single rapid intravenous injection resulted in ovulation in 33% and 75% of animals respectively. Totally ineffective ovulatory doses ranged from 16 - 200 ng/kg body wt. Evidence of stimulated follicles with these sub-ovulatory doses were nevertheless found (Plate 47). This was expected since synthetic LH-RH preparations like the native material are known to stimulate the release of both LH and FSH. Administration of the vehicle (0.15 M NaCl) alone was, as expected, without any effect.

The minimum effective dose of LH-RH required to induce ovulation in 100% of urethane-anesthetized animals when administered systemically was 400 ng/kg body wt (Table II). This dose is one-third larger than the equivalently effective dosage required in the unanesthetized but otherwise identically treated rabbit (Table I). Despite differences in the quantity of synthetic decapeptide required to induce ovulation in conscious and anesthetized groups, differences in the times at which ovulation occurred following LH-RH administration were not observed. In urethane anesthetized rabbits, as in conscious rabbits, ovulation occurred between 13 and 16 hours after injection precluding the possibility of a delayed ovulatory response with the anesthetic. Supraovulation following administration of large exogenous doses of LH-RH in anesthetized

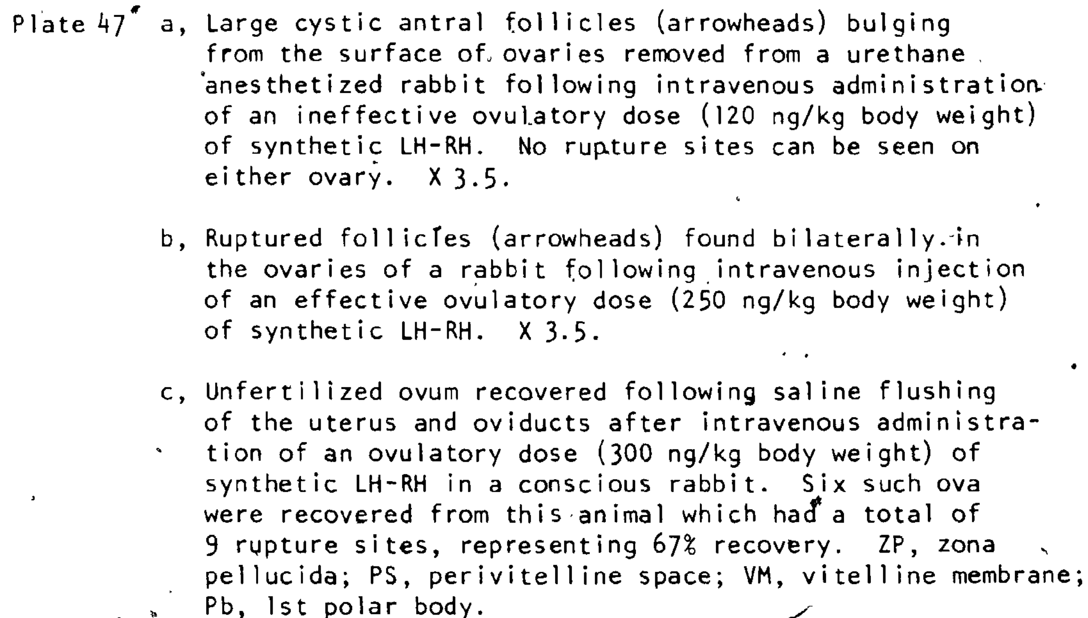


Plate 47 a, Large cystic antral follicles (arrowheads) bulging from the surface of ovaries removed from a urethane anesthetized rabbit following intravenous administration of an ineffective ovulatory dose (120 ng/kg body weight) of synthetic LH-RH. No rupture sites can be seen on either ovary. X 3.5.

b, Ruptured follicles (arrowheads) found bilaterally in the ovaries of a rabbit following intravenous injection of an effective ovulatory dose (250 ng/kg body weight) of synthetic LH-RH. X 3.5.

c, Unfertilized ovum recovered following saline flushing of the uterus and oviducts after intravenous administration of an ovulatory dose (300 ng/kg body weight) of synthetic LH-RH in a conscious rabbit. Six such ova were recovered from this animal which had a total of 9 rupture sites, representing 67% recovery. ZP, zona pellucida; PS, perivitelline space; VM, vitelline membrane; Pb, 1st polar body.

TABLE II

INDUCTION OF OVULATION IN URETHANE ANESTHETIZED RABBITS FOLLOWING

INTRAVENOUS INJECTION OF SYNTHETIC LH-RH

Dose of LH-RH (ng/kg body wt.)	Body Wt. (kg) ($\bar{x}$±S.D.)	No. of Rabbits Ovulated/N	Total No. of Ruptured Follicles	No. of Ruptured Follicles per Rabbit ($\bar{x}$±s.e.m.)
Saline Controls	3.7±0.6	0/3 (0%)	0	0
16-200 *	4.2±0.1	0/10 (0%)	0	0
250	3.9±0.4	1/3 (33%)	7	7
300	5.1±0.8	3/4 (75%)	14	4.7±2.7
400	4.8±1.4	3/3 (100%)	17	5.7±0.9
1150	5.4	1/1 (100%)	1	1
2970	4.2	1/1 (100%)	1	1

*None of the rabbits receiving doses < 200 ng/kg body wt. ovulated; they were therefore grouped together. Included within this group were 2 animals that received 16 ng/kg, 2 at 37 ng/kg, 1 at 63 ng/kg, 3 at 120 ng/kg and 2 animals that received 200 ng/kg.

rabbits (Table II) was likewise not apparent. An abrupt decrease in the average number of rupture sites with increased dosage may even be suggested, although the sample is too small to justify any definitive conclusions. Overstimulation of the ovary with a resultant decrease in the number of ruptured follicles and oocytes released is not uncommon following administration of large exogenous doses of gonadotropins in the rabbit (Parkes, 1943; Wilson and Zarrow, 1962; Fox and Krinsky, 1968) or in rats treated with urethane (Lincoln and Kelly, 1972; Blake and Sawyer, 1972). In most cases, the number of ova recovered bilaterally from the uterus and oviducts of rabbits in this group (urethane anesthetized-intravenously administered LH-RH) closely paralleled the number of ruptured follicles that were identified (Plate 47).

ii) Intraventricular Administration of Synthetic LH-RH in Urethane Anesthetized Rabbits

Data demonstrating the efficacy of intraventricularly administered synthetic LH-RH (Plate 48) in inducing ovulation in the anesthetized rabbit is presented in Table III. The MED_{100} of LH-RH producing ovulation by this route of administration was 100 ng/kg body wt. This dose is 25% that of the equivalent minimum effective dose required systemically (Table II), thus indicating that systemically ineffective amounts of synthetic LH-RH when injected into the lateral cerebral ventricle are able to induce ovulation in urethane anesthetized rabbits. Ovulation did not occur in control rabbits that received 10 μl of 0.15M NaCL only. As with the systemic injections, increasing dosages of LH-RH administered intraventricularly did not result in significant increases in the number of ruptured follicles.

TABLE III

INDUCTION OF OVULATION IN URETHANE ANESTHETIZED RABBITS FOLLOWING INTRAVENTRICULAR INJECTION OF SYNTHETIC LH-RH

Dose of LH-RH (ng/kg body wt.)	Body Wt. (kg) ($\bar{x}$±S.D.)	No. of Rabbits Ovulated/N	Total No. of Ruptured Follicles	No. of Ruptured Follicles per Rabbit ($\bar{x}$±s.e.m.)
Saline Controls	3.3±0.3	0/3 (0%)	0	0
50	4.7±0.1	2/3 (67%)	8	4.0±3.0
100	4.5±1.2	3/3 (100%)	22	7.3±2.9
200	4.9±0.3	2/2 (100%)	19	9.5±2.5
400	3.8	1/1 (100%)	10	10

Plate 48 X-ray (taken in the A-P plane) of a rabbit skull while secured in the Kopf stereotaxic frame illustrates the *in situ* locus of the microliter cannula (arrow) at the time of injection. It is inserted into the left lateral ventricle at a level approximately 3 mm rostral to the interventricular foramen. (Stereotaxic co-ordinates A 2.0, L 2-2.5, H 4.0-6.0.) Accurate placement at the time of injection was found to be invariably associated with a positive reflux of CSF. At the end of each experiment, nevertheless, the position of the cannula in the ventricle was verified by gross inspection with the aid of a dissecting microscope. nc, nose clamp; no, nasal cavity; hp, hard palate; orc, oral cavity; z, zygoma; m, superimposition of maxillary and mandibular archs; za, zygomatic arch; o, occipital bone; fm, foramen magnum; oc, orbital cavity.

The results of this investigation clearly demonstrate the effectiveness of synthetic LH-RH administered systemically in inducing ovulation in both the conscious and the urethane anesthetized rabbit. Our observations, like those of Humphrey et al. (1973) and Dermody, Humphrey and Reel (1972) however, fail to verify a significant graded increase in the number of ova released with increasing dosage of LH-RH as reported by Amoss, Blackwell and Guillemin (1972).

In the present study, the dose of LH-RH required to induce ovulation in 100% of conscious rabbits was 300 ng/kg body wt (Table I) and in 60% of the animals, two-thirds of this dose (200 ng/kg body wt) was equally effective. These values are in close agreement with that (200 ng/kg body wt) required to produce ovulation in the constant estrous rabbit (Amoss et al., 1972) and more recently the 250 ng/kg body wt dose of synthetic LH-RH required in unprimed rabbits of the same strain (Kanematsu, Scaramuzzi, Hilliard and Sawyer, 1974). In the latter report, however, the dose (250 ng/kg body wt) administered by Kanematsu et al. (1974) actually represents something less than the minimum requirement since ovulation occurred in only 73% of rabbits so treated. There is less correspondence, however, with the results of Dermody et al. (1972) and Humphrey et al. (1973) in which the minimally effective systemic dose of LH-RH required to induce ovulation in Dutch-Belted estrous rabbits was determined to be 880 ng/kg body wt. On the other hand, Yanihara, Sakurai, Okinaga and Arai (1973) found that intravenous injection of as much as 1 μg (approximately 300-500 ng/kg body wt according to our calculation) of synthetic LH-RH was totally ineffective in inducing ovulation in untreated virgin rabbits. This variability in the ovulatory response to synthetic LH-RH as recorded

in the literature may be attributable to such factors as strain differences, variation in the physiological state of the animal and a lack of uniformity in the biological potency of the LH-RH preparations used.

The threshold dosage of systemically administered synthetic LH-RH required to induce ovulation in 100% of urethane anesthetized rabbits was 400 ng/kg body wt (Table II). This value is 33% greater than the equivalently effective dose required in the unanesthetized but otherwise identically treated rabbit (Table I). This suggests that the sensitivity of the ovulatory response to exogenously administered LH-RH in the rabbit is altered but not blocked by a standard anesthetic dose of urethane. Whether this is due to a central effect of urethane on the hypothalamus or a peripheral effect on the ovary is not revealed by our investigation. It is known, however, that anesthetic doses of urethane when administered prior to the critical period of proestrus in the rat, do block spontaneous ovulation (Haller and Barraclough, 1968; Terasawa, Whitmoyer and Sawyer, 1969; Lincoln and Kelly, 1972). Although there is no unanimous agreement as to the site or mechanism of urethane's blocking action, a number of suggestions have been advanced. Blake and Sawyer (1972) reported that neither intraperitoneal nor intravenous urethane altered rat pituitary responsiveness to exogenous LH-RH appreciably and suggested that its principal action was blockade of endogenous LH-RH release from the brain. Intraperitoneal administration also decreased the sensitivity of the ovary to exogenous LH, suggesting an additional peripheral blocking action exerted directly on the ovary or its blood supply. Lincoln and Kelly (1972) however, found the rat ovary sensitive to exogenous LH after urethane and proposed that urethane acted by

blocking, delaying or reducing the ovulatory release of LH.

A potentiating effect of ether anesthesia, on the other hand, may be a plausible alternative. Anesthetics are known to produce changes in peripheral hormone levels. Ether anesthesia elicited increases in serum gonadotropins (LH, FSH and prolactin) in male rats (Krulich, Hefco, Illner and Read, 1974; Euker, Metes and Riegle, 1975; Morris and Knigge, 1976). In addition to this effect on gonadotropin release, Morris and Knigge (1976) have reported changes in endogenous levels of hypothalamic and CSF LH-RH in response to ether anesthesia in rats. Immediately after ether exposure there was a significant decline in the hypothalamic content of endogenous LH-RH followed by an increase at 30 min. A concomitant rise in CSF LH-RH concentration was observed 10 min after ether exposure. The initial decline in the hypothalamic content of LH-RH may be of particular significance in the context of our results, since it could indicate a rapid release of endogenous LH-RH into the portal circulation.

The data presented in Table III show that ineffective amounts of systemically administered synthetic LH-RH do, in fact, induce ovulation when injected into the lateral cerebral ventricles of urethane anesthetized rabbits. The MED_{100} by this route of administration was only 25% of the equivalently effective dose required systemically. A comparison of the responses obtained by the two modes of administration suggests that intraventricular injections may be 4-5 times as potent as intravenous injections in inducing ovulation. Assuming that LH-RH is transported from the CSF across the ME directly into the portal vasculature without reaching the peripheral circulation, it would be reasonable to expect that a smaller effective dose would be required

intraventricularly than systemically. A greater concentration of LH-RH, however, would also likely reach the anterior pituitary by the intraventricular route of administration than by the intravenous route. Opinion expressed in the literature is divided regarding the relative effectiveness of these two modes of administration. Ben-Jonathan _et al._ (1974) determined that LH-RH administered intraventricularly in rats was more effective over a prolonged period of time in stimulating LH release than when given intravenously. Uemura, Asai, Nozaki and Kobayashi (1975), however, reported that intraventricularly injected LH-RH induced almost the same amount of discharge of LH and FSH from the pituitary as that induced by intravenous injection. In contrast, administration of LH-RH by the intraventricular route to ovariectomized estrogen-progesterone primed rats was shown by Weiner _et al._ (1971b, 1972) to be less effective than intravenous injections in increasing serum LH. Similarly Kendall _et al._ (1971, 1972) were unable to demonstrate that the intraventricular administration of TRH in rats was more effective in inducing TSH secretion than was TRH given by the intravenous route. Furthermore, Gordon _et al._ (1972) reported that the intraventricular administration of TRH was less effective in stimulating TSH secretion than injection into either the ME or anterior pituitary.

Meaningful comparison of the two routes of administration are not without unavoidable difficulties. In addition to problems of assessing relative dilution of the releasing hormones both in plasma and CSF, the extent of their inactivation in each milieu is also an important consideration in evaluating the efficacy of these modes of administration. There is, however, clearly a need for additional

documentation in this area. Of the small amount of available literature on the subject incubation of TRF with plasma has been shown to inactivate the hormone (Bowers, Schally, Enzmann, Bøler and Folkers, 1970; Kendall et al., 1971) whereas preincubation with CSF did not affect its activity (Kendall et al., 1971). Cramer and Barraclough (1975), however, reported that an LH-RH artificial CSF mixture allowed to stand for 4 hours at 24-28°C resulted in biological inactivation of the decapeptide but with reduced exposure time (30 min) some biological activity was retained. Notwithstanding these difficulties of interpretation involved in the comparison of these two routes of administration, the findings of the present investigation are consistent with the view that LH-RH introduced into the ventricular system can readily reach the anterior pituitary gland and is clearly capable of eliciting the release of its appropriate pituitary hormone(s). What remains to be determined, however, is the route by which this is accomplished (i.e., by traversing the ME directly and thus gaining access to the portal circulation) and whether there may be ependymal mediation in this transfer across the ME. The latter consideration is addressed in a subsequent section (Chapter 4.4iv).

It is apparent that no directevidence has been provided to indicate that LH-RH traverses the ME by active transport or by passive diffusion. Some support for this assumption, however, may be found in several recent studies. Ondo, Mical and Porter (1972) for example found that substances of large molecular weight such as LH, prolactin or bovine hemoglobin when injected into the third ventricle were recoverable in hypophysial portal blood within 30 minutes but not in the peripheral arterial blood thus eliminating the latter as a significant

source of these substances in stalk blood. LH-RH injected intraventricularly in rats was detectable in portal blood within 10-15 minutes (Ben-Jonathan et al., 1974). Although it was concluded that it could be transported from the CSF to the hypophysial portal blood in significant quantity there were also indications of its slow and prolonged entry into the systemic vasculature from the CSF. Results supporting the view that TRH is likewise able to cross the ME from the CSF into hypophysial portal blood have recently been documented by Oliver, Ben-Jonathan, Mical and Porter (1975). They reported that intraventricular injection of H^3-TRH in rats resulted in a peak concentration of the tripeptide in portal blood within minutes with only a lower level of activity detectable in arterial blood. Cramer and Barraclough (1975), on the other hand, were unable to detect the presence of LH-RH in peripheral plasma following prolonged ventricular infusion (200 minutes). The LH-RH which nevertheless reached the pituitary to elicit an LH discharge therefore seemingly passed via the ME to the portal capillaries. Despite the compelling, yet somewhat conflicting evidence, favouring the translocation of active principles from the CSF to the pituitary gland via the ME, it is apparent that the possibility that they may reach the peripheral circulation and act secondary to absorption there cannot be entirely excluded. Our own attempts to determine the effective spread of LH-RH from the ventricular site of injection reinforces this conclusion. Direct observation of the distribution of 15 µl (approximately 1.5x the volume of LH-RH injected) of marker dye was made at 10 minute, 30 minute, and 11-13.5 hour intervals following intraventricular administration. Within 10 minutes after injection, traces of the marker dye were readily and consistently seen in

the lateral ventricle at the level of the injection site, however no indication of its presence anywhere within the opposite lateral ventricle was ever found. While also found diffusely throughout the third ventricle including the ventral wall, floor and infundibular stalk, greater concentrations were usually seen within the interventricular foramen, at the third ventricle-mesencephalic junction and within the cerebral aqueduct itself. Comparatively little trace of dye was seen within the fourth ventricle although it was seen within the subarachnoid space on the lateral and dorsal surfaces of the medulla where it appeared to emanate from the paired lateral foramina. By 30 minutes, however, significant quantities of the marker dye had already gained access to the expansions of the subarachnoid space at the base of the brain with little trace of its presence remaining anywhere within the ventricular system. At subsequent intervals (11-13.5 hours), traces of the dye were almost entirely confined to the subarachnoid space on the anterior surface of the brain stem, within the adventitia of the cerebral blood vessels there and radiating for variable distances onto the lateral convexities of the cerebrum, cerebellum and brainstem. It may not be entirely appropriate to analogise the flow of marker dye with LH-RH injected intraventricularly for several reasons, not the least of which may be the presumed capacity of the ME to selectively extract and concentrate solutes such as the latter. Nevertheless the observations do suggest: 1) that marker dye and possibly synthetic decap[illegible]de is rapidly conducted (within 10-30 minutes) from the injection site in the lateral ventricle to the subarachnoid space, 2) that such substances may also very rapidly reach, and quite possibly traverse, the ME to the portal vasculature, but since the CSF is such a dynamic fluid and the flow possibly abetted by ciliary action, it is

likely that such intraventricularly-injected substances contact the ME only briefly. The indication being that this occurs in less than 10 minutes, and 3) that significant quantities may also reach the systemic circulation via the dural sinuses and the cerebral circulation directly via the subarachnoid space. Capillaries in the subarachnoid spaces have been suggested as sites of CSF reabsorption (Dandy and Blackfan, 1913, 1914). In this context, Ondo and coworkers (1973) have observed that while LRF placed in the third ventricle promptly reached the portal vasculature by traversing the ME bypassing the general circulation, LRF placed in the cisterna magna also stimulated LH release after a delay following injection which was believed to reflect the time necessary for CSF in the cisterna magna to enter the subdural sinuses and achieve an effective LH-RH concentration in arterial blood. It is interesting to note that although LH-RH injected intraventricularly was found in portal blood 10-15 minutes after injection, maximal transport occurred 20-60 minutes after introduction into the CSF (Ben-Jonathan et al., 1974) corresponding with a slow and constant passage of LRF into the systemic blood. While intraventricular injection of [^{3}H] TRH similarly resulted in radioactivity in hypophysial portal blood that was detectable within minutes, peak concentrations occurred 20-30 minutes after injection during which time however, the concentration of radioactivity in arterial blood was negligible (Oliver et al., 1975).

At the present time, therefore, it is not possible to state unequivocally that the CSF serves as a vehicle for the delivery of hypophysiotropins to the adenohypophysis via the median eminence.

iii) Morphology of the Ependymal Lining of the Rabbit Third Ventricle Following Synthetic LH-RH Induced Ovulation

The ependymal surface of the ventrolateral wall and floor of the third ventricle was examined in all conscious rabbits that received synthetic LH-RH in doses ranging from 100-5950 ng/kg body wt by the intravenous route of administration. To ensure that equivalent areas of the ventricle were selected from each animal for comparison, this small region of the ventricle was arbitrarily subdivided into three rostrocaudal levels (a-c) as shown in Plate 57. The region of the ventricle designated (a) in Plate 57 is equivalent to the rostral IR. The middle one-third of the IR corresponds to the region designated (b) and the area designated (c) is equivalent to the caudal one-third of the IR. In conscious rabbits that received ovulatory dosages (200 or 300 ng/kg body wt) of synthetic LH-RH intravenously (Plate 49a-c, 50a-c), the ependymal surface of their respective infundibular recesses did not differ significantly in morphology from one another at the time of ovulation. They were likewise indistinguishable from equivalent regions of the ventricle in animals that had received either LH-RH or saline but failed to ovulate (Plate 49d-f). Dose related changes in morphology were not evident even with relatively large amounts of LH-RH (8x or 20x the minimum effective dosage) similarly administered (Plate 50d-f).

Essentially the same results were obtained in urethane anesthetized rabbits that received LH-RH by the intravenous and intraventricular routes of administration. Significant dose related differences in morphology were not evident either within (Plate 51, 52) or between treatment groups (Plates 49-50 vs 51 vs 52).

Unlike the cyclic changes in ependymal morphology observed during

Plate 49 a-c, Scanning electron micrographs representative of three different rostrocaudal levels of the IR. The conscious rabbit from which the micrographs were taken received an ovulatory dose (200 ng/kg body weight) of LH-RH that was administered intravenously. Within 13-16 hours of injection, the animal ovulated.

a, Rostral IR. X 2700.

b, Mid IR. X 2200.

c, Caudal IR. X 2200.

d-f, Scanning micrographs of the IR as in figs. a-c above 13-16 hours following intravenous administration of LH-RH (200 ng/kg body weight) in a conscious rabbit that failed to ovulate. Compare with figs. a-c above. The absence of significant differences is self evident.

d, Rostral IR. X 2150.

e, Mid IR. X 2700.

f, Caudal IR. X 2150.

(Camphene sublimatin method)

Plate 50 a-c, Scanning electron micrographs taken at three different rostrocaudal levels through the IR of a conscious rabbit 13-16 hours following intravenous injection of the minimum effective ovulatory dose (300 ng/kg body weight) of LH-RH. Compare with Plate 49 and 51.

a, Rostral IR. X 2150.

b, Mid IR. X 2100.

c, Caudal IR. X 2050.

d-f, Scanning micrographs of the IR as in figs. a-c above 13-16 hours following intravenous administration of approximately 8 X the minimum effective ovulatory dose (2450 ng/kg body weight) of LH-RH in the conscious rabbit. Compare with figs. a-c above. Equivalent regions of the IR are morphologically indistinguishable from one another.

a, Rostral IR. X 2250.

b, Mid IR. X 2200.

c, Caudal IR. X 2200.

(Camphene sublimation method)

Plate 51 a-c, Scanning electron micrographs taken rostrocaudally through the IR 13-16 hours following intravenous administration of LH-RH (300 ng/kg body weight) in a urethane anesthetized rabbit that failed to ovulate. Comparison with Plate 49 d-f shows no significant differences in morphology between treatment groups.

a, Rostral IR. X 2700.

b, Mid IR. X 1500.

c, Caudal IR. X 2250.

d-f, Scanning micrographs of the IR as in figs. a-c above 13-16 hours following intravenous administration of the minimum effective ovulatory dose (400 ng/kg body weight) of LH-RH in a urethane anesthetized rabbit. Compare with figs. a-c above. Significant dose related differences in morphology were not evident within treatment groups.

a, Rostral IR. X 2250.

b, Mid IR. X 2250.

c, Caudal IR. X 2250.

(Camphene sublimation method)

Plate 52 a-c, Scanning electron micrographs taken rostrocaudally through the IR of a urethane anesthetized rabbit 13-16 hours following intraventricular administration of a minimum effective ovulatory dose (100 ng/kg body weight) of LH-RH. The appearance of the ependymal surfaces of the IR is indistinguishable from the equivalent surface in intravenously injected (Plate 51) but otherwise identically treated rabbits.

a, Rostral IR. X 1500.

b, Mid IR. X 2000.

c, Caudal IR. X 1800.

d, Mid IR only as in fig. b above from a rabbit that received the same dose (100 ng/kg body weight) of LH-RH intraventricularly illustrating the similarity in the morphological appearance of the two surfaces. X 2000.

e, Mid IR as in figs. b and d above from a rabbit that was given 200 ng/kg body weight of LH-RH intraventricularly. No significant qualitative difference in the morphology of the ependymal surface from either figs. b or d above is discernible. X 1500.

f, Mid IR from a rabbit that received 400 ng/kg body weight of LH-RH intraventricularly. Compare with figures b, d and e above. X 1800.

(Camphene sublimation method)

different stages of the rat (Brawer _et al._, 1974) skunk (Hagadoorn, 1965) and monkey (Anand Kumar, 1968a, b; Knowles and Anand Kumar, 1969) sexual cycles, the character of the ependymal surface of the IR in the rabbit did not change with the treatments used in this investigation. This suggests an absence of ependymal cell responsiveness in a reflex ovulator such as the rabbit to the changing levels of circulating gonadotropins and gonadal steroids occasioned by LH-RH induced ovulation.

iv) Morphology of the Ependymal Lining of the Rabbit Third Ventricle Immediately Following Intraventricular Administration of Synthetic LH-RH

In control rabbits, 5 or 15 minutes after intraventricular injection of 10 µl of 0.15M NaCl alone, the ependymal surface of the lateral wall and floor of the infundibular and mammillary recesses appeared as it does normally in untreated animals (Chapter 4.2). The ependymal surface of the recesses was irregularly contoured and typically nonciliated except for the occasional solitary cilium. The apices of most ependymal cells bulged conspicuously into the ventricular lumen, their lateral borders demarcated by deep furrows (Plate 53b-d). Also found, however, were flattened and more smoothly contoured areas in which cellular outlines were less distinct (Plate 53a). Individual ependymal cells differed considerably in their surface morphology. Numerous knob- or bleb-like protrusions extended from the apical surface of most cells (Plate 53b-d; 57a). Although variations in the size (0.2-1.4 µm diam.), shape, distribution and frequency of these profiles were noted, they were nevertheless the most common type of surface specialization encountered. Some cells, however, often within the same field, exhibited

Plate 53

Scanning micrographs taken from the third ventricle of a control rabbit that received 10 ul of 0.15M NaCl intraventricularly and which was sacrificed 5 minutes after injection. The micrographs are representative of each of the four correspondingly labelled rostrocaudal levels of the ventricle diagrammed in Plate 57.

a, Rostral one-third of the lateral wall and floor of the IR. The ependymal surface is relatively flat and non-ciliated except for the occasional solitary cilium. Some cells are largely smooth surfaced while others exhibit a variety of surface eruptions. X 3500.

b, Middle one-third of the IR. The ependymal surface is irregularly contoured with the apices of most cells bulging conspicuously into the ventricle. Note the two isolated cells (arrows) whose surface profile contrasts markedly with the surrounding field. X 2000. Inset, isolated ependymal cell like that shown by the arrows whose surface exhibits numerous microvilli. X 4900.

c, Caudal one-third of the lateral wall and floor of the IR. Individual ependymal cells vary greatly in their surface morphology. Numerous knob- or bleb-like protrusions (arrowheads) emerge from the luminal surface of most ependymal cells. Conspicuous by its isolation is a solitary cell (arrow) whose surface (as in fig. b above) exhibits numerous microvilli. X 3000.

d, Lateral wall and floor of the MR. Note that the morphology of the ependymal surface is not significantly unlike adjacent regions of the IR. X 3600.

(Critical point drying method)

a smooth structureless surface which was largely devoid of specializations while the luminal surface of other cells projected only a feltwork of microvilli (Plate 53b, c) or some combination of all of the above.

In marked contrast, 5 minutes following intraventricular administration of LH-RH (200 ng/kg body wt) the ependymal surface of the ventrolateral wall and floor of the ventricle changes significantly in a manner suggestive of increased surface activity. The myriad of surface profiles encountered in controls (Plate 53a-d; 57a) were largely supplanted by numerous microvilli which now pervaded the area, extending throughout the rostrocaudal extent of the IR and also into the MR (Plate 54, 55, 57b). Cylindrical processes (0.07-0.12 μm in diam.) of irregular length extended from the surface of most ependymal cells (Plate 54, 55, 57b) and represented the dominant type of specialization. As a consequence, ependymal cells that were either devoid of surface specialization or that exhibited only knob- or bleb-like surface profiles were far less conspicuous. Such was the case in all animals that received the same treatment.

Examination of the same regions of the ventricle in animals sacrificed 15 minutes after intraventricular administration of the same dose of LH-RH, revealed that the villiform eruptions of the ependymal surface which were prominent 5 minutes after injection, were no longer evident (Plate 56, 57c). The ependyma now exhibited morphological features which were similar to and as diverse as those found in saline controls.

Prior to this study, morphological changes in ependymal cells induced by intraventricularly administered LH-RH had not been reported.

Plate 54

Scanning micrographs taken from the third ventricle of a rabbit that received synthetic LH-RH (200 ng/kg body weight) intraventricularly and which was sacrificed 5 minutes after injection. The micrographs are representative of each of the four correspondingly labelled regions of the ventrolateral wall and floor of the ventricle illustrated in Plate 57. The luminal ependymal surface of the lateral wall and floor of the infundibular and mammillary recesses is now characterized by the eruption of numerous microvilli.

a, Rostral IR. X 4200.

b, Middle IR. X 4500.

c, Caudal IR. X 2100. Inset, higher magnification of a portion of this field shown in figure c. X 3000.

d, Mammillary recess. X 4000.

(Critical point drying method)

Plate 55

Scanning micrographs taken from the ventrolateral wall and floor of the third ventricle (as illustrated in Plate 57) of a rabbit that was given a single intravenous injection of synthetic LH-RH (200 ng/kg body weight) and sacrificed 5 minutes later. The luminal surfaces of most ependymal cells are highly irregular due to the eruption of numerous irregular finger-like processes. Compare with Plate 54. Note the close morphological correspondence of equivalent regions of the luminal surface in animals identically treated.

a, Rostral IR. X 4300.

b, Middle IR. X 2200. Inset, higher magnification of an area shown in figure b.

c, Caudal IR. X 3200.

d, Mammillary recess. X 4100.

(Critical point drying method)

Plate 56

Scanning micrographs taken from the ventrolateral wall and floor of the third ventricle (equivalent to the levels diagrammed in Plate 57) of a rabbit that received synthetic LH-RH intraventricularly (200 ng/kg body weight) and which was sacrificed 15 minutes after injection. The transient eruption of microvilli, which were prominent 5 minutes after injection, are no longer evident. The ependymal surface of the lateral wall and floor of the IR and MR now exhibit morphological features which are more comparable to those noted in saline controls (see Plate 53).

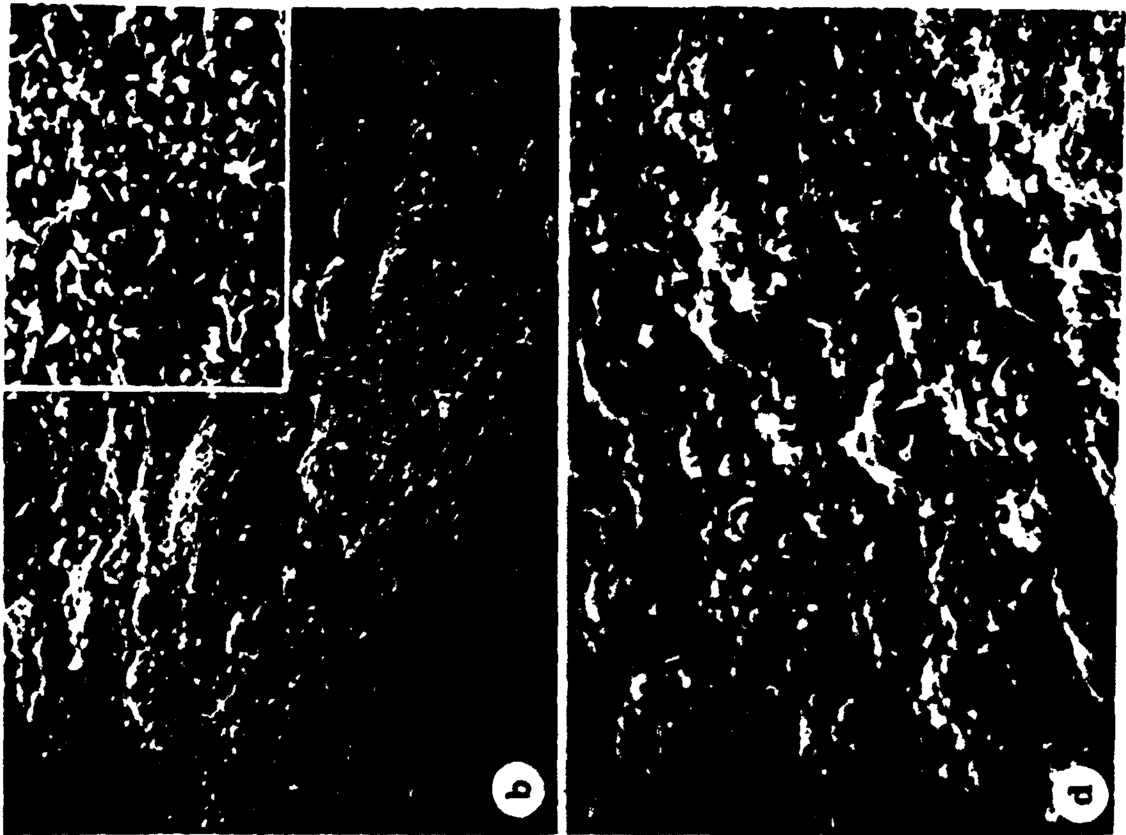
a, Rostral IR. X 2600.

b, Middle IR. X 2900.

c, Caudal IR. X 3600.

d, Mammillary recess. X 4000.

(Critical point drying method)



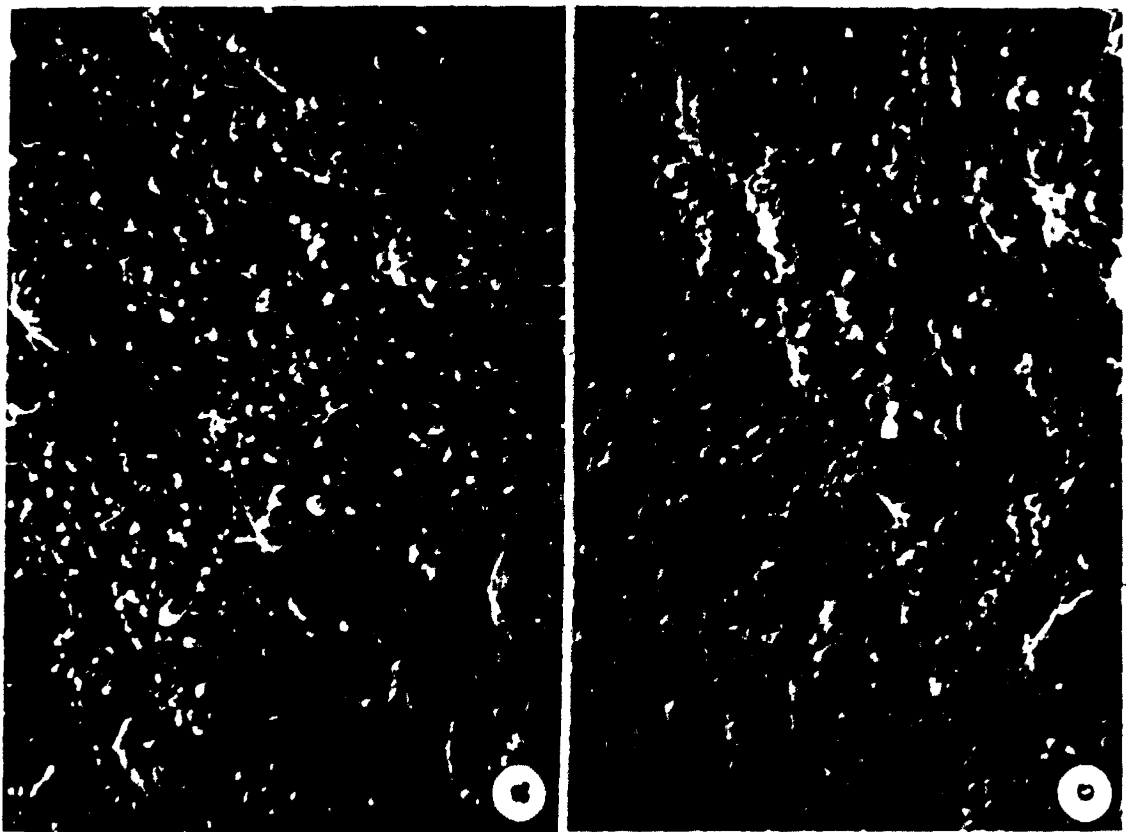


Plate 57

Diagram (top left) of the hemisected rabbit diencephalon illustrating the exposed surface of the third ventricle in midsagittal section as viewed with the SEM. The regions designated a-d of the ventro-lateral wall and floor of the ventricle correspond to those illustrated in the accompanying scanning electron micrographs. AC, anterior commissure; OCh, optic chiasma; MM, mammillary body; PC, posterior commissure; MTN, midline thoracic nuclei; a-c, infundibular recess; d, mammillary recess.

a, Saline control sacrificed 5 minutes after injection. The thick epon section is taken from the middle one-third of the IR (area b in the accompanying diagram). Note that the convex apical surface of tanycytes bulging conspicuously into the ventricle are provided primarily with bleb-like structures (arrowheads). Azure II - Methylene Blue. X 1500.

b, LH-RH treated rabbit which was sacrificed 5 minutes post-injection. The thick epon section is taken from the region of the ventricle designated b in the accompanying diagram. Note the fuzzy striated apical border (arrowheads) of the cells which corresponds with the delicate villiform processes seen at the SEM level. Azure II - Methylene Blue. X 1640.

c, An area similar to that shown in the two figures above from an LH-RH treated rabbit which was sacrificed 15 minutes after injection. The apical ependymal surface although irregular (arrowheads) is largely devoid of microvilli. It is more comparable to the appearance noted in saline controls. Azure II - Methylene Blue. X 1660.

Scott et al. (1974) described alterations of the ependymal surface in the rat IR 10 minutes following the ventricular infusion of dopamine. Schechter and Weiner (1972) have similarly reported changes within circumscribed regions of the ependymal surface of the rat third ventricle five minutes but not at 15 minutes after intraventricular administration of epinephrine and dopamine in amounts known to stimulate the secretion of releasing factors. Although the time at which morphological changes were observed following injection is comparable to that of the present study the nature of the changes, however, are not. We have been unable to confirm, for example, the eruption of increased numbers of bleb-like protrusions observed by these investigators on the ependymal surface of the floor of the ventricle five minutes after injection. Schechter and Weiner (1972) believed this response to most likely represent a secretory process in which fragments of ependymal cell cytoplasm were discharged into the CSF. The nature of the surface modifications observed in the present investigation, however, suggests that they represent transient differentiations which may signify increased absorptive activity and thus are thought to be more consistent functionally with absorption across the ependymal-CSF interface. Furthermore, the surface modifications noted by Schechter and Weiner (1972) in response to intraventricular administration of catecholamines were found to be restricted to the ependymal surface of the floor of the ventricle only; a region in which the ependymal surface was described, in control animals, as largely smooth surfaced with only infrequent surface eruptions. In areas characterized by a rather more complex surface morphology (i.e., the lateral recess and adjacent ventricular wall) marked changes in the ependymal surface were not observed. Our data, in contrast, indicates

a general or uniform responsiveness to LH-RH encompassing the ventrolateral wall and floor of the ventricle throughout its rostrocaudal extent.

At the present time, we do not have sufficient information to justify any definitive conclusions regarding the functional significance of the microvillus eruptions of the ependymal surface. It is worth noting, nevertheless, that the morphological changes observed in this investigation occurred at a time which corresponds closely to reported times at which similarly administered hypophysiotropins are detectable in the ME or effluent stalk blood (Weiner et al., 1972; Ben-Jonathan et al., 1974). The time course of TRH uptake by normal or cultured ME in vitro was found by Knigge (1973) to be both rapid and biphasic. Phase one equilibrium is reached within 10 minutes and phase II within 50-90 minutes. Similar results were obtained by Vaala and Knigge (1974) for the uptake of LH-RH by ME in vitro. More recently Kobayashi (1975) and Uemura et al. (1975) have demonstrated the presence of LH-RH within ependymal perikarya and their processes in the ME of the rat immediately following extended infusion into the third ventricle. Considered against this background of information, the transient eruption of microvilli revealed in our study is morphologically compatible with the suggestion that ependymal components of the ME may absorb LH-RH from the ventricular fluid, transporting it for release into the hypophysial-portal vasculature.

Several investigators have reported the selective concentration of a variety of compounds in the ME or in the ependymal cells of the ME: HRP injected into the ventricle or subarachnoid space (Kobayashi et al., 1972; Kobayashi, 1972; Léranth and Schiebler, 1974), corticotro-

phin, thyroxine and corticosterone intraventricularly administered (Kendall _et al._, 1972), TRH (Kendall _et al._, 1972; Joseph _et al._, 1973; Knigge _et al._, 1974), endogenous neurophysins (Robinson and Zimmerman, 1973), intravascularly administered thyroxine and triiodothyronine (Stumpf, 1973 cited in Brawer _et al._, 1974) and estradiol intramuscularly injected (Anand Kumar and Knowles, 1967; Anand Kumar and Thomas, 1968). It would appear unlikely therefore that any specificity exists with regard to the selective absorption and/or transport of LH-RH from the CSF. It seems rather more plausible that these cells may be found to have a non-specific affinity for, or responsiveness to, any substances that gain access to the ventricular fluid. The possibility therefore that the ependymal lining of the IR may be reshaped transiently in accordance with the introduction of a variety of non-specific agents in the CSF is a consideration that cannot be readily excluded.

5. SUMMARY AND CONCLUSIONS

In this study, regional differences in the morphology of the ependymal lining of the cerebral ventricles of normal female white New Zealand rabbits were systematically investigated with the light microscope. Attention was focussed primarily on the third ventricle and on the distribution and structural characteristics of tanycyte ependymal cells in particular. A total of 12 animals was used. Serial paraffin sections were cut from the brains of six of these rabbits and stained with chrome alum hematoxylin phloxine. An equal number of brains were prepared using the tungstate modification of Golgi-Cox method, celloidin embedded and sectioned serially. The results obtained from this study are summarized as follows:

1) The walls of the lateral and third ventricles are lined for the most part by a single continuous layer of ciliated cuboidal cells. This observation is in agreement with descriptions provided of ependyma in this location in a number of other species.

2) In Golgi-Cox preparations it was possible to identify two different forms (Types I and II) of ciliated cuboidal cells based on the structure of their basal or abluminal surface. Because of the capriciousness of the staining method we cannot be certain that perhaps all such ciliated-cuboidal cells have basal processes.

3) Tanycyte ependymal cells were rare in the lateral ventricles, but represented the main cell type on the ventrolateral

wall and floor of the third ventricle.

4) Tanycytes occurred sporadically amid the ciliated cuboidal ependymal cells of the lateral walls of the supraoptic recess of the third ventricle.

5) Tanycyte processes project into the subjacent neuropil for variable distances and commonly terminate either on vessel walls in association with neurons or glial cells or indeterminately.

6) Although dissimilarities among tanycytes were found, they could not be related regionally to any particular hypothalamic location nor could they be subclassified. These observations agree with those of Bleier (1971, 1972) but they fail to comply with those of either Millhouse (1971, 1975) or Sharp (1972).

7) Gomori-positive material was not observed in typical mural ependymal cells or tanycyte ependymal cells or their processes, in contrast to previous reports of selective and non-selective gomori-positive staining of ependyma.

8) With transmission electron microscopy, the ciliated cuboidal ependymal cells and the tanycytes can be distinguished as two fundamentally distinct types of cells lining the ventricles.

9) In the dorsolateral wall of the third ventricle, the ependymal cells were separated from the underlying neuropil by a zone of glial cells and processes. The basal cytoplasmic processes of cuboidal cells themselves were closely interwoven with the glial cell processes in this zone.

10) Tanycytes of the ventrolateral wall and floor of the third ventricle by contrast rest directly upon the underlying neuropil. Here the subependymal glial zone is lacking.

11) Like those of the rat third ventricle (Millhouse, 1972) tanycytes in the rabbit do not exhibit the cytological features of active secretory cells.

12) The relationship of tanycyte terminal processes to blood vessels along the ventrolateral wall and floor of the third ventricle is very much like that of perivascular astrocytes elsewhere along the ventricle. In view of this similarity and relative paucity of subependymal astroglia in this region it is suggested that tanycytes may merely assume the role of astrocytes in this region of the ventricle.

The surface characteristics of ependymal cells lining the cerebral ventricles of 12 rabbits, 6 rats, 6 mice, one rat kangaroo and 12 humans were examined with the scanning electron microscope. Attention was focussed on the third ventricle although for comparative purposes some samples of selected regions of the lateral ventricles were examined as well. The results of this investigation are summarized as follows:

1) With minor interspecies differences the ependyma of the third ventricle exhibits a consistent pattern of regional variation in surface morphology.

2) On the basis of this variation, the third ventricle can be divided into three distinct regions: dorsolateral wall, transitional zone and ventrolateral wall and floor (infundibular and mammillary recesses).

3) The largest expanse of the ventricular surface, the dorsolateral wall (or upper two-thirds) of the third ventricle is distinguished by the presence of a homogenous population of densely-ciliated cells.

4) The luminal surface of ependymal cells forming the ventrolateral wall and floor (or lower one-third) of the third ventricle is distinct in appearance from adjacent ventricular surfaces and is characteristically non-ciliated.

5) The transitional zone which is interposed between the ciliated dorsolateral wall and the non-ciliated ventrolateral wall of the third ventricle exhibits surface features common to both regions as one is gradually transformed into the other.

6) The region of the ventrolateral wall and floor of the third ventricle with its unique topography and apical membrane specializations corresponds to the region of greatest tanycyte concentration as determined in a previous study.

7) This regional variation in the structural organization of the ependymal surface is consistent with the view that morphologically different regions may possess different functional capacities. We are unable however to shed any new light on what these might be.

A number of previous investigators have reported changes in ependymal morphology in response to castration. In this investigation the third ventricles of 18 ovariectomized rabbits were studied with the SEM at various post-operative intervals. Laparotomized rabbits (N = 8) served as controls. The results were as follows:

1) No significant changes in ependymal morphology were found that could be directly ascribed to castration at any of the time periods studied. In this respect this study agrees with similar findings by Brawer (1971) and by Sharp (1972).

2) The morphological changes in ependyma that have been reported in the literature in response to castration appear to

fall within the boundaries of normal variation in morphology commonly observed within the ventrolateral walls and floor of the third ventricle. The sampling needed for TEM may either miss or over-emphasize the significance of variation in a given area.

The efficacy of synthetic LH-RH in inducing ovulation in the rabbit was determined for intraventricular and intravenous administration and compared. Thirteen conscious rabbits were given LH-RH in doses ranging from 0.1 to 6.0 µg/kg body weight as a single injection in the marginal ear vein. Twenty-two urethane anesthetized rabbits received doses of LH-RH from 0.016 to 3.0 µg/kg body weight by the same route of administration. Nine urethane anesthetized rabbits received LH-RH in doses from 0.05 to 0.4 µg/kg by injection into the lateral cerebral ventricle. Identically treated controls (N = 9) received vehicle (0.15 M NaCl) only. The results were as follows:

1) In conscious rabbits the dose of LH-RH necessary to induce ovulation in 100% of the animals when given intravenously was 300 ng/kg body weight. Two-thirds of this dose (200 ng/kg) was effective in 60% of the animals so injected. Similar values have been reported by Amoss et al. (1972) and Kanematsu et al. (1974) in rabbits of the same strain.

2) Our findings failed to verify a significant increase in the number of ova released with increasing doses of LH-RH as reported by Amoss et al. (1972).

3) The minimum effective dose of LH-RH which was required to induce ovulation in 100% of urethane anesthetized animals when administered intravenously was 400 ng/kg body weight. This was

one-third larger than the equivalent dose required in unanesthetized but otherwise identically treated rabbits.

4) Ovulation was neither delayed nor blocked by urethane anesthesia.

5) The minimum effective dose of LH-RH required to induce ovulation in 100% of animals when given intraventricularly was 100 ng/kg body weight. The intraventricular route may be as much as 4-5 times more effective than the intravenous route in this regard. The possible reasons for this are discussed. The evidence nevertheless indicates that LH-RH introduced into the ventricular system is clearly capable of eliciting the release of its appropriate pituitary hormones.

6) Observation of the movement of marker dye following intraventricular injection suggests that although substances given intraventricularly contact the ME and may reach the portal vasculature by traversing the ME, significant quantities may also reach the systemic circulation via the dural sinuses and the cerebral arterial circulation via the subarachnoid space.

7) Administration of vehicle alone in conscious and in urethane anesthetized rabbits by either intravenous or intraventricular routes was without effect.

Electron microscopic examination of the ventrolateral wall and floor of the third ventricle in both conscious and anesthetized rabbits which received LH-RH by either the intravenous or intraventricular routes of administration revealed that:

1) The ependymal surface of the IR of conscious and anesthetized rabbits that ovulated in response to LH-RH did not differ

significantly in morphology one from the other at the time of ovulation (13-16 hours after injection).

2) The morphology of the IR in those animals that ovulated was indistinguishable from that of the same region of the ventricle in identically treated rabbits that received either LH-RH or saline but failed to ovulate.

3) Essentially the same results were obtained regardless of whether the LH-RH was given intravenously or intraventricularly.

4) Morphological changes were not observed on the ependymal surface of the IR that could be correlated with ovulation in the rabbit.

To determine ependymal responsiveness to LH-RH, 12 female rabbits were used. A dose of LH-RH (200 ng/kg body weight) which was equal to twice the predetermined minimum effective ovulatory dose was given intraventricularly to 6 anesthetized rabbits. These animals were sacrificed 5 or 15 minutes after injection. An equal number of controls received the 0.15 M NaCl only and were sacrificed at the same time intervals. The results were as follows:

1) Pronounced changes in morphology of the ependymal surface of the ventrolateral wall and floor of the third ventricle were observed in rabbits that were sacrificed 5 minutes after injection of LH-RH.

2) The changes took the form of microvillouseruptions on the luminal surface of the ependymal cells.

3) Similar changes were not observed at 15 minutes after LH-RH injection or in controls which received isotonic saline only.

4) The nature of the changes suggested that they are transient

modifications of the surface which may signify an increased absorptive activity.

5) These observations provide some morphological basis for the hypothesis that ependymal components of the ventrolateral wall and floor of the third ventricle are implicated in the uptake and possibly the transfer of LH-RH to the portal vasculature. These observations however do not as yet allow definitive conclusions to be drawn regarding functional significance.

aspects of apical ependymal secretion were investigated by Rodriguez (1970a) in the toad SCO. Although ependymal cells were found to contain numerous electron-dense (200-400 nm) and pale (130-260 nm) granules concentrated in the apical and perinuclear regions, no evidence supporting either apocrine secretion or exocytosis was found. The author did feel, however, that the presence of two types of secretory granules indicated that the SCO may also be involved in secretion into the ventricle of some additional principle unrelated to Reissner's fibre. The presence of numerous microvilli projecting from the ependymal surface into the ventricle, and the large number of vesicles in the supranuclear cytoplasm indicated that the SCO may also have an absorptive function.

Despite numerous studies of this region of the ependyma, its functional significance is still largely unknown. Sargent (1900) maintained that the SCO and Reissner's fibre constituted a neural mechanism for the transmission of optic reflexes from the spinal cord to the optic tectum and vice versa. Some evidence has been advanced to suggest that the SCO may secrete aldosterone and serve as a volume receptor (Gilbert and Glaser, 1961). A role for the SCO in water-electrolyte metabolism is supported by the reduction in water intake and intense dehydration that has been demonstrated to ensue in the rat following electolytic coagulation of the SCO (Gilbert, 1956). His hypothesis was that the SCO secretes a hormone in response to osmotic pressure changes in the blood or CSF which acts on neural end organs to alter water consumption and accordingly the fluid composition of the body.

cat SCO examined with the scanning electron microscope, which they believed to represent a secretory product released by these cells into the CSF. Non-fenestrated capillaries are distinguished within the subependymal neuropil of the SCO. They are surrounded by a distinctive perivascular space enclosed by a vascular and parenchymal basement membrane (Schmidt and D'Agostino, 1966; Weindl and Joynt, 1972b; Mollgård, 1972). Basal processes of SCO ependymal cells terminate in a cuff around the external perivascular basement membrane of these subependymal capillaries. Some ependymal processes have also been reported to terminate on basement membrane lined cavities thought to be extensions of the perivascular space of these capillaries (Rodriguez, 1970a, b). Unlike either the organum vasculosum of the lamina terminalis or the area postrema, a blood-brain barrier was found to be present in the SCO (Gröntoft, 1954; Weindl and Joynt, 1971, 1972b). The permeability properties of the capillaries of the SCO following either intravenous or intraventricular injection of horseradish peroxidase were found to be the same as those of typical brain capillaries (Weindl and Joynt, 1971, 1972b)

A secretory function for the modified ependymal component of the SCO has been proposed by many investigators. Histochemical studies have demonstrated the presence of secretory granules within the ependymal cells of this organ that stain selectively with PAS and CAHP methods (Stutinsky, 1950; Wislocki and Leduc, 1952; Bargmann and Schiebler, 1952). The 'ependymosecretion' is considered to be a mucopolysaccharideprotein complex (Teichmann, 1967). There is some suggestion, however, that the SCO may produce more than one type of

secretory product. Three different Gomori-positive stainable granules were distinguished within the guinea pig SCO (Teichmann, 1967; Vigh, Rohlich, Teichmann and Aros, 1967a). Two types of granules, identified as a protein containing a small amount of polysaccharide and a protein bound mucopolysaccharide with possibly a lipid component, were localized within the cytoplasm of the ependymal cells and were believed to represent two different 'ependymosecretory' substances. The third variety of granule (a glycolipoprotein) was localized in the hypendymal glial cells of the organ and believed to be phagocytosed 'ependymosecretion' or developing lipofuscin. In the rabbit SCO, electron-dense secretory material was concentrated in the perinuclear and basal portions of the ependymal cells, and their basal cytoplasmic processes abutting on capillaries contained a second morphologically distinct variety of secretory granules (Schmidt and D'Agostino, 1966). Although this suggested that two secretory products may be elaborated, morphological evidence was presented only for basal secretion into the extracellular space, supporting an endocrine function for the SCO of the rabbit. The fate of the secretory material of the SCO is still a source of controversy. The localization of secretory material within the basal part of the ependymal cells and within their basal processes argues in favor of release into the vasculature and/or subarachnoid space (Rodriguez, 1970b). Alternatively, the preferential apical localization of secretory material within ependymal cells is interpreted as evidence for its secretion into the CSF where it is assumed to form Reissner's fibre, although there is not unequivocal evidence that it is in fact a secretory product of the SCO. Ultrastructural

aspects of apical ependymal secretion were investigated by Rodriguez (1970a) in the toad SCO. Although ependymal cells were found to contain numerous electron-dense (200-400 nm) and pale (130-260 nm) granules concentrated in the apical and perinuclear regions, no evidence supporting either apocrine secretion or exocytosis was found. The author did feel, however, that the presence of two types of secretory granules indicated that the SCO may also be involved in secretion into the ventricle of some additional principle unrelated to Reissner's fibre. The presence of numerous microvilli projecting from the ependymal surface into the ventricle, and the large number of vesicles in the supranuclear cytoplasm indicated that the SCO may also have an absorptive function.

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The presence of sulphur rich protein has been noted in the ependyma of the SCO (Talanti, 1958) and is also believed to be a part of the secretory product of the organ as well (Talanti, 1971). Incorporation of ^{35}S-labelled cysteine in the ependyma of the SCO has been reported (Sloper, Arnott and King, 1960; Sterba, Ermisch, Freyer and Hartmann, 1967; Leatherland and Dodd, 1968). A change in thyroid function in rats treated with thiouracil or excess thyroxine is accompanied by a change in the incorporation of ^{35}S-labelled cysteine in the SCO (Talanti and Pasanen, 1968). Following thyroidectomy, intense incorporation of both ^{35}S-labelled cysteine and methionine occurs in the ependyma of the rat SCO (Talanti, 1971). Enzyme histochemical investigations of the SCO indicate the presence of glycoproteins and abundant glycogen in the ependyma and the hypendyma (Shimizu, 1955; Mollgard, 1972). The surface coat covering the ependyma was considered to be a sialic acid containing glycoprotein (Møllgård, 1972) similar to that found in Reissner's fibre (Sterba and Wolf, 1969). The presence of non-specific esterase, acid and alkaline phosphatase enzymes and their suspected association with secretion granule formation and active transport across cell membranes suggested that the activity of the human fetal SCO is related to an exchange of neurohormones from the SCO to the blood in addition to an absorptive and secretory function carried out between the CSF and the SCO (Møllgård, 1972).

ii) Organum Vasculosum of the Lamina Terminalis

Another area of specialized ependyma, the organum vasculosum of the lamina terminalis (OVLT), is found at the rostral end of the

third ventricle in the mid-sagittal plane between the anterior commissure and the optic chiasma. The OVLT (also referred to as the supraoptic crest or the medial vascular prechiasmatic gland) is a thin richly vascularized septum situated between the prechiasmatic cistern and the preoptic recess of the third ventricle. Although all circumventricular organs (CVO) are generally well vascularized, a unique angioarchitectonic pattern of vascularization, as in the median eminence, is distinguished in the OVLT (Weindl, 1965; Weindl and Joynt, 1972a). Loops of its external capillary network are closely related to the subarachnoid space and the internal (subependymal) capillary network are separated from the ventricular CSF only by the layer of ependymal cells and their processes. Duvernoy, Koritké and Monnier (1969) identified an arterial portal system in the human OVLT. However, significant data supporting either a portal relationship or anastomoses with the vessels of the adenohypohpysis has not yet been provided.

The capillaries of the OVLT have the same fine structural features as those of the median eminence, neural lobe, pineal gland and area postrema (Weindl and Joynt, 1972a). They were found to have fenestrated endothelium surrounded by wide perivascular spaces (Weindl, Schwink and Wetzstein, 1967; Weindl and Joynt, 1972a). The permeability properties of the capillaries of the CVO have recently been reviewed by Weindl and Joynt (1972a). Following intravenous injection of HRP, reaction product was found in the area postrema, ME and OVLT but not in typical brain tissue (Weindl et. al., 1967; Weindl, 1969; Weindl and Joynt, 1972a, b). In contrast, when peroxidase or labelled amino acids of much smaller molecular weight are

injected intraventricularly, penetration of the OVLT like the ME does not occur, however, they readily enter the neuropil underlying neighbouring ciliated ependyma (Weindl and Joynt, 1969, 1972a, b). Usui (1968) reported the presence of both zonula occludens and adherens near the apices of adjacent ependymal cells in the rat OVLT. However, unlike ependyma elsewhere in the rat brain (Brightman and Palay, 1963), zonula occludens were frequently encountered as the first luminal junction in the OVLT.

Recent SEM investigations of the OVLT in a variety of species reveal that the pleomorphic ependymal cells of the organ, unlike major portions of the ventricular wall, are generally non-ciliated. Small irregular protrusions of the plasmalemma, however, commonly project from the ependymal surface. Supraependymally lying cells with branching processes closely resembling small neurons were also occasionally evident (Weindl and Joynt, 1972a, b; Scott et al., 1973, 1974b). In the rat, two distinct types of ependymal protrusions, a fin-like thin fold of the apical cytoplasm akin to a marginal fold and a bulbous protrusion of irregular size and shape presumed to be a transient formation indicative of increased surface activity, were distinguished on the surface of these cells (Usui, 1968). The close association of vesicles and pits or caveolae with these structures and with the apical cytoplasm was interpreted by this investigator as indicative of a potential absorptive function for this organ. Weindl and Joynt (1972a) however, reported that a homogenous granular material is discharged from the apical surface of certain ependymal cells of the rabbit OVLT into the ventricular fluid.

Ultrastructural investigations of the OVLT have also

revealed that a number of ependymal cells have single stout basal processes which extend to the peripheral basal lamina of the perivascular space and/or pia mater. A predominence of these tanycyte endfeet have been observed over terminals of neurosecretory processes within the OVLT—a relationship of unknown significance (Weindl and Joynt, 1972a). A variety of dense core vesicles are commonly observed in the perikarya and axon terminals of neurons within the parenchyma of the OVLT (Weindl et. al., 1967; Rohlich and Wenger, 1969; Weindl and Joynt, 1972a). The contents of these DCV, presumed to be neurosecretory material or humoral controlling factors, are thought to be released into the capillaries of the organ as in the ME. Since only a moderate number of neurosecretory cells could be found in the OVLT of the cat and monkey, Weindl (1969) and Weindl and Joynt (1972a, b) favored a hemoneural transport and a central receptive function for the organ. Similar unmyelinated nerve endings containing DCV abutting on pericapillary spaces were described in the rat medial vascular prechiasmatic gland (Le Beux, 1972), however, their content was presumed to be monoamines although the possibility that they contained peptide hormones wasn't ruled out. A few of these processes were found to course between ependymal cells enlarging in bulb or knob-like endings in the preoptic recess of the ventricle. Whether these free endings function as receptors, reacting to changes in CSF composition or whether they actively secrete into the ventricle in a hemostatic or endocrine capacity is uncertain.

A similar though presumably unrelated area of catecholamine containing neurons in contact with the CSF has been distinguished in

the roof and walls of the preoptic recess of amphibians (Vigh-Teichmann, 1968; Vigh-Teichmann, Vigh and Aros, 1969a; Vigh-Teichmann, Rohlich and Vigh, 1969b; McKenna and Rosenbluth, 1971). Although it has been designated the 'organon recessus preopticus' (periventricular organ), it likely corresponds to the subependymal periventricular component of the preoptic nucleus (McKenna and Rosenbluth, 1971; Knowles, 1972).

Secretory characteristics of specialized ependymal cells in the medial basal supraoptic and/or prechiasmatic recess of the rat and mouse have also been described (Lévêque, Stutinsky, Porte and Stoeckel, 1967). The ependymal cells of this region, like those of a similar region distinguished in the IR, contain PAS-positive material unrelated to CAHP and aldehyde fuchsin-positive neurosecretory material (Lévêque and Hofkin, 1961, 1962). Their distinctive morphology, their arrangement in a discrete area and their association with a fenestrated vasculature was suggestive of a glandular formation with synthetic activity for which the authors tentatively proposed the name 'medial prechiasmatic gland'. Although the ependymal cells of the zone are reported to be associated with the capillary network of the OVLT, it appears that a structure distinct from, though possibly related to, the OVLT may be intended. Recently, cytological changes in the ependymal cells of this zone have been examined in the rat under conditions in which the secretory activity of LH was either enhanced or inhibited (Lévêque, 1972). The results indicate that in the androgen sterilized rat, the quantity of PAS stainable substance accumulated during the first 15-20 days of age is

much greater than in controls. The peak attained at the 20th day is followed by a precipitous drop in quantity of substance until puberty unlike the plateau established in controls. In light of these results, it was suggested that ependymal cells of the medial basal preoptic area may be responsible for synthesis of LH-RH. Weindl and Joynt (1972a) have indicated that the location of the OVLT in the medial preoptic area, a region generally associated with releasing hormone synthesis and release from the ME suggests a functional interaction with the ME.

LH-RH has been found in significant quantity in the OVLT using immunocytochemical techniques (Zimmerman et al., 1974; Barry et al., 1974) although the cellular elements containing LH-RH within the OVLT are unknown. Kizer, Palkovits and Brownstein (1976) confirmed that the OVLT from the rat brain contained significant quantities of LH-RH. The concentration of LH-RH in the OVLT was 58% of that found in the ME. In addition TRH was found in the OVLT and LH-RH and TRH were found in the SFO, AP and SCO suggesting that releasing factors may be present in the CVO as a general phenomenon. No change in the levels of LH-RH was found in the OVLT of either male or female rats following surgical isolation of the hypothalamus (Brownstein, Palkovits and Kizer, 1975). Thus LH-RH in the OVLT likely does not originate from axons arising from cell bodies elsewhere in the hypothalamus. Palkovits, Brownstein, Arimura, Sato, Schally and Kizer (1976) have recently reported that all CVO in the rat had measurable amounts of somatostatin. The highest amounts were found in the OVLT and the lowest were found in the SFO although concentrations were only 1/4-1/5 of the concentration measured in the arcuate nucleus. The possible significance of this

however, was not given.

iii) Paraventricular Organ

The specialized ependymal region referred to as the "paraventricular organ" (PVO) by Roussy and Mosinger (1938) was first described as a glandular ependymal organ situated bilaterally in the lateral walls of the third cerebral ventricle of fish, reptiles, birds and some mammals (Ariens Kappers, Huber and Crosby, 1920/21). It was distinguished morphologically by the presence of elongated non-ciliated ependymal cells, a richly vascularized subependymal region and the presence of an albuminous substance believed to be of ependymal origin within the ventricle overlying the organ. Its presence along the upper margin of the IR has subsequently been demonstrated in a variety of vertebrate species (Charlton, 1925, 1928; Legait and Legait, 1956; Fleischhauer, 1957). However, its precise boundaries and its vascularization vary. Although the function of the PVO is unknown, on the basis of its morphology, a secretory function has been suggested. In recent studies, the PVO has been variously designated as the organon vasculosum hypothalami (Fleischhauer, 1957; Braak, 1968; Braak and Hehn, 1969) and "area vasculosum ependymalis hypothalami" (Hofer, 1958). In a comparative study of the PVO in 18 different vertebrates from fish to mammals Vigh, Aros, Zarand, Tork and Wenger (1962) revealed that although somewhat variable in structure, it characteristically consisted of an ependymal and hypendymal portion. The ependymal component consisted of a radial arrangement of multiple rows of elongated ciliated cells with basally situated nuclei. The hypendymal component was comprised of the basal processes of the ependymal cells, glial cells and numerous capillaries. Gomori-positive granules within the ependyma and an albuminous

Gomori-negative coagulate in the ventricle overlying the PVO were a consistent finding in each species examined. The authors inferred from these observations that the ependymal cells produced two types of secretion ("ependymosecretion") which in all probability were discharged into the ventricle. In amphibians some neurosecretory neuronal processes of the preoptic hypothalamic nucleus extend to the lumen of the third ventricle within the paraventricular organ (Vigh, 1967). Dehydration of these amphibia was followed by an increase in Gomori-positive material elaborated by these neurosecretory fibres in the PVO and in the ventricular lumen. Although dendrites of preoptic neurosecretory cells containing elementary granules and which extend through the ependyma into the third ventricle have similarly been described in Zoarces viviparous L. (Öztan, 1967), Hyla regilla (Smoller, 1965) and Rana temporaria (Dierickx, 1962), no anatomical relationship with the PVO, however, was indicated. A group of neurons referred to as the "nucleus organi paraventricularis," distinct from either the ependymal or neurosecretory neuronal components of the PVO, have also been distinguished (Vigh, 1967; Vigh, Teichmann and Aros, 1967b). Processes of these neurons also protrude as bulb-like endings between the ependymal cells of the organ into the ventricular lumen. As in the "organon recessus preopticus," catecholamines and monoamines have been demonstrated in these neurons (Röhlich and Vigh, 1967; Teichmann, Vigh and Aros, 1968). It is suggested, therefore, that the PVO possesses a double innervation and that the relationship between the "nucleus organi paraventricularis" and the ependyma indicates a sensory function for the organ (Vigh, 1967). The presence of a neuronal component in the PVO distinguishes it from a simple ependymal differentiation, such as

the subcommissural organ. The PVO of *Xenopus Laevis* tadpoles is characterized by an ependymal lining with a sparcity of cilia and two distinct types of nerve cells (Peute, 1969). Both types of neurons, which are distinguishable primarily on the basis of their vesicle content, have processes protruding into the lumen of the ventricle. Type I neurons, found deep in the hypendymal region contain typical dense-cored catecholamine containing vesicles with a diameter of 800-1100 Å. The variety designated type II, located in the immediate vicinity of the ependyma, contain larger (1350 Å) vesicles resembling elementary granules of neurosecretory cells. Although a secretory function into the CSF is suggested for both cell types, Peute (1969) postulated that the presence of unusual cilia (8 + 1 and 9 + 1 type) and the absence of synaptic contacts on type I neurons qualified them for a possible sensory function. Similar monoamine containing neurons have been reported in the organon vasculosum hypothalami of *Lacerta viridis* (Braak, 1968), *Rana Temporaria* (Braak and Hehn, 1969) and *Xenopus Laevis* tadpoles (Goos and Van Halewijin, 1968).

The observations of Takeichi (1967) indicate that the paraventricular ependymal organ in the turtle is of comparable structure. Multiple rows of columnar ependymal cells possessing bulbous cytoplasmic expansions with many microvilli and a few cilia at the free surface were in evidence. No evidence, however, of secretory or absorptive activity could be found in these cells except for very small vesicles dispersed within the cytoplasm. The so-called albuminous substance of earlier LM reports was not considered by this author to be an apocrine secretion at all, but rather corresponded to the terminal club-like expansions within the ventricle of hypendymal neuronal processes. Their terminal

expansions contained two distinct types of vesicles, the clear synaptic type vesicle (500-1600 Å diam.) and the cored vesicle (elementary granule) measuring 600-1500 Å in diameter. Although only one type of neuron containing two kinds of vesicle was described, they could be divided into two groups according to the quantity of the respective vesicle type dispersed within them. Formation of these vesicles occurred within the terminals of the neuronal processes, and their contents, presumably neurosecretory material, was discharged into the CSF by a microapocrine secretion.

In the sparrow, the PVO is well developed as it is in birds in general (Röhlich and Vigh, 1967). It is characterized by well developed ependyma; tall, cylindric, devoid of cilia with many microvilli and basal processes that pierce the underlying neural and glial feltwork ending by pedicle-like enlargements on capillaries. As in the turtle (Takeichi, 1967), the fine structure of the ependyma does not provide any morphological evidence that would suggest that it plays a specific and important role in the function of the PVO. Between the discontinuous ependymal cell layer, "light cells" of indeterminant neuronal or glial origin and ventricular processes of basally situated neurons are interposed. The apical part of the "light cells" protrude from the ependymal surface into the ventricular cavity frequently with club-shaped endings similar to those of neurons. A slender basal process was also seen to emerge from those cells. The most specific component of the PVO in the sparrow, however, is the two types of nerve cells which have also been identified. Their correspondence to similar elements described above is apparent. The more superficial cells (type I) have a dendrite-like process which terminates within the ventricle in

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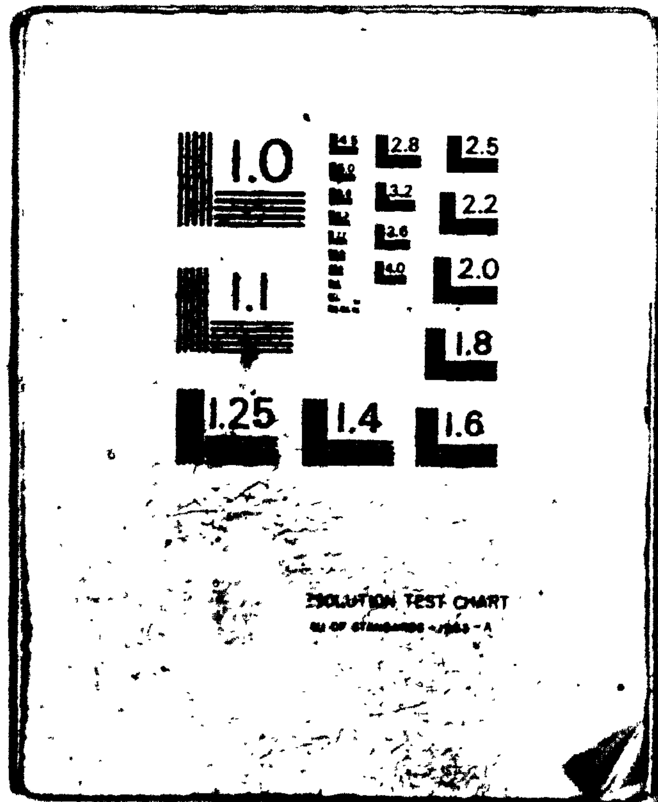

a bulbous swelling. Within the whole neuron monoamine containing dense-core vesicles of 840 Å average diameter were found. Large type II neurons are situated in deeper layers and rarely contain dense-core vesicles. An effector or receptor function for these neuron terminals could not be ascertained with certainty. Morphological evidence suggestive of possible PVO involvement in pituitary pars intermedia function is recently reviewed by Knowles (1972).

iv) Subfornical Organ

The subfornical organ or intercolumnar tubercle is a small hemispherical nodule protruding from the rostrodorsal wall of the third ventricle. It is a midline structure situated above the anterior commissure in the region of the interventricular foramen between the diverging columns of the fornix. Its presence has been demonstrated in a wide variety of mammalian, avian and reptilian species as well as in some amphibians (Akert, Potter and Anderson, 1961; Kohno and Usui, 1966; Weindl and Joynt, 1972b; Leonhardt and Lindemann, 1973; Phillips, Balhorn, Leavitt and Hoffman, 1974; Weindle and Schinko, 1977). The SFO is composed of a body, a dorsal stalk to which the choroid plexus is attached and a ventral stalk which ends near the anterior commissure (Akert *et al.*, 1961; Kohno and Usui, 1966; Phillips *et al.*, 1974). It is known to share certain characteristics with the other CVO's such as a rich vascularity, location outside the blood-brain barrier and contact with the CSF (Dellmann and Simpson, 1975).

Leonhardt and Lindemann (1973) and Dellmann and Simpson (1975) have described the presence of ependymal tanycytes in the SFO. Ependymal channels lined by ciliated ependymal cells penetrated the SFO to varying

extents (Dellmann and Simpson, 1975). Such channels were not found, however, in either the rabbit (Leonhardt and Lindemann, 1973) or the rat (Phillips et al., 1974).

Nerve cell processes containing giant vacuoles constituted one of the most remarkable features of the SFO in the rabbit (Leonhardt and Lindemann, 1973); the colloidal contents of these vacuoles were believed to be secreted into the ventricle. No evidence of this was noted in the rat (Phillips et al., 1974) and the idea was not favoured by Dellmann and Simpson (1975) even though giant vacuolated nerve cells were found by them.

Dense networks of sinusoidal capillaries both fenestrated and non-fenestrated have been reported in the SFO (Dellmann and Simpson, 1975). Spoerri (1963) drew attention to the link between the sinusoidal capillaries and glomerular loops of the SFO and the vasculature of the choroid plexus. A vasular connection of the SFO and the supraoptic crest was also suggested. Duvernoy and Koritke (1969) emphasized the relationship between the capillary plexus of the SFO and the ventricular cavity, noting their conspicuous lack of connections with neighbouring regions. The similarity of the vasculature of the SFO and the AP has been alluded to by Koella and Sutin (1967) and Duvernoy and Koritke (1969).

Dellmann and Simpson (1975) have found typical cholinergic and aminergic axonal terminals in the SFO but rarely have observed peptidergic (neurosecretory) axons. The presence of a cholinergic plexus within the SFO was documented by Akert (1969). The SFO like the OVLT and AP has been shown to be rich in biogenic amines and their related enzymes (Saavedra et al., 1976).

A variety of functions have been ascribed to the SFO. It has been speculated that the SFO may have an absorptive function, a sensory or a neurosecretory function (for review see Kaella and Sutin, 1967). Recent evidence has implicated the SFO with the possible regulation of drinking behaviour and body fluid regulation. Simpson and Routtenberg (1973) called attention to the possibility that the central dipsogenic receptors for one of the known physiological stimuli of drinking behaviour (Angiotensin II) may reside within the SFO. Their results showed that Angiotensin II applied directly to the SFO elicited short-latency drinking in water sated rats while ablation of the body of the SFO blocked drinking induced by Angiotensin II.

Kucharczyk, Assaf and Mogenson (1976) contend that the SFO represents one of multiple central receptor sites for the dipsogenic effect of Angiotensin II. This view, however, is not supported by Hoffman and Phillips (1976). The investigations of Thornborough and Passo (1973) have implicated the CVO of the third ventricle and particularly the SFO in the regulation of sodium metabolism. Lesions of the SFO were found to block the natriuresis that occurs following infusion of hypertonic NaCl. They postulated that the SFO may function as a receptor for sodium ion concentration in blood and CSF. It is obvious that the precise function of the SFO remains to be elucidated.

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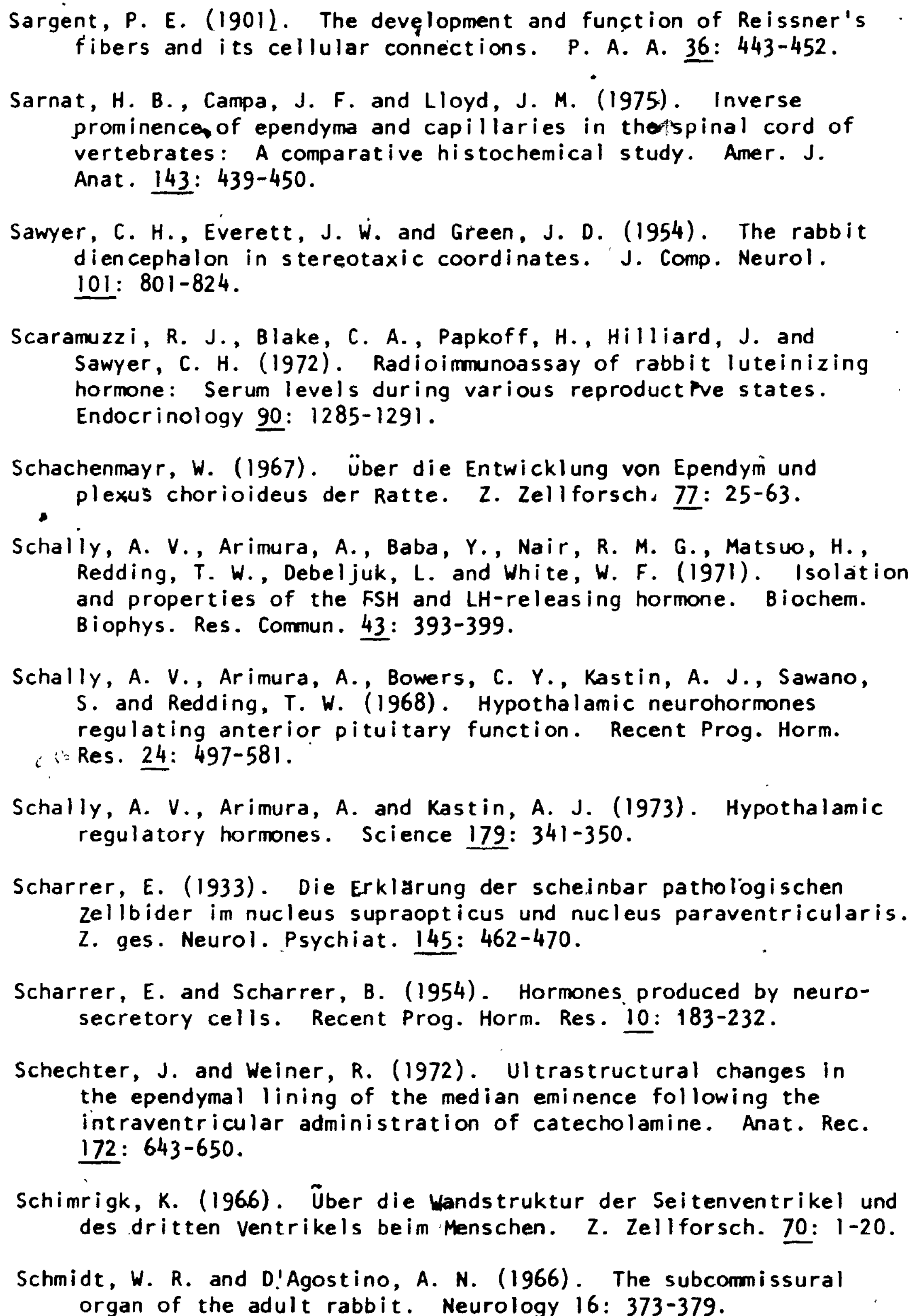

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ADDENDUM

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Abstracts

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Invited Lectures

(1) Scanning and transmission electron microscopy of the ependymal lining of the third ventricle. Delivered at the Cdn. Assoc. Anat. Symp., Functional Morphology of the Hypothalamus, CFBS Annual Meeting, University of Saskatchewan, Saskatoon, June, 1973.