The Immunocytochemical Localization of Taurine in Developing and Adult Rabbit Retina and Optic Nerve

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July, 1993

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree Master of Science.

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Commerce Business	0505
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History	0509
Labor	0510
Theory	0511
Folklore	0358
Geography	0366
Gerontology	0351
History	
General	0578
Ancient	0579
Medieval	0581
Modern	0582
Black	0328
African	0331
Asia, Australia and Oceania	0332
Canadian	0334
European	0335
Latin American	0336
Middle Eastern	0333
United States	0337
History of Science	0585
Law	0398
Political Science	
General	0615
International Law and Relations	0616
Public Administration	0617
Recreation	0814
Social Work	0452
Sociology	
General	0626
Criminology and Penology	0627
Demography	0938
Ethnic and Racial Studies	0631
Individual and Family Studies	0628
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Theory and Methods	0344
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Food Science and Technology	0359
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Plant Culture	0479
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Plant Physiology	0817
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Biology	
General	0306
Anatomy	0287
Biostatistics	0308
Botany	0309
Cell	0379
Ecology	0329
Entomology	0353
Genetics	0369
Limnology	0793
Microbiology	0410
Molecular	0307
Neuroscience	0317
Oceanography	0416
Physiology	0433
Radiation	0821
Veterinary Science	0778
Zoology	0472
Biophysics	
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Medical	0760

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Physical Geography	0368
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Nutrition	0570
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Pharmacology	0419
Pharmacy	0572
Physical Therapy	0382
Public Health	0573
Radiology	0574
Recreation	0575
Speech Pathology	0460
Toxicology	0383
Home Economics	0386

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General	0485
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Biochemistry	0487
Inorganic	0488
Nuclear	0738
Organic	0490
Pharmaceutical	0491
Physical	0494
Polymer	0495
Radiation	0754
Mathematics	0405
Physics	
General	0605
Acoustics	0986
Astronomy and Astrophysics	0606
Atmospheric Science	0608
Atomic	0748
Electronics and Electricity	0607
Elementary Particles and High Energy	0798
Fluid and Plasma	0759
Molecular	0609
Nuclear	0610
Optics	0752
Radiation	0756
Solid State	0611
Statistics	0463

Applied Sciences

Applied Mechanics	0346
Computer Science	0984
Engineering	
General	0537
Aerospace	0538
Agricultural	0539
Automotive	0540
Biomedical	0541
Chemical	0542
Civil	0543
Electronics and Electrical	0544
Heat and Thermodynamics	0348
Hydraulic	0545
Industrial	0546
Marine	0547
Materials Science	0794
Mechanical	0548
Metallurgy	0743
Mining	0551
Nuclear	0552
Packaging	0549
Petroleum	0765
Sanitary and Municipal	0554
System Science	0790
Geotechnology	0428
Operations Research	0796
Plastics Technology	0795
Textile Technology	0994

PSYCHOLOGY

General	0621
Behavioral	0384
Clinical	0622
Developmental	0620
Experimental	0623
Industrial	0624
Personality	0625
Physiological	0989
Psychobiology	0349
Psychometrics	0632
Social	0451

Taurine in Developing and Adult Rabbit Retina and Optic Nerve

ABSTRACT

The retina of a newborn rabbit is not fully mature in terms of morphology, chemical composition or capabilities. Taurine is the predominant component of the free amino acid pool of vertebrate retinas. In the rabbit it is present at birth and increases four-fold by maturity reaching levels close to 50mM in the adult. However, until the present studies its cellular localization was unknown. This study confirmed the postnatal (PN) increase in retinal taurine levels and examined PN changes in the immunocytochemical localization of retinal and optic nerve taurine. I have localized taurine immunoreactivity (taurine-IR) of developing and adult retinas and optic nerves using a highly specific antibody that was developed in our laboratory.

In the immature retina taurine-IR expression was associated with the onset differentiation of neuroblasts which showed a sequential order of development from central to peripheral, and inner to outer retina. The precocious development of horizontal cells was matched with early taurine-IR. In some cell types taurine-IR was a transient phenomenon while others retained taurine-IR to adulthood. Taurine-IR in the adult rabbit retina was localized to (i) photoreceptor cell outer segments, the myoid region of their inner segments and their synaptic terminals, (ii) a subset of bipolar and horizontal cells, and (iii) a small sub-population of amacrine cells. Several synaptic lamina of the inner plexiform layer (containing bipolar and amacrine terminals) were also taurine-IR. One striking localization of taurine-IR was in ganglion cell axons lying within the retina, during the first PN week. When I examined the developing optic nerve, the bundles of small axons had high levels of taurine-IR, while the large intervening glial cell bodies were negative or only weakly stained. In contrast, in adult optic nerve, taurine-IR was most prominent in glia.

My localization results indicate that the PN quadrupling of taurine content is due not only to photoreceptor cell development but also to the transient and/or stable expression of taurine-IR in other cell types as they differentiate. Particularly striking is the localization in horizontal cells where it is a candidate for a trophic factor in early PN synaptic organization, and in adult retinas as the yet unidentified inhibitory transmitter. The transient localization of taurine-IR in ganglion cell axons within the retina and optic nerve corresponds in timing to the "critical period" for the formation of retinogeniculate connections.

RESUME

La rétine d'un lapin nouveau-né n'est pas complètement mature quant à sa morphologie, sa composition chimique ou de ses possibilités. La taurine est chez les vertébrés la composante majeure des acides aminées libres de la rétine. Chez le lapin, elle est présente dés la naissance et augmente jusqu'à quatre fois sa quantité initiale pendant la maturation, atteignant des niveaux de près de 50 mM chez l'adulte. Cependant, jusqu'ici, son site cellulaire était inconnu. La présente étude confirme l'augmentation post-natale (PN) de la taurine rétinienne et examine les changements PN dans la localisation immunocytochimique de la taurine au niveau de la rétine et du nerf optique durant leurs dévélopements en utilisant un anticorps fortement spécifique mis au point dans notre laboratoire.

Dans la rétine immature, l'expression de l'IR-taurine était associée avec le début de la différentiation des neuroblastes qui montrent une sequence de dévélopement débutant de la région centrale vers la périphérie et de la rétine interne vers la rétine externe. Le dévélopement précoce des cellules horizontales coincide avec la première présence de l'IR-taurine. Dans certains type de cellules, l'IR-taurine se manifeste d'une façon transitoire alors que d'autres types de cellules retiennent l'IR-taurine jusqu'à la maturité. L'IR-taurine a été localisée dans la rétine du lapin adulte aux endroits suivants : (i) dans les segments externes des photorecepteurs, dans la région myoidale des segments internes et leurs terminaux synaptiques, (ii) dans un sous-ensemble de cellules bipolaires et horizontales et (iii) dans une petite sous-population de cellules amacrines. Plusieurs couche synaptiques de la couche plexiforme interne (comprenant des terminaisons bipolaires et amacrines) démontraient aussi de l'IR-taurine. L'observation la plus remarquable est sans aucun doute la présence d'IR-taurine dans les axones des cellules ganglionnaires de la rétine interne durant la première semaine PN. Lorsque j'ai examiné le nerf optique en dévélopement, un groupe de petits axones a demontré une forte quantité d'IR-taurine tandis que de larges cellules gliales adjacentes avaient une coloration faible ou négative. En comparaison, dans le nerf optique adulte, l'IR-taurine était plus proéminente dans les cellules gliales.

Mes résultats sur la localisation immunocytochimique de la taurine indique que le quadruplage de sa quantité PN est due non seulement au dévélopement des photorecepteurs mais aussi à sa présence de nature transitoire ou permanente dans certains types de cellules lors de leur différentiation. Toutefois ce qui est particulièrement remarquable est sa

présence dans les cellules horizontales où la taurine est candidate pour un rôle de facteur trophique au début de l'organisation synaptique PN et, dans les rétines adultes, comme transmetteur inhibiteur, dont la modalité n'a pas encore été identifiée. La présence transitoire de l'IR-taurine dans les axones des cellules ganglionnaires de la rétine interne et du nerf optique correspond chronologiquement à la "période critique" de formation des connections rétino-géniculées.

ACKNOWLEDGEMENTS

I would like to thank Dr. Norma Lake for her constant guidance and support. I would also like to thank Carole Verdone-Smith and Luisa de Marte for their technical assistance. As well, I would like to thank Carole Verdone-Smith and Dr. Pierre Lachapelle for the french translation of the abstract. Finally I would like to thank my husband Gino for his unrelenting love and support.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

ABBREVIATIONS

A	amacrine cell
ABTS	2,2-azino-di-3-ethylbenzthiazolinsulfonate
ß	beta
Bp	bipolar cell
bv	blood vessel
BSA	bovine serum albumin
CNS	central nervous system
CSA	cysteine sulfinic acid
CSAD	cysteine sulfinic acid decarboxylase
℃	degrees celsius
DNA	deoxyribonucleic acid
EM	electron microscopy
EM-ICC	electron microscopy immunocytochemistry
ERG	electroretinography
e	ellipsoid
E	embryonic
ELISA	enzyme-linked immunosorbent assays
GABA	gamma aminobutyric acid
G	ganglion cell
GCL	ganglion cell layer
G	glutaraldehyde
GAR-FITC	goat anti-rabbit fluorescein isothiocyanate
GAR-HRP	goat anti-rabbit immunoglobulin coupled to horseradish peroxidase
HPLC	high-pressure liquid chromatography
H	horizontal cell
INL	inner nuclear layer
IPL	inner plexiform layer
IS	inner segments
kg	kilogram
LGN	lateral geniculate nucleus

L	L-isomer
$MgCl_2$	magnesium chloride
µg	microgram
µm	micron
mg	milligram
ml	milliliter
mm	millimeter
mM	millimolar
M	molar
m	myoid
nm	nanometer
NFL	nerve fiber layer
OLM	outer limiting membrane
ONL	outer nuclear layer
OPL	outer plexiform layer
OS	outer segments
PAP	peroxidase anti-peroxidase
PBS	phosphate buffered saline
PR	photoreceptor
PE	pigment epithelium
PN	postnatal
SC	superior colliculus
Taurine-IR	taurine immunoreactivity

I. INTRODUCTION

A) Physical Characteristics of Taurine

Taurine is a metabolically inert sulfur-containing amino acid with a molecular weight of 125 daltons. At physiological pH, it exists as a zwitterion which gives it high water solubility and low lipophilicity, making it difficult for taurine to diffuse through membranes (Huxtable, 1992). It is not present in cells as a substrate for energy production or peptide synthesis since it does not contain the required carboxylic acid group, but has a sulfonic acid group instead. Taurine exists in the free form in the cytoplasm (Hayes, 1976 ; Gaull, 1989) and is not evenly distributed throughout all tissues but is particularly concentrated in millimolar amounts in excitable tissues such as skeletal muscle, myocardium, brain and retina, whereas intracellular or plasma levels are in the low micromolar range (Jacobsen and Smith, 1968).

B) Biochemical Functions of Taurine

One of the well documented biochemical functions of taurine in man is in the liver where it conjugates bile acids, to form taurocholic acid, which is important in lipid and fatty acid absorption. Bile acids conjugated with taurine are more effective than glycine conjugates in aiding intestinal absorption and metabolism of lipids. The excretion of bile acids conjugated with taurine constitutes an important means of removing cholesterol from the body (Jacobsen, 1980).

Recently taurine has been shown in vitro to inhibit the enzyme methyltransferase that is involved in the metabolism of phosphatidylethanolamine to phosphatidylcholine (Hamaguchi et al, 1991). This would regulate the phospholipid composition of membranes if it occurred in vivo, but that has not yet been directly demonstrated. However Huxtable et al (1989) have shown that there is a tight correlation between the taurine content and the phosphatidylethanolamine : phosphatidylcholine ratio of brain synaptosomes (isolated nerve terminals) during the normal course of postnatal development.

C) Actions of Taurine at the Cellular Level

Cell volume regulation in response to changes in osmolality is an important biological function in all animal species. In mammals, brain osmoprotection is designed to maintain cerebral cell volume constant (and hence ion gradients and excitability) when disturbances in serum tonicity occur. Taurine has been shown to act as an osmoprotective molecule in the cat and mouse ie. it moves to preserve cell volume, and is important in the defense against brain dehydration during chronic hypernatremia (Trachtman et al, 1988). Chronic hypernatremic dehydration induced in developing mice by water deprivation and salt loading for four days, increased sixteen of the nineteen amino acids measured in the brain. Taurine accounted for over one half of the total increase. This would maintain osmotic equilibrium and limit the loss of cell water (Thurston et al, 1980). This phenomenon may also be causally related to the cerebral edema that develops during too rapid rehydration of infants and children with chronic hypernatremic dehydration.

There is abundant evidence that cytoplasmic taurine modulates the entry of calcium into the cell. There seems to be a three way interaction of taurine, calcium and membrane phospholipid components. Zwitterionic taurine is analogous to the charged head groups of the neutral phospholipids, phosphatidylcholine and phosphatidylethanolamine. It binds to the neutral phospholipids in both biological proteolipid and artificial phospholipid membranes via a low affinity process. This results in the alteration of the high affinity binding of calcium to acidic phospholipids, and the binding of taurine modifies calcium binding sites so as to reduce the number of sites (some calcium binding sites are masked due to the conformational changes induced by membrane expansions, leading to a reduction in binding capacity) and markedly increase the affinity of the remaining sites. The direct effects of taurine on the phospholipid membranes modify other lipid-dependent phenomena, such as the operation of ion channels, regulation of membrane bound enzymes and protein phosphorylation processes (Huxtable, 1989). Changes in the phospholipid content of the membrane would modify the binding profile of taurine therefore altering its effect on calcium binding. A linear relationship between the phosphatidylethanolamine /phosphatidylcholine ratio of the synaptosomal fraction of developing rat brain and taurine content has been reported (Huxtable et al, 1989).

D) Taurine in the Central Nervous System (CNS)

It has been suggested that taurine may be a neurotransmitter or neuromodulator in the CNS. That is, in the CNS, taurine satisfies many of the criteria essential for a neurotransmitter role, but the findings are sometimes ambiguous : [1] It has been found within nerve endings and associated with synaptic vesicles, but it is co-localized with many other putative transmitters. [2] It has a potent depressant glycine-like action when applied to CNS neurons and is antagonized by strychnine, and in some regions by bicuculline, suggesting that it acts at glycine and GABA receptors. However some evidence suggests that there are also separate taurine receptors (Simmonds, 1983). The receptor isolation work is not yet very convincing (Frederickson et al, 1978 ; Haas and Hosli, 1973 ; Wu et al, 1990). [3] High affinity uptake mechanisms capable of removing taurine from the synaptic site have been demonstrated (Borg et al, 1979 ; Huxtable, 1981). A high affinity transport system for taurine exists in brain and the transport is sodium-dependent, energy-requiring and specific (Collins, 1977 ; Hruska et al, 1978). [4] The enzyme that synthesizes taurine, cysteine sulfinic acid decarboxylase (CSAD), is found within some nerve endings, but the distribution of enzymic activity correlates poorly with the distribution of taurine. In many instances, taurine levels and functions are maintained more by transport than by local synthesis, and marked abnormalities can occur when transport is curtailed even when synthesis is maintained. The major pathway for taurine synthesis in the brain is probably the cysteine sulfinic acid pathway whereby cysteine is transformed to cysteine sulfinic acid, which is decarboxylated by CSAD to hypotaurine, and the latter is finally oxidized to taurine (Madsen, 1990).

E) Taurine in Development

Taurine is present in fetal and newborn mammalian brain in high concentration and decreases slowly after birth reaching the concentrations found in the adult animal by the time the offspring is weaned. This reduction in the concentration of taurine during postnatal development is in contrast to the pattern for most transmitter amino acids in the brain. That is, they either increase, or change very little during development. It should be noted that during the period of postnatal development, although the concentration of taurine is decreasing, the total brain content of taurine is

increasing rapidly. That is, during the period of very rapid brain growth, the brain conserves whatever taurine is present and supplements this with taurine accumulated from the mother's milk, from other parts of the body and with increased de novo synthesis of taurine to supply the taurine pool during this period of development. These mechanisms result in a rapid increase in total taurine in the brain during the neonatal period but at the same time, there is a more rapid increase in brain weight thus resulting in a decrease in taurine concentration during neonatal life.

Taurine is found in and released from growth cones (Taylor and Gordon-Weeks, 1989 ; Taylor et al, 1990) and is also found in developing synaptosomes (Huxtable et al, 1989). In vitro, exogenous taurine enhances neurite outgrowth and the proliferation of cytoskeletal components in neuroblastoma [a tumour cell line] (Spoerri et al, 1990a,b) and neurite outgrowth from regenerating ganglion cell axons following crush (Lima et al, 1988).

It is a common feature of brain development that neurons are produced in a germinative zone and migrate to their final functional position. Taurine corrects the granule cell migration deficit in weaver mutant mice (an autosomal recessive mutation resulting in an almost complete loss of cerebellar granule cells that fail to migrate and die in the germinative zone). In a normal co-culture of granule cells and Bergmann glia the granule cells migrate along the glia. If the granule cells and glia are from the weaver mutant mouse, the granule cells do not approach the glia. If one adds taurine to this system, then the granule cells will migrate along the glia (Trenkner, 1990).

In the liver and brain from fetuses and newborn monkeys and man, taurine biosynthesis is limited by extremely low or absent CSAD activity. How does one explain the high taurine content found in developing brain when little taurine is being synthesized early in development ? A) Transport of dietary derived taurine : Several studies have shown that taurine passes slowly through the blood-brain barrier of adult rats whereas it passes rapidly through the fetal rat blood-brain barrier. When ^{35}S taurine is injected intraperitoneally into pregnant rats, the maximum specific activity in fetal brain is reached within 24 hours. In maternal brain the specific activity reached is one third that in the fetal brain and occurs after one week. After birth, when the concentration of taurine is decreasing during development, the total brain taurine is increasing, apparently by a combination of reduced turnover, increased synthesis and

uptake from the circulation. ^{35}S taurine injected intraperitoneally into lactating rats a few hours after birth is secreted in the milk and is transferred to the pups. This suggests that the neonatal rat brain undergoes changes in taurine concentration, total taurine content, taurine turnover and taurine uptake during development, and that separate pools of taurine may exist in neonatal rat brain that differ from those in mature brain (Sturman, 1979 ; Lake, 1983).

B) Axonal transport : Studies in the rabbit have shown that exogenous taurine injected into the eye is transported to the lateral geniculate axonally in optic axons to a greater extent in developing nerves than in mature nerves (Sturman, 1978). That is, more taurine is transported axonally during development, prior to and during the period of synapse formation, than is transported at a later time.

Studies of axonal taurine transport have used the visual system which is suited for such studies because the eye is very accessible for injection of substances into the vitreous chamber which then has easy access to the cell bodies of the retinal ganglion cells, the axons of which form the optic nerve. This clearly defined bundle of fibers terminates in easily recognized brain nuclei, in the optic tectum in lower vertebrates, and in the lateral geniculate nucleus (LGN) or superior colliculus (SC) in mammals. Radioactive taurine may be injected into one eye of, for example, the goldfish, rabbit or rat. Since the optic axons of goldfish cross completely at the optic chiasm and all but a few of the optic axons of the rabbit and rat cross at the chiasm to reach the contralateral optic tract, LGN and SC, the amount of radioactivity migrating via the optic nerve can be calculated by subtracting the radioactivity present in the ipsilateral optic tract, LGN and SC, from that present in the contralateral components, to correct for radioactivity arriving by the general circulation.

In addition, in the visual system of the developing rabbit, estimated rates of transport were intermediate between those of the fast and slow components of protein transport thus suggesting that axonal taurine transport is not directly linked to the axonal transport of proteins (Sturman, 1978). Taurine is present in the free form in the cytoplasm and is transported "free", not as a constituent of protein.

Taurine deficiency during development

Studies of taurine deficiency during pregnancy have revealed in the cat an

increased reproductive loss, fetuses are frequently resorbed or aborted, kittens at term are stillborn or of low birth weight, and the live kittens have poor survival rate, grow slower, have photoreceptor degeneration and exhibit neurological abnormalities such as spastic gait and poor motor control (Hayes et al, 1975 ; Sturman et al, 1985).

Morphological studies of the cerebellum of such taurine-deficient kittens show that the granule cells fail to migrate from the site of neurogenesis to the cortex, reminiscent of the weaver mutant mouse. Many mitotic figures are present in the cells in the external granule cell layer of 8 week old kittens indicating that cell division is still occurring, a process which in normal kittens ends at 3 weeks. The persistence of cells in the external granule cell layer of cerebellum observed in such kittens suggests that taurine participates in the postnatal ontogeny of cerebellar maturation, however no mechanisms have been demonstrated.

The visual cortex of kittens is also affected by taurine-deficiency. One sees structural abnormalities in the dendritic arbors in visual cortex. At birth, neuroblasts at both the ventricular and pial zones have failed to complete their differentiation and have not migrated into the cortical plate. Subsequent arborization is poor and much organization is lost (Wright et al, 1986 ; Palackal et al, 1988 ; Sturman et al, 1987). These studies indicate that taurine is an essential nutrient for developing cats but its location or precise mode of action remain unknown.

Taurine is essential for normal visual development in primates also. There is evidence of a functionally significant loss of vision in taurine-deficient infant primates (Neuringer and Sturman, 1987). Impairments in visual acuity were observed as early as 4 weeks of age and cone photoreceptor structure was disorganized in the foveal region of the retina of such primates. The retina and visual system of human infants are less developed at birth than in rhesus monkeys, and therefore they might be expected to be even more vulnerable to the effects of taurine deficiency. Another study provides evidence that dietary taurine is essential for maximum growth, as measured by weight gain, of infant nonhuman primates fed a soy protein milk formula (devoid of taurine and low in its metabolic precursors) for 5 months (Hayes et al, 1980). These findings indicate that the feeding of commercially available synthetic formulas, which contain little or no taurine, to human infants may result in similar delayed cerebellar maturation, as human infants also require exogenous taurine to maintain

their body taurine pools (Sturman et al, 1985).

Concern about the possible taurine depletion of human infants fed commercial infant formula containing much lower levels of taurine than breast milk was raised by the evidence that taurine depletion of cats and kittens results in a number of neurological disorders, including visual dysfunction, retinal and tapetal degenerations and impairment of cerebellar and visual cortex development. Human infants and children nourished totally by parenteral nutrition, with the usual solutions containing no taurine, indeed have low plasma taurine concentrations accompanied by ERG abnormalities and granularity of the pigment epithelium [PE] (Gaull, 1989). These symptoms can be corrected by taurine supplements. These findings led a number of manufacturers of human infant formulas in the US, Canada, Japan and Europe to add taurine to their products, up to the level found in human breast milk (30 mM).

These studies and others point to the importance of taurine during development but few mechanisms have been proposed. We do not even know its cellular location, since until recently there has been no histochemical technique for taurine.

F) Taurine in the Retina

Taurine is present in retina in large amounts and in mature retina is the free amino acid present in the greatest concentration. The taurine concentrations range from 10 mM in the frog retina to about 50 mM in the rat and rabbit retina (Voaden et al, 1981). The concentration of taurine in retinas from all the species studied thus far (rats, cats and rabbits) increases during postnatal development.

The enzymes responsible for taurine biosynthesis seem to be of low activity or absent in the retina. Instead the levels of taurine found in the retina appear to be mainly dependent on the transport of preformed taurine out of the blood and across the PE. In theory the source of blood taurine is from endogenous biosynthesis, the diet, or both. In rats, blood taurine can be supplied by endogenous biosynthesis from precursors in the liver. However, cats, monkeys and man depend on preformed taurine from the diet because their liver capacities for taurine synthesis are very low (Rassin, 1981).

In the mammalian retina, taurine appears to play an important role in maintaining the integrity of the retina and the viability of photoreceptor cells. The

absence of dietary taurine in cats or blockade of taurine transport in rats leads to a reduction in the concentration of taurine in the retina, followed by defects in the ERG, the degeneration of the photoreceptors and eventual blindness (Hayes et al, 1975 ; Lake, 1986 ; Lake and Malik, 1987).

G) General descriptions of the functional anatomy of the adult retina, the developing retina, the developing and adult optic nerve

A general description of retinal morphology is given here to facilitate interpretation of the immunocytochemical studies.

The Adult Retina

The mature retina is a multilayered neural structure at the back of the eye which exhibits a high degree of order and organization (see Figure 1). In addition to the light-sensitive photoreceptor neurons (PR), the retina consists of five classes of neurons and three types of glial cell. These neurons are organized into three cellular layers [outer nuclear (ONL), inner nuclear (INL) and ganglion cell layers (GCL)], which are separated by two synaptic layers [outer plexiform (OPL) and inner plexiform layers (IPL)]. As seen in Figure 1, the ONL consists of the cell nuclei of the rod and cone PRs. The INL contains the horizontal, bipolar, amacrine and interplexiform perikarya. The cell bodies of the ganglion cells are found along the inner margin of the retina and make up the GCL. Some amacrine cells (displaced) are also found in this layer. The axons of the ganglion cells run along the inner portion of the retina forming the nerve fiber layer and exit the eye through the optic disc to form the optic nerve.

Pigment Epithelium (PE)

The retinal PE is a single cell layer which separates the retina from the blood capillaries of the choroid. The PE serves multiple functions essential for proper retinal function : [1] It is vital to the integrity of rod and cone PRs and it participates in the process of continuous renewal of their outer segments by phagocytosis of their shed discs. [2] Its tight junctions (zona occludens) act as a barrier to free diffusion from the choroidal blood supply and control the composition of fluids present in the extracellular space. [3] A major portion of the required substrates such as taurine, other amino

acids, glucose and vitamin A, reach the retina from the blood through specific transport processes located in the retinal PE. [4] It is involved in the visual cycle which isomerizes the all-trans form of the vitamin A chromophore (made non-functional as a result of light absorption) back to the 11-cis form which combines with opsin protein to remake the light sensitive visual pigment eg. rhodopsin. The PE also serves as a storage depot for the chromophore. [5] The PE synthesizes melanin granules which absorb excess light energy and reduce light scatter, resulting in better resolution of visual images. [6] The PE is involved in the synthesis of glycosaminoglycans, found in the subretinal space that are thought to contribute to the adhesion of the retina to the PE (Marc, 1986), necessary for retinal survival.

Outer Nuclear Layer (ONL)

A) Photoreceptor Cells (PR)

The PR cells, lying outermost, are adjacent to the retinal PE. Two major types of PRs transduce a light stimulus on the retina into an electrical signal : [1] rods respond to dim light and mediate scotopic vision and [2] cones operate in bright light and are responsible for color vision. The rabbit retina is a rod-dominated retina. The rod cell is an elongated cell whose outer segment is connected to the inner segment by a ciliary process (Figure 2). The rod outer segment consists of the plasma membrane enclosing a stack of flattened disk membranes that contain the visual pigment, rhodopsin. The inner segment contains the major metabolic machinery of the cell. The ellipsoid (closest to the outer segment) contains mitochondria which provides energy for both the inner and outer segments. The myoid region (proximal portion of the inner segment) consists of bundles of actin filaments and microtubules. Rough and smooth endoplasmic reticulum and the Golgi complex (sites of protein synthesis) lie adjacent to the nucleus in the myoid. The PR axon proceeds to the cell synaptic terminal the site of synaptic connections with second-order bipolar and horizontal neurons. Cones often have a shorter outer segment, a thicker inner segment and a larger terminal than rods (Marc, 1986).

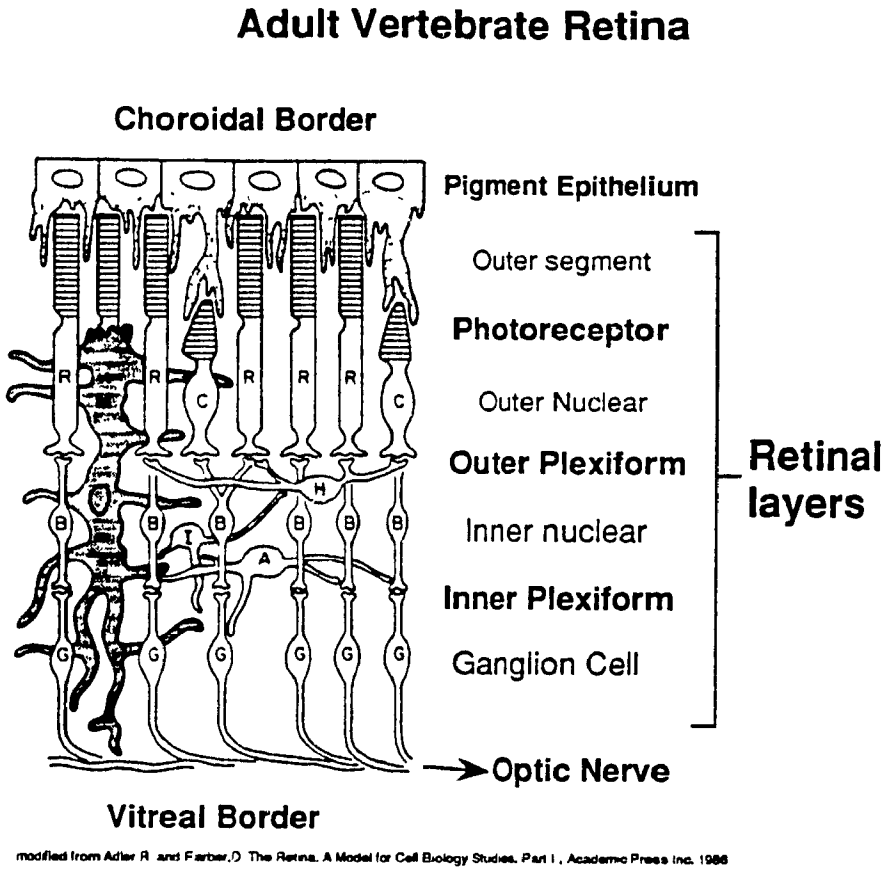
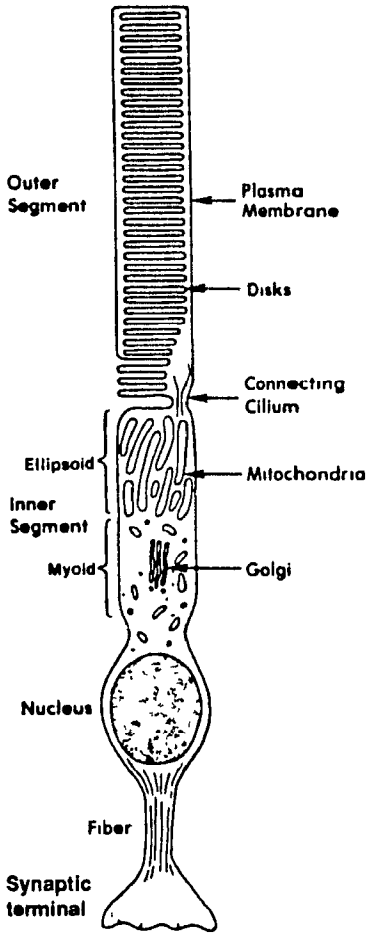


Figure 1 : A schematic representation of an adult vertebrate retina. The pigment epithelium lies between the photoreceptors (PRs) and their major blood supply, the choroid capillaris. The neural retina is comprised of 3 cellular layers (the outer nuclear layer consisting of rod [R] and cone [C] nuclei, the inner nuclear layer consisting of horizontal [H], bipolar [B] amacrine [A] and interplexiform [I] neurons and the ganglion cell [G] layer, and 2 synaptic layers (outer plexiform layer and inner plexiform layer. The axons of the ganglion cells (G) form the nerve fiber layer along the vitreal border of the retina and together exit the eye as the optic nerve. Muller cells (M) [glia] span the retina.

Schematic diagram of vertebrate rod cell



modified from Adler,R and Farber D The Retina A Model for Cell Biology Studies Part I, Academic Press Inc. 1986

Figure 2 : A schematic diagram of the structure of a vertebrate rod cell (modified from Adler and Farber, 1986). Further description is found in the text.

Outer Plexiform Layer (OPL)

Synaptic contacts occur in defined layers of the retina. The OPL contains synapses of the PRs mentioned above, and those horizontal cells onto bipolar cells. The axons of interplexiform cells which terminate on horizontal cells are also found in this layer.

Inner Nuclear Layer (INL)

A) Horizontal Cells

Horizontal cells are neurons whose cell bodies sit along the outer margin of the INL and that extend processes laterally in the OPL. Many mammalian retinas, such as from rabbits or cats, contain two types of horizontal cells : [1] an axonless cell [large or type-A horizontal cell] and [2] a cell with a short axon [small or B-type horizontal cell] (Dowling and Boycott, 1966). Electron microscopy has shown that the processes of the axonless cell and the proximal dendritic processes of the short axon cell contact exclusively cones, whereas the axon terminal processes of the short axon cell receive input from the rod terminals (Dowling, 1991).

B) Bipolar Cells

Bipolar cells are neurons whose cell bodies lie in the INL and extend processes both in the OPL and IPL. They are the output neurons of the OPL carrying information to the IPL. There are two major types of bipolar cells in the mammalian retina : [1] rod bipolar and [2] cone bipolar cells. Both types have their dendritic tree in the OPL where they receive input signals from rods and cones, respectively, and an axon in the IPL that provides output onto amacrine or ganglion cells. In the Golgi-impregnated rabbit retina, two different types of bipolar cells have been shown. The first type has a dense bush of short dendrites fanning out from the cell body through the OPL while an unbranched vitreal process extends to the innermost portion of the IPL (sublamina b). There it ends in a conical arborization consisting of a few large terminal expansions. These endings are approximately 3-5 μm in diameter and appear to lie close to the ganglion cell somata. This type of bipolar cell closely resembles the rod bipolar cells of Cajal's classification. These endings have been identified in the electron microscope as the terminal expansions of the rod bipolars according to their size, location and synaptic

ribbon content (Raviola and Raviola, 1967). The rod bipolar endings are rarely found in contact with the ganglion cell somata. Their prevalent synaptic output is onto narrow-field, bistratified (AII) amacrines (Raviola and Raviola, 1967; Strettoi et al, 1990).

The dendrites of the second type of bipolar cell spread out from the cell body at an obtuse angle and at the vitreal limit of the OPL adopt a tangential course, while in the IPL, their vitreal processes give rise to many branches. Their arborizations spread at different levels but they have never been observed in the innermost portion of the IPL. This type of bipolar cell closely resembles Cajal's cone bipolars (Raviola and Raviola, 1967; Strettoi et al, 1990). Cone bipolars show a physiological dichotomy in that one class (ON cells) depolarize and the other (OFF cells) hyperpolarize to a light stimulus projected onto their receptive field center. In rod-dominated retinas, such as those of rabbits and cats, the majority of the bipolar cells are rod bipolar cells. In rabbit retina, an individual rod bipolar cell can receive input from as many as 100 rods.

C) Amacrine Cells

Amacrine cells are axonless neurons whose processes lie in the IPL, making synapses with the processes of bipolar, ganglion and other amacrine cells. There are two populations : [1] one with perikarya at the inner margin of the INL and [2] another with perikarya in the ganglion cell layer (GCL) known as displaced amacrine cells. There may be as many as thirty types of amacrine cells in mammalian retina on the basis of differential morphology and immunohistochemical characterization (Dowling, 1987).

D) Interplexiform Cells

The interplexiform cell is a retinal interneuron. These cells are arranged with their dendrites in the IPL and their axons in the OPL. These cells extend processes to the INL where they make synaptic contacts with horizontal and bipolar cells. They may feedback to control horizontal cell coupling and participate in light adaptive responses (Dowling, 1987).

Inner Plexiform Layer (IPL)

The IPL of the vertebrate retina is the region of synaptic interplay of the bipolar,

ganglion and amacrine cells. A prominent feature of the IPL in the rabbit is a row of large nerve endings, 2-4 μm in diameter, lying at the boundary between the inner plexiform layer and the ganglion cell layer.

The IPL is subdivided into a distal (sublamina a) and a proximal (sublamina b) portion, proximal meaning closer to the optic nerve and the brain. In mammalian retina, photopic (cone) and scotopic (rod) vision are served in part by different neuronal pathways. There is a direct pathway from the cone PRs to the cone bipolar cells to the ganglion cells. All cones depolarize at light OFF and this signal is transmitted to OFF-center or ON-center bipolar cells. The axonal arbors of the OFF-center bipolars stratify in sublamina a (the OFF layer) of the IPL where they synapse with the dendrites of OFF-center ganglion cells. The ON-center bipolars synapse with ON-center ganglion cells in sublamina b (the ON layer). The rod PRs connect to one type of rod bipolar cell. The synapses of the rod bipolars contact AII amacrine cell processes in sublamina b (Vaney et al, 1991), and via them influence the dendrites of ganglion cells.

Retinal Glia

The vascularized retina of adult vertebrates contains three macroglial cell types : Muller cells, oligodendrocytes and astrocytes. The predominant type of glial cell in the retina is the Muller cell. Muller cell nuclei are found in the INL and their processes are oriented radially between the outer retina, where they form tight junctions with each other and PR cells at the level of the inner segments in an arrangement known as the outer limiting membrane (OLM), and the inner retina (inner limiting membrane) where their endfeet border the vitreous cavity (see Figure 1). The nuclei and processes of the astrocytes are usually found closely associated with the ganglion cell bodies or their axons in the nerve fiber layer (NFL) [Lewis et al, 1988]. Oligodendrocytes myelinate axons within the CNS, and peculiar to the rabbit, are found in the NFL of the retina. The astrocytes and oligodendrocytes are confined to the medullary ray region of the retina and are absent from the greater part of the NFL (Schnitzer, 1985 ; Robinson and Dreher, 1989).

In the rabbit, the central part of the retina consists of the medullary ray and visual streak regions, and the peripheral part refers to the remainder of the retina. The visual streak (below the optic nerve head) contains the highest density of retinal

ganglion cells [comparable to the fovea in primates]. These regions are specialized for high visual resolution (Vaney and Hughes, 1976). The retinal medullary ray area is a region in the posterior pole, superior to the optic nerve head through which the ganglion cell axons leave the retina. The rabbit is unusual among mammals in that the ganglion cell axons are myelinated within the nerve fiber layer (NFL) of the retina, in the medullary ray area, whereas in most other mammals, myelination of the optic nerve occurs only external to the eye.

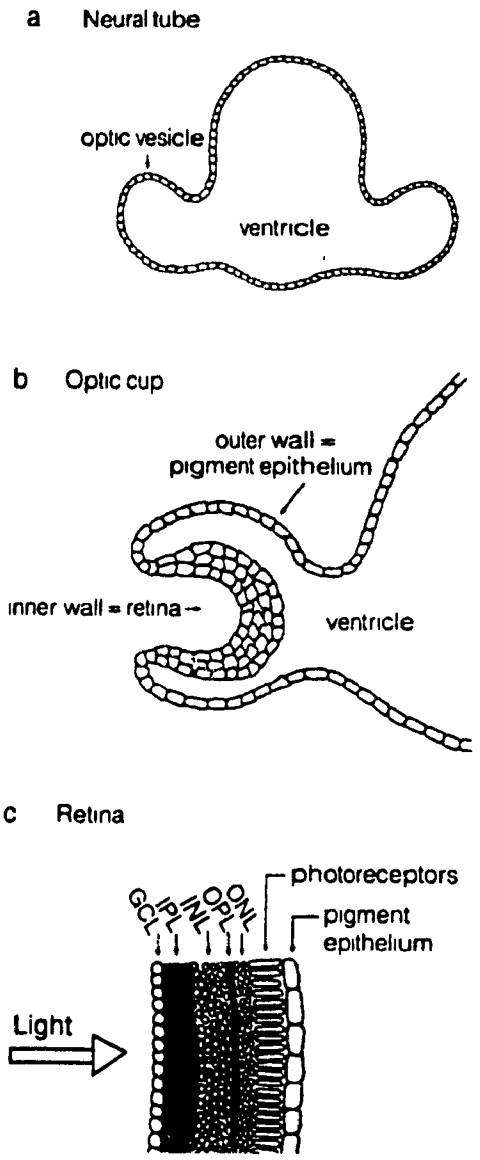
The Developing Retina

The retina is a thin layer of neural tissue lining the back of the eye. It derives from the neural tube and has the same embryonic origin as the brain. The neural tube evaginates to form two optic vesicles in the head region of the embryo (Figure 3). Each optic vesicle subsequently invaginates to form an optic cup that eventually becomes the retina. Both walls of the optic cup are one cell thick, but the cells of the inner wall divide to form a neuroepithelial layer many cells thick. These cells or neuroblasts differentiate into all of the retinal cells (Dowling, 1987).

The vertebrate retina provides an appropriate CNS tissue for developmental studies. As a model of neural development, the retina has been popular because it is accessible and, in many experimental animals such as the rabbit, much of its structure is formed postnatally (Barnstable et al, 1988).

Retinal Thickness and Layering

With the exception of the myelinated region, in general, immature retinae are thicker than more mature ones. The thickness of the retina decreases with postnatal development because the eye increases in size and the retinal cells do not continue to increase in number with the exception of the nerve fiber layer astrocytes (see below), but instead spread out. This decrease in thickness is more pronounced in the periphery than in the center : the visual streak retains higher cellular densities and higher visual acuity than the periphery. It is known that the first axons to reach the optic chiasm come from ganglion cells in this region of the retina, and they lay out the crossing and non-crossing paths for subsequent fibers (Stone et al, 1985). The mature retina may be divided into a "central" area of the retina which refers to the visual streak



Modified from Dowling, J. E., **The Retina**:
An approachable part of the brain
Harvard University Press 1987

Figure 3 : Embryonic (a, b) and postnatal (c) development of the mammalian retina. A schematic diagram showing (a) the optic vesicles which develop from the neural tube, (b) the invagination of an optic vesicle to form an optic cup, and the layers of the retina that develop from the cells of the inner wall of the optic cup, (c) the fully differentiated retina in which the ventricle space between the neural retina and the pigment epithelium has disappeared.

(characterized by a horizontally oriented concentration of ganglion cells spanning most of the width of the retina and a matching concentration of cones [Hughes, 1971]) and medullary ray region (a region superior to the optic nerve head through which the ganglion cell axons leave the retina), whereas the designation "peripheral", refers to the regions of the retina outside the central area.

The mammalian retina undergoes phases of mitotic activity : [1] an early phase producing both neurons and glial cells and then [2] a late phase producing glial cells (in the NFL, for instance). It seems likely that the late phase within the INL is glial as well, that is, consists of dividing Muller cells (Reichenbach et al, 1991). In rabbits, two independent studies reached the conclusion that by the end of the first postnatal week, proliferation of stem cells is completely finished. One of these studies was based on counting mitotic figures in the ventricular layer (Stone et al, 1985), a method which fails to detect cells in other phases of replication. However, there is massive astroglial proliferation within the rabbit retinal NFL, up to at least four weeks postnatal (Schnitzer, 1988a, 1990).

Tritiated thymidine studies have determined when cell populations undergo their final mitotic division. Birth dates of horizontal cells are among the earliest in the mammalian retina and they appear to differentiate at the same time as ganglion cells (Messersmith and Redburn, 1990). Next bipolar cells are produced and finally PRs (Carter-Dawson and LaVail, 1979 ; Rapaport and Stone, 1983 ; Walsh and Polley, 1985). Rod PR cell growth and differentiation begins after that of the cones (Messersmith and Redburn, 1990).

Autoradiographic analysis shows that in the PN day 1 rabbit retina, horizontal cells are labeled with ^{3}H-GABA but in the adult animal, these cells are no longer labelled (Redburn and Madtes, 1986). If GABA is a horizontal cell transmitter at early but not at late developmental stages, this suggests that horizontal cells have the ability to switch from one transmitter to another during ontogenesis. Another possibility is that GABA may not be a transmitter in these inhibitory interneurons but may be playing a neurotrophic role during the development of the retina, promoting organization and layering of neuronal elements (Schnitzer and Rusoff, 1984).

As mentioned for the adult, the rabbit is unusual among mammals in that the ganglion cell axons are myelinated within the NFL of the retina, whereas in most other

mammals, myelination of the optic nerve occurs only external to the eye. This myelination begins on PN day 10.

The Developing and Adult Optic Nerve

The optic nerve does not contain any neuronal cell bodies but is composed of the axons of retinal ganglion cells that project from the eye to the brain, two major classes of macroglial cells (oligodendrocytes and astrocytes), microglia, vascular cells and leptomeningeal cells that ensheathe the whole nerve. During postnatal development the oligodendrocytes extend processes that wrap around most axons to form an insulating myelin sheath. Less than 5% of axons in the adult nerve are unmyelinated. The myelin sheath is interrupted by gaps called nodes of Ranvier, where excitation is confined : the action potential travels along the axon by jumping from node to node, a process that increases the efficiency and rate of nerve impulse propagation. The functions of the astrocytes are less clear. They extend processes to the periphery of the nerve forming a glia limitans, and to blood vessels, forming a perivascular sheath ; both structures are thought to participate in the blood-nerve barrier. Projections to nodes of Ranvier (Raff, 1989) are speculatively linked with modulation of conduction properties (Figure 4). In addition some astrocytes extend a process to the axon at the nodes of Ranvier, the gaps in the myelin sheath.

Establishment of the retinal projection to the brain involves an overproduction and subsequent elimination of large numbers of retinal ganglion cells and their axons (Provis and Penfold, 1988). The first optic axons in the rat are generated and grow toward the brain between E14 and E15.5 (Horsburgh and Sefton, 1986) at the time of closure of the optic fissure. More axons are added to the developing optic nerve over a period of days or weeks. The average axon diameter increases from 0.18 µm at birth to 0.80 µm in the adult rat (Sefton and Lam, 1984) and the size, type and number of glial cells increases (Sturrock, 1975). The development of the rat's visual system during the first five days of life is associated with a loss of 60% of the axons present in the optic nerve at birth (Crespo et al, 1985 ; Crespo and Viadero, 1989). In the rabbit, the pattern is similar. The number of optic nerve axons reach the maximum of 750,000 at E23-24. By E31/PN day 1, this number decreases by almost half, to about 350,000 axons. During the postnatal period, a further 15% decrease to adult numbers

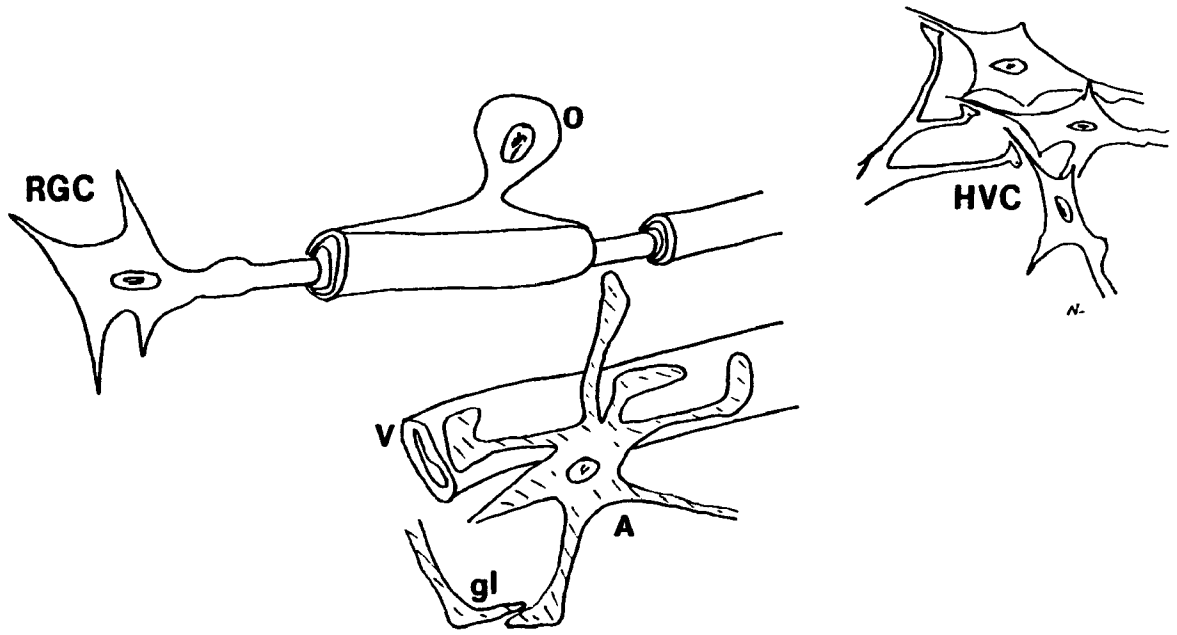


Figure 4: Diagram of optic nerve and macroglia. The axons of retinal ganglion cells (RGC) travel across the retina in the nerve fiber layer and exit through the optic disc or optic nerve head area where they collectively form the optic nerve. The optic nerve consists of these axons and two major types of macroglia, the oligodendrocytes (O) that myelinate the majority of axons in mature nerve, and the astrocytes (A). Astrocytic processes form blood-nerve barrier structures, the glia limitans (gl) at the nerve periphery and through their endfeet that surround blood vessels (V). They also send a process to the axons at the nodes of Ranvier, the gaps in the myelin sheath. In mature animals the axon terminals are thought to release glutamate onto neurons in higher visual centers (HVC) such as the lateral geniculate nucleus and the superior colliculus.

occurs, with adult values, about 294,000, being reached between PN day 30 and PN day 50 (Robinson et al, 1987).

Two mechanisms are postulated to account for the decrease in axon numbers during development : [1] Axonal loss may be due to the "pruning" of axonal collaterals without the loss of the parent cell, as has been reported for developing callosal projections in mammalian neocortex (O'Leary et al, 1981). [2] Loss of axons may accompany the elimination of those parent cells which have failed to establish sustaining connections with their target neurons.

The reasons why axon loss and cell death occur during the development of the CNS are not well understood. It has been suggested that in the developing neuromuscular system, motorneuron afferents may compete for a trophic factor which is only available from target cells. Motorneurons projecting to appropriate sites may have a competitive advantage over inappropriately projecting axons and those which fail to contact target neurons may be eliminated. A similar suggestion has been advanced to account for the loss of ganglion cells and their axons during development. Axon loss in the developing rabbit optic nerve is almost certainly due to the elimination of the parent ganglion cells (Robinson et al, 1987) since the number of ganglion cells are similar to the number of axons during the period of axonal loss (Robinson et al, 1987).

During the first two postnatal weeks, the diameters of most axons in the rabbit optic nerve increases substantially from 0.3 μm to 2.5 μm excluding myelin ; glial processes interdigitate between most axons. Thereafter the number of axons decreases to the adult number of 294,000. Axon diameters in the adult optic nerve range from about 0.3 μm to 4.5 μm excluding the myelin sheath. The axons are unmyelinated at birth. Myelination begins at about day 10 in the epiretinal portion of the rabbit optic nerve and is complete by about day 22.

Axonal Transport of Taurine

Experiments have shown that taurine is axonally transported in goldfish and mammalian visual systems. For example, ^{35}S-taurine was injected into the vitreous humor of the eye in developing and young adult rabbits. Considerably more ^{35}S-taurine is transported axonally in the visual system of neonatal rabbits than in that of

adult rabbits (Sturman, 1978). In a similar study done in the rat, the animals were injected at 1, 4, 7, and 11 days after birth (prior to and during the major period of synapse formation in the lateral geniculates), and the amount of taurine transport was higher than that observed in young adults. In contrast, the amount of taurine transport in rats injected 15 days after birth (after the period of synaptogenesis in the lateral geniculates) was not significantly different than that seen in adult animals. It appears that the amount of taurine axonally transported along neonatal rat optic axons prior to and during the formation of synaptic connections is severalfold higher than along young adult axons (Politis and Ingoglia, 1979).

The concentration of taurine in the optic nerve is about two times greater than that found in the optic tract (post optic chiasma), lateral geniculate nucleus and superior colliculus, both in the developing and adult rabbit.

H) Question addressed by this thesis

My thesis project has been to provide a description of the localization of taurine in the retina and optic nerve in the adult, and during the postnatal development of the rabbit. Our laboratory has successfully developed an antibody to taurine which is useful for the immunocytochemical localization of retinal taurine (Lake and Verdone-Smith, 1989). This antibody was used to localize endogenous taurine in glutaraldehyde-fixed retinas and optic nerves of developing and adult rabbits in order to delineate or map its distribution within the different cells of the postnatal rabbit retina and the optic nerve, as a function of age.

II. MATERIALS AND METHODS

Antibodies to taurine

Taurine is a small molecule, and thus is not immunogenic. However, small molecules can be conjugated to peptide carrier molecules and thus be rendered immunogenic haptens. Synthesis of our immunogen was based on the method described by Campistron (Campistron et al, 1986). In brief, taurine was coupled via glutaraldehyde (G) to carrier proteins, either poly-L-lysine or bovine serum albumin (BSA), by mixing the reactants together in test tubes. This generated the hapten complexes taurine-G-poly-L-lysine or taurine-G-BSA which were isolated from the reactants by a series of dialyses, reoxidation and centrifugation steps. The lyophilized immunogen was reconstituted in sterile saline, emulsified in Freund's complete adjuvant and injected intramuscularly into male New Zealand white rabbits. The rabbits were injected in rotation with taurine-G-poly-L-lysine and taurine-G-BSA in incomplete adjuvant every ten days and 10 ml aliquots of blood were collected monthly via the ear vein. The blood was refrigerated overnight ; then the serum was separated by centrifugation, aliquotted in 0.5 ml volumes and frozen until needed. Prior to enzyme linked immunosorbent assays (ELISA) testing or immunocytochemistry, an aliquot of serum was mixed with 1 mg each of lyophilized G-poly-L-lysine and G-BSA overnight at 4° C to absorb any carrier-specific reactivity. In some cases the serum was absorbed overnight with 2 mg GABA-G-BSA to remove reactivity to GABA, when indicated by the initial ELISA. The primary antiserum was obtained as the supernate following a centrifugation step at 15,000g for 10 minutes.

ELISA characterization

The specificity of our primary antiserum was tested using ELISA. ELISA characterization involved coating microtiter wells with lyophilized antigen-G-BSA, reconstituted in carbonate-bicarbonate buffer, pH 9.6, at concentrations ranging from 0.01 µg/ml to 100 µg/ml, or carrier only (G-BSA) blanks. The antigens used for cross-reactivity testing included BSA-G conjugates of glycine, ß-alanine, L-aspartate, GABA, cysteine sulfinic acid (CSA), hypotaurine and taurine prepared as described above for taurine. The wells were coated with the target antigen solutions overnight at

4° C, then rinsed 3 times with 0.05% Tween in phosphate buffered saline (PBS), pH 7.4. To block non-specific binding sites, the wells were treated with 1% BSA in PBS-Tween for 1 hour at 37° C, followed by three PBS-Tween rinses. Primary antiserum was diluted to 1:10,000 in PBS-Tween/BSA and pre-incubated at 37° C for 1 hour and then was added to the wells and the plate was kept at 4° C overnight. Following 3 PBS-Tween rinses, the wells were refilled with goat anti-rabbit immunoglobulin coupled to horseradish peroxidase (GAR-HRP) at a concentration of 1:5000 in 1% BSA in PBS-Tween for 3 hours at 37° C. After rinsing the wells thoroughly with PBS-Tween, color formation was developed using 0.1M ABTS (2,2-azino-di-3-ethyl-benzthiazolinsulfonate) and the plate was read after 5 minutes at a wavelength of 410 nm on a Dynatech EIA reader. The same procedure was undertaken to test the commercially available taurine antiserum. The ELISA characterizations have revealed that our sera are highly selective and reactive for taurine compared to the other related amino acids such as glycine, ß-alanine or GABA, which are also found in the retina. The commercially available antiserum (Seralab AES 137, Dimension Labs) has considerable cross-reactivity with GABA and ß-alanine and lower reactivity to CSA, aspartate and glutamate. The reactivity of our sera to GABA when it is present, can be entirely removed without attenuating the reactivity to taurine, by pre-incubating with GABA-G-BSA overnight. This method is not financially feasible with the commercial antiserum that costs in excess of $500 for 250 µl.

Preparation of retinal and optic nerve sections

Retinal tissue and optic nerve were obtained from pigmented New Zealand white rabbit pups at postnatal days 1 (n=3), 3 (n=2), 7 (n=3), 10 (n=2) and 16 (n=2). Mature tissue was also examined The pups were administered an overdose intraperitoneal injection of Nembutal (40 mg/kg body weight). The eyes were immediately enucleated, the cornea was perforated with a needle and the eye and attached optic nerve stump were immersed at room temperature in a fixative of 3.5% glutaraldehyde in 0.1M sodium cacodylate/0.06M $MgCl_2$ at pH 7.2. The eyes and optic nerves were placed in aldehyde fixative in order [1] to create the antigen (taurine-G-protein) to which the antibody was raised, and [2] to prevent the loss or redistribution in the tissue of the soluble amino acids. Studies have shown that for the

latter, high concentrations of glutaraldehyde fixatives are required (Ottersen and Storm-Mathisen, 1985; Ottersen, 1988; Battista and Lake, unpublished). Following 15 minutes of fixation with constant agitation, the cornea was cut away and the lens removed. The eyecup was then placed back in fresh fixative for one hour. Subsequently, the back of the eye with the retina was cut in small wedges and the optic nerve isolated, using a sharp razor blade and the pieces of tissue were returned to fixative for the remaining fixation time (a total of 3 hours). Following fixation, the pieces of tissue were rinsed in a cacodylate-sucrose buffer and placed in 2% osmium tetroxide for 1 hour. After a buffer wash, the tissues were transferred to a solution of 1% uranyl acetate for 30 minutes and subsequently dehydrated in increasing alcohol concentrations and infiltrated in Epon. The tissues were embedded in flat molds and cured in a 60° C oven overnight. The blocks of tissue were hand-trimmed with a razor blade and sectioned on a Reichert ultramicrotome. One µm sections were mounted with a drop of distilled water on glass slides and dried on a 40° C warming tray overnight. Some sections were stained with 1% toluidine blue to permit observations concerning quality of fixation, orientation of the section etc.

Immunocytochemistry

For light microscope observations, I have used two visualization methods : PAP and immunofluorescence. The standard peroxidase anti-peroxidase (PAP) method of indirect labelling (Sternberger et al, 1970) relies on a bridging of antibodies and enzymatically produces a chromogen at the site of antigenic activity. I used this method less frequently but it generated permanent slides. I have used the immunofluorescent method more frequently because it generated better (higher contrast) black and white photographs, however it does not produce permanent slides. For either method, to increase penetration of the immunochemicals, Epon was removed from the sections by incubating the slides in a freshly prepared solution of 8% sodium methoxide for 15 minutes at room temperature, followed by a 30 second incubation in 3% hydrogen peroxide in methanol The slides were rinsed in PBS-0.1% Triton X-100, pH 7.4 and transferred to a humid chamber where the sections were covered with 4% BSA in PBS-0.1% Triton X-100 for 1 hour to reduce non-specific labelling. Following this blocking step, the sections were exposed to the primary taurine antiserum at dilutions of

1:500 to 1:1000 in 2% BSA in PBS-0.1% Triton X-100 and left overnight at 4° C. The following day, the slides were rinsed 3 times, 10 minutes each time, in PBS-0.1% Triton X-100. The slides were then incubated in goat anti-rabbit fluorescein isothiocyanate (GAR-FITC) diluted 1:10 in PBS-0.1% Triton X-100, for 4 hours at 37° C, shielded from light with a black cloth. Following rinsing in PBS-0.1% Triton X-100, the slides were mounted using 0.1% para-phenylenediamine in glycerol-PBS mounting medium, pH 9.6. Fluorescence was observed with a Nikon fluorescence microscope using dichroic filters for blue excitation and emission at 450-490. Photomicrographs were taken using Kodak Tmax 400 black and white film and an automatic exposure system.

Control sections were processed in parallel, but substituted for the primary antiserum was either taurine antiserum pre-adsorbed with the taurine conjugates [that is, the antigen], or buffer containing 2% BSA. These showed a complete lack of reactivity indicating that our sera likely do not react with anything other than taurine in these sections.

Biochemistry

Biochemical analysis was carried out on retinas from pups at 1 and 7 postnatal days of age. One eye from each animal was used for morphology, and the other for determination of DNA and taurine content. The retinas were homogenized in 85% ethanol, and placed in the freezer overnight. After centrifugation, the pellet was used for estimation of DNA content (Yates et al, 1974) while the supernatant was dried under a stream of nitrogen gas, and resuspended in 0.525 ml of distilled water. A 0.5 ml aliquot was passed over a dual bed ion-exchange column (0.8 x 2 cm of Dowex 50, H^+ form, layered over 2 cm of Dowex 1, Cl^- form). The column was washed with 3 ml of distilled water and the effluent assayed for taurine using ninhydrin (Troll and Cannan, 1953) and a spectrophotometric method. HPLC studies have shown that amino acids other than taurine are retained by the column (Larsen et al, 1980). All samples were assayed in triplicate and compared to standards. Since there have been several published studies of the increases in retinal taurine content during development, these studies were included mainly for their value in learning the analytical techniques [taurine/DNA (nmol/mg) : PN1 , 1131.6 and PN7 , 1561.8].

III. RESULTS

A) THE ADULT RETINA

Localization of taurine immunoreactivity (taurine-IR) in the retina of the adult rabbit

In the accompanying Figures (5-9) that are photomicrographs of sections of the adult rabbit retina, specific taurine immunofluorescence appears white, and most attention is directed to regions of high reactivity. Control sections processed with taurine antiserum pre-adsorbed with the taurine conjugates (antigen) showed a complete lack of reactivity which photographs as dark grey or black (for example Figure 9).

Distal retina

There are only low levels of taurine-IR in the pigment epithelium, except for some inclusion bodies which may be phagosomes (Figure 5). Phagosomes are the shed photoreceptor outer segment tips phagocytosed by the pigment epithelium, and the outer segments when attached to the photoreceptor cells are indeed highly immunofluorescent.

The photoreceptor cells were the most numerous taurine-IR neurons found in the adult retina (Figures 5-8). Marked taurine-IR was found in their outer segments and in the myoid, but not the ellipsoid, region of the inner segments. Taurine-IR was also very pronounced in the synaptic terminals of the photoreceptors (Figures 5-8). In some cone rich regions, the cones appear more immunoreactive than the rods (Figures 5, 7, 8). It is known that cones are scarce outside the visual streak region (Raviola and Raviola, 1967).

Proximal retina

Taurine-IR was seen in a sub-population of the horizontal cells in the peripheral area of the adult rabbit retina (Figure 5).

Immunoreactivity was also seen in most bipolar cells (Figures 5, 7). Our one micron sections facilitate resolution, but decrease the probability of seeing all of a cell's projections in one field. However, in some sections, taurine-IR bipolar axons can be

followed into the region of their synaptic terminations (Figures 5, 7).

Taurine-IR was seen in a sub-population of amacrine cells in the inner nuclear layer (Figures 5, 7, 8) and in displaced amacrine cells present in the ganglion cell layer (Figure 5).

The inner plexiform layer has many punctate deposits of taurine-IR which are likely to be the synaptic endings of taurine-IR bipolar and amacrine cells (Figures 5, 7, 8). Numerous large taurine-IR processes resembling terminals are also seen close to the ganglion cell bodies (Figures 5, 7, 8). These are in the position of bipolar terminals to the ON ganglion cells. Taurine-immunoreactive somata in the ganglion cell layer are rare, and are most likely displaced amacrine cells (Figure 5).

In general the glial Muller cells are devoid of taurine-IR throughout most of their perikaryal cytoplasm and some appear as black silhouettes (Figures 5, 8). In some sections, the Muller cells do show some background low levels of taurine-IR (Figure 7). There are bright fluorescent spots of taurine-IR in the region of the tight junctions of the outer limiting membrane which will require EM analysis to determine if their origin is photoreceptor, glial, or both (Figures 5, 6, 8).

A summary of the distribution of taurine-IR in the adult retina appears on Table 1.

Table 1 : Taurine-IR in the Adult Rabbit Retina

Cell Type	Taurine-IR level
PE	– ; but inclusions (PR OS) are +++
Rod	+++ especially OS, synaptic terminals
Cone	+++
Bipolar	++ somata and synaptic terminals
Horizontal	small subpopulation (somata and OPL processes ?)
Amacrine	small subpopulation (somata and IPL processes)
Interplexiform	?
Ganglion Cell	–
Muller cells	usually – ; infrequently +
Astrocytes	?
Oligodendrocytes	?

Key

PE	pigment epithelium
PR OS	photoreceptor cell outer segments
OS	outer segments
OPL	outer plexiform layer
IPL	inner plexiform layer
+++	high
++	moderate
+	low
–	nil
?	insufficient data

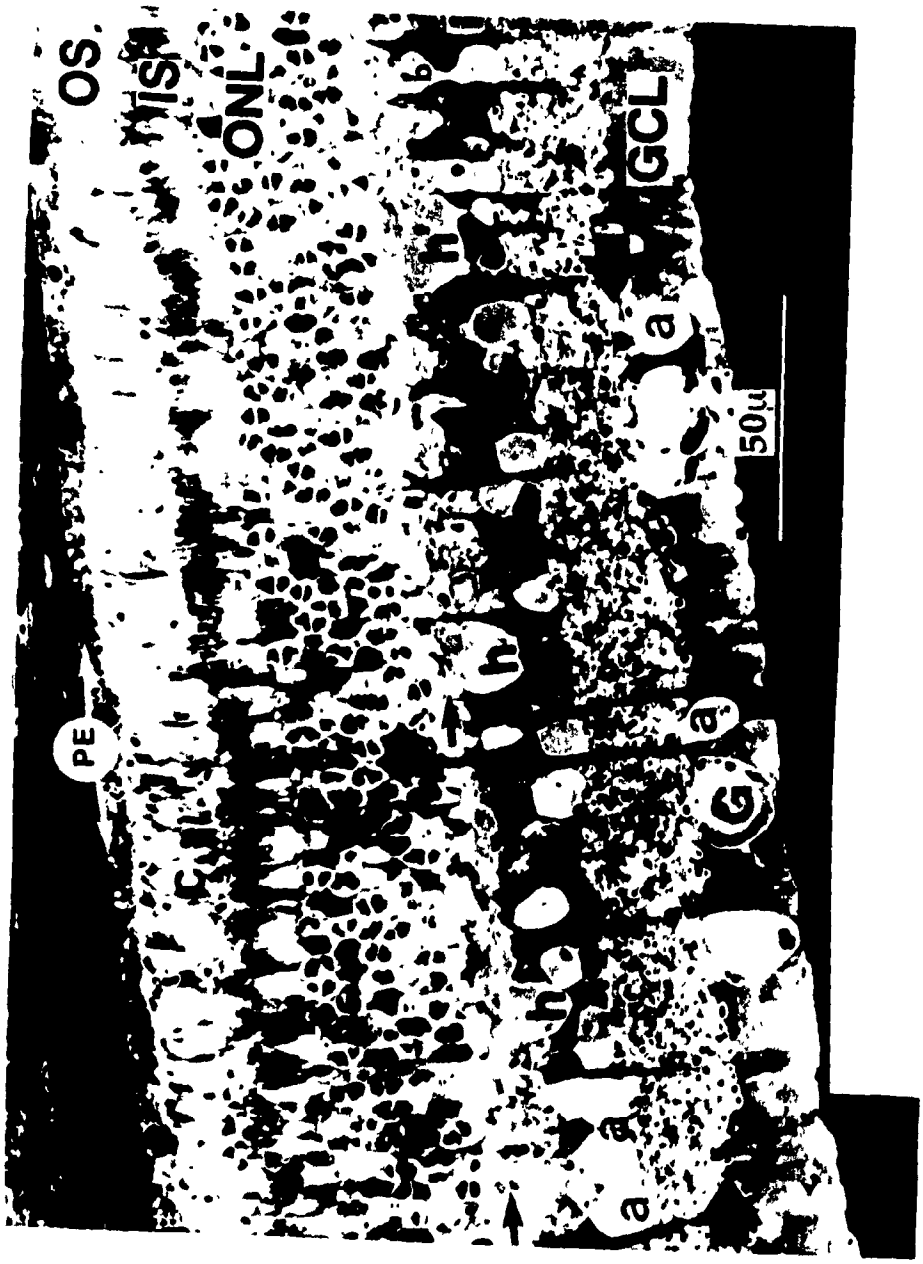

OS.
IS
ONL
GCL
PE
50μ

Figure 5 : Taurine-IR in a 1 μm section of adult rabbit retina. There are only low levels of taurine-IR in the pigment epithelium (PE) except for some inclusion bodies, which may be phagosomes (shed PR outer segment [OS] tips). PRs occupy the top half of this section. Their OS are highly immunofluorescent as are their IS myoids and synaptic terminals (small black arrows). Taurine-IR was seen in sub-populations of amacrine cells (a) in the inner nuclear layer and in displaced amacrine cells in the ganglion cell layer (GCL). Some bipolar cells (b) were taurine-IR, while a subset of horizontal cells (h) showed reactivity outlining their plasma membranes. In this same layer can be seen many Muller cells whose cell bodies and projections towards the GCL appear as black silhouettes due to their unreactivity.

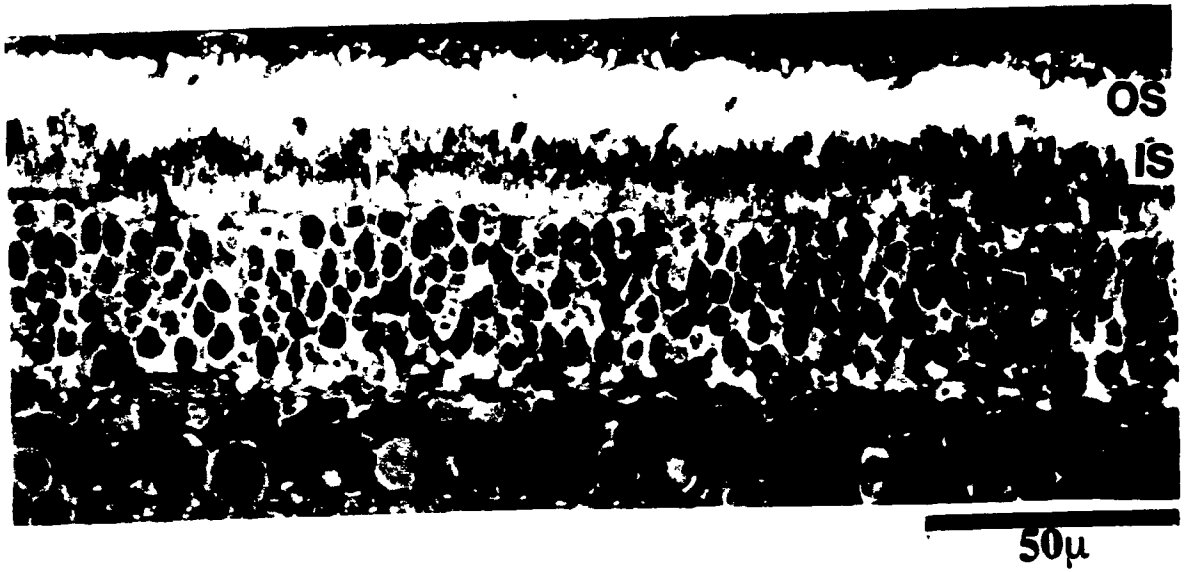


Figure 6 : Taurine-IR in the distal adult rabbit retina. This section was selected to show that when the overall immunoreactivity is kept low by antiserum dilution, the most taurine-IR parts of the photoreceptor are the outer segments and the synaptic terminals.

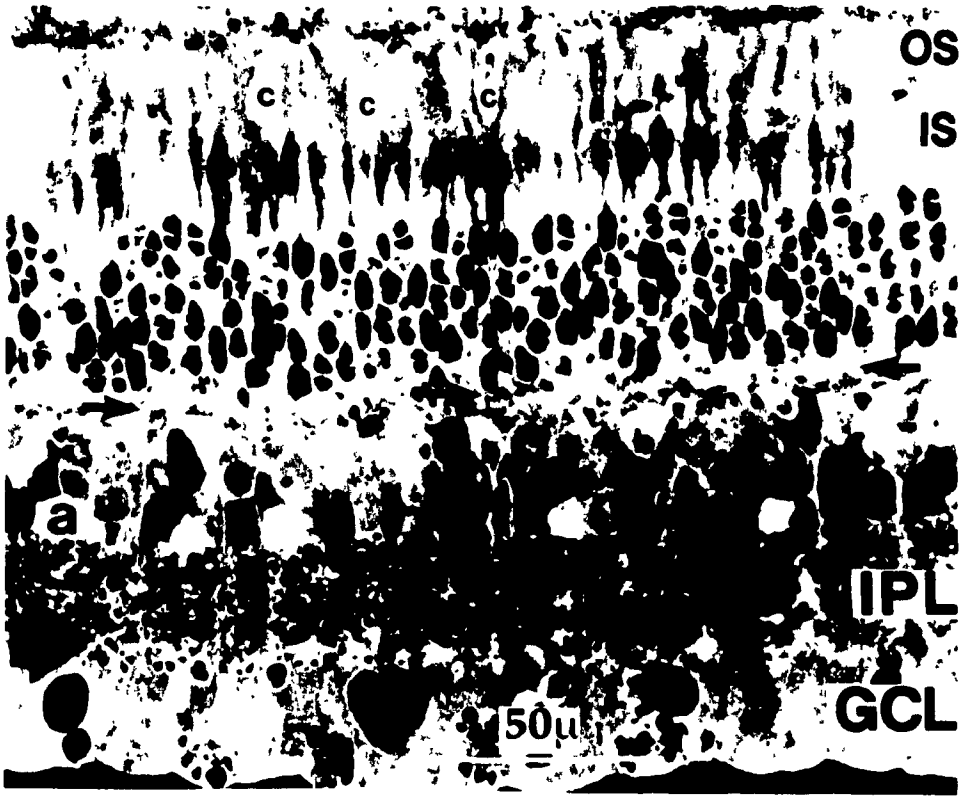


Figure 7 : Taurine-IR in the adult rabbit retina. This section was chosen because it is a cone rich region. Note that the larger cone outer segments (c) appear more immunofluorescent than the intervening thinner rod OS. A second feature of this section is the large number of taurine-IR bipolar cells (b) in the inner nuclear layer, some with fluorescent axons projecting into the inner plexiform layer (IPL) and giving rise perhaps to some of the taurine-IR terminals at the border of the GCL. A number of taurine-IR amacrine cells (a) are seen at the other edge of the IPL.

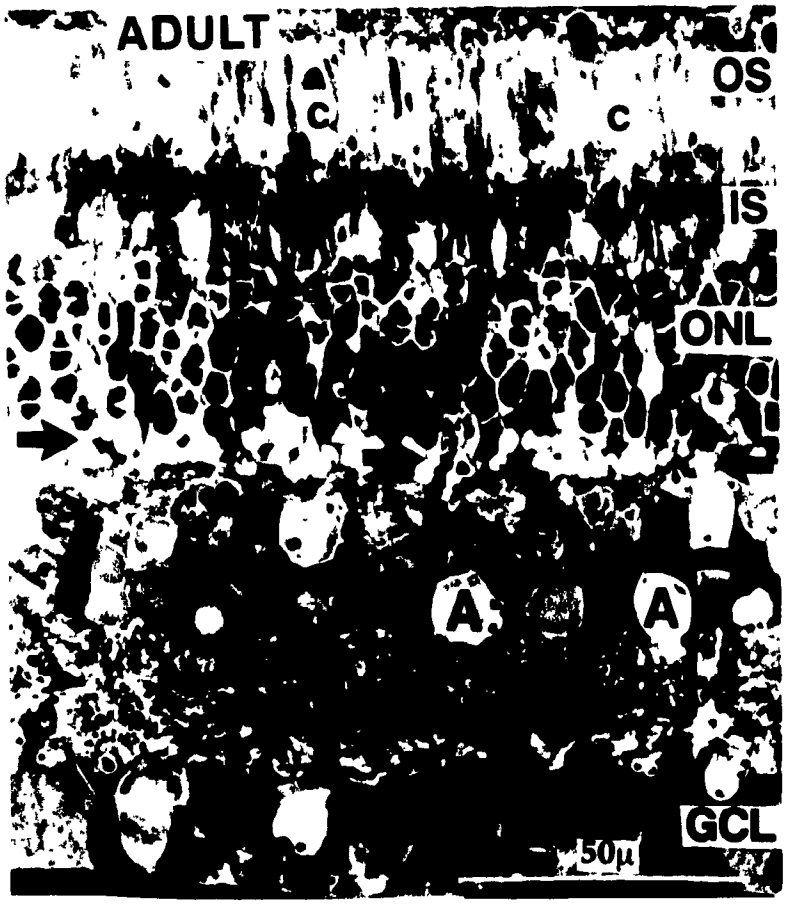


Figure 8 : Taurine-IR in the adult rabbit retina. Immunofluorescence appears white in this 1 μm transverse section. Refer to Figure 9 for the immunological control section. The most intense and numerous taurine-IR neurons were the photoreceptors that occupy the top half of this section. Taurine-IR was pronounced in their outer segments (OS), especially those of cones (c), in the myoid, but not the ellipsoid region of their inner segments (IS) and in their synaptic terminals (small black arrows). Marked taurine-IR is seen in 2 amacrine cells (A) and in the horizontal cell above the right A cell. A number of taurine-IR terminal-like processes (large black arrow) are seen close to the ganglion cell layer (GCL). The Muller cells are unreactive ; their cell bodies in the middle of the inner nuclear layer and their vertical processes projecting down to the GCL are seen as black silhouettes.

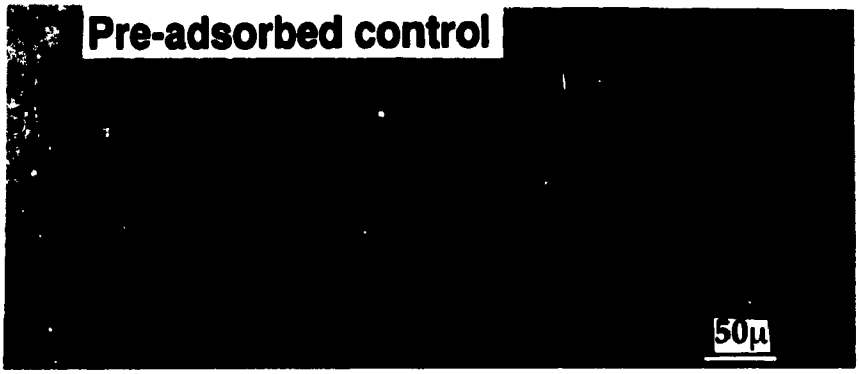


Figure 9 : Immunocytochemical control. This section of adult rabbit retina was processed with taurine antiserum pre-adsorbed with taurine conjugates and shows lack of immunoreactivity. The bright white specks are contaminating dust particles.

B) THE DEVELOPING RABBIT RETINA

Localization of taurine immunoreactivity (taurine-IR) in the retina of the developing rabbit

Immunocytochemistry was carried out on material from developing rabbit pups from PN 1 to PN 16. Examples of each observation time are given in Figures 10-17. Figures 12 and 14 show immunocytochemical control sections. Retinal glia (Muller cells) were not identified except in adult retinas and so are not discussed.

PN day 1 and PN day 3 (Figures 10-12)

At PN day 1, the outer retina is comprised of a thick layer of undifferentiated neuroblasts mostly destined to become photoreceptors. Mitotic figures are seen close to the outer limiting membrane (OLM) indicating continuing neurogenesis. Positioned in the middle of the outer neuroblast region are the cell bodies and processes of the type-A horizontal cells. These horizontal cells differentiate earlier and appear mature compared to the neighbouring neuroblasts. The position of the type-A horizontal cell lateral processes delineates where the future synapses of the outer plexiform layer will be (Polley et al, 1989).

The inner retina is composed of mature somata in the ganglion cell layer, a narrow cell free plexiform layer and a few mature amacrine cell bodies on its distal side.

At PN day 1, the neonatal retina taurine-IR is especially marked in the horizontal cells and their lateral processes, in selected somata in the position of amacrine cells, in punctate deposits in the inner plexiform layer (which are likely amacrine cell growth cone processes) and in the axons of ganglion cells in the nerve fiber layer, but not in their somata (see Figure 10). However at PN day 3 the ganglion cell somata are also taurine-IR (Figure 11).

Messersmith and Redburn (1992) suggest that the inner and outer plexiform layers are linked at birth by the processes of the interplexiform cell. I noted several taurine-IR cells with morphological characteristics of the interplexiform cell at PN day 1 and PN day 3 (see Figures 10A and 11A).

PN day 7 (Figures 13-15)

At this stage, the retina shows more maturation. Although the photoreceptor cell bodies in the outer nuclear layer have achieved a columnar organization they still display immature profiles with compact elongated nuclei. Mitotic figures are no longer observed. Taurine-IR is seen in their distal developing inner and outer segments and in their synaptic terminals.

Taurine-IR is also seen in horizontal cells and now in bipolar cells, including taurine-IR bipolar cell projections towards the inner plexiform layer. Several taurine-IR synaptic strata can be seen in the inner plexiform layer. A punctate distribution of taurine-IR close to the ganglion cells is seen and these may be the large bipolar terminals, which have previously been identified as such, using EM immunocytochemistry in the adult rat (Lake and Verdone-Smith, 1989). A few amacrine cells are still immunoreactive, but the ganglion cell somata are mostly unreactive at this stage.

Taurine-IR is still pronounced in the unmyelinated axons of the nerve fiber layer (NFL) [see Figures 13 and 15]. Many unreactive glial cell bodies can be seen in the NFL (see Figures 13B, 15B), however we have not yet determined if they are astrocytes, which are known to be present at this time, or immature oligodendrocytes, there in readiness for myelination.

PN day 10 (Figure 16)

The inner and outer segments of the photoreceptors have grown considerably as shown in the two sections of a PN 10 rabbit retina. The photoreceptor synaptic terminals are seen in the outer plexiform layer and they are markedly taurine-IR. The horizontal and bipolar cells still remain immunoreactive and one also can see displaced amacrine cells which are taurine-IR (see Figure 16A). Punctate taurine-IR is seen in several strata of the inner plexiform layer and these terminal-like structures are especially numerous close to the ganglion cell somata.

Taurine-IR in ganglion cell axons appears diminished when compared to the earlier ages, and while glial cell somata in the NFL were previously unreactive, now some show taurine-IR (see Figure 16B).

PN day 16 (Figure 17)

At this time in development, the eyes have usually been open for a couple of days. The inner and outer segments of the photoreceptors are almost fully developed. The absence of taurine-IR from the ellipsoid region of the inner segments (the ellipsoids contain mitochondria and provide energy for both the inner and outer segments) is evident, in contrast to the high levels found in the inner segment myoids (which contain bundles of actin filaments, microtubules, rough and smooth endoplasmic reticulum and Golgi complex) and in the outer segments (Figure 17).

Table 2 summarizes a time line of the postnatal appearance of taurine-IR in the rabbit retina and nerve fiber layer constructed from my examination of slides and photomicrographs.

Table 2 : Taurine-IR in the Rabbit Retina

	AGE					
CELL	**PN1**	**PN3**	**PN7**	**PN10**	**PN16**	**Adult**
PE	–	–	–	–	–; (+++, inclusions)	same as PN 16
Outer segments			++	++	+++	+++
Inner segments			++	++	– (e) +++ (m)	– (e) +++ (m)
PR terminals			+++	+++	+++	+++
Horizontal cell	+++	+++	++	+++	+	+
Bipolar cell			++	+++	++	++
Amacrine cell			+	+	+	+
Interplex. cell	++	++	?	?	?	?
IPL	puncta	puncta	puncta	puncta	puncta	puncta
Ganglion cell		+++	–	–	–	–
NFL axons	+++	+++	+++	+	–	–
NFL glia			–	++	?	?

Key

Levels of taurine-IR were rated as follows :

+++	high
++	moderate
+	low
–	nil
puncta	particle-like
?	insufficient data
blank	cell type has not differentiated sufficiently to be identified
PE	pigment epithelium
PR terminals	photoreceptor terminals
Interplex. cell	interplexiform cell
IPL	inner plexiform layer
NFL	nerve fiber layer
e	ellipsoid
m	myoid

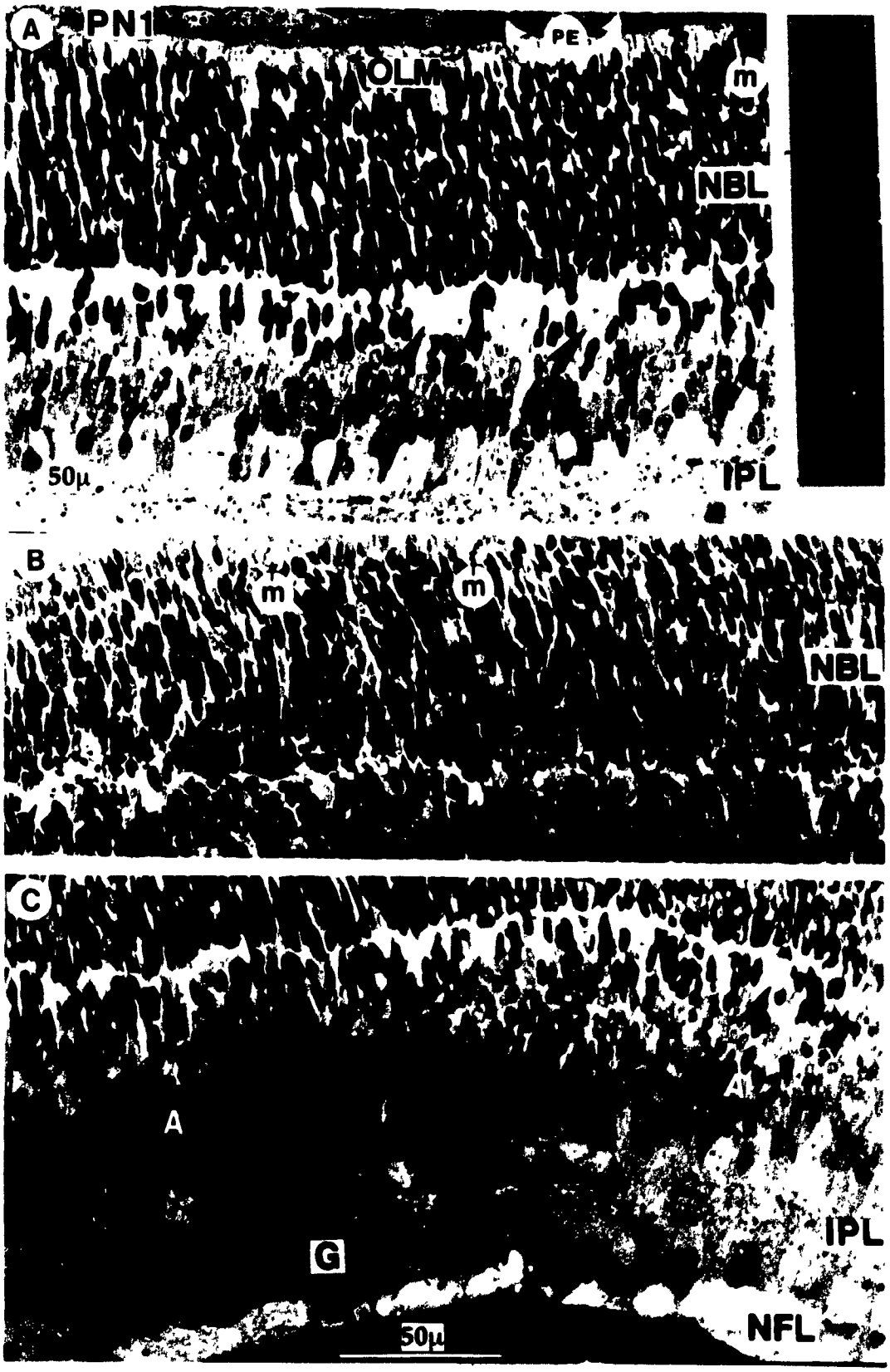

A
PN1
PE
OLM
m
NBL
50μ
IPL
B
m
m
NBL
C
A
A
G
IPL
50μ
NFL

Figure 10 : Taurine-IR in the developing rabbit retina. Three one micron sections (A-C) of PN day 1 rabbit retina. In A, one sees the pigment epithelium (PE) to the inner plexiform layer (IPL) and B shows outer retina. C shows inner retina. Scale bar is the same for A and B. The retina is immature at this age. The neuroblast layer (NBL) in A and B of the outer retina is comprised of immature nuclear profiles (dark, elongated nuclei), most destined to become photoreceptors. Note the mitotic figures (m in A and B) close to the outer limiting membrane (OLM), indicative of ongoing neurogenesis. In this same region the growth cones of the developing photoreceptors show taurine-IR (white), most easily seen in A as small vertical processes pushing up against the OLM and the unreactive PE. On the central side of the NBL lie the horizontal cells (arrowheads in A, B, C) which are comparatively more mature and most of which express taurine-IR in their somata and lateral processes. These processes delineate where the future synapses of the outer plexiform layer will be. In panel A some other cells of the inner nuclear layer that show taurine-IR : (1) somata that have ascending and descending processes may be interplexiform cells [see also Figure 11] and (2) on the border of the IPL there are cell bodies in the position of the future amacrine cells (see also panel C, white A). The ganglion cell axons forming the nerve fiber layer (NFL) contain high levels of taurine-IR whereas their somata (G) are devoid of taurine-IR. This region is only shown in panel C. The large black holes between G and the NFL are artefacts which arose during processing.


A
PE
PN3
OLM
NBL
INL
i
A
A
IPL
50µ

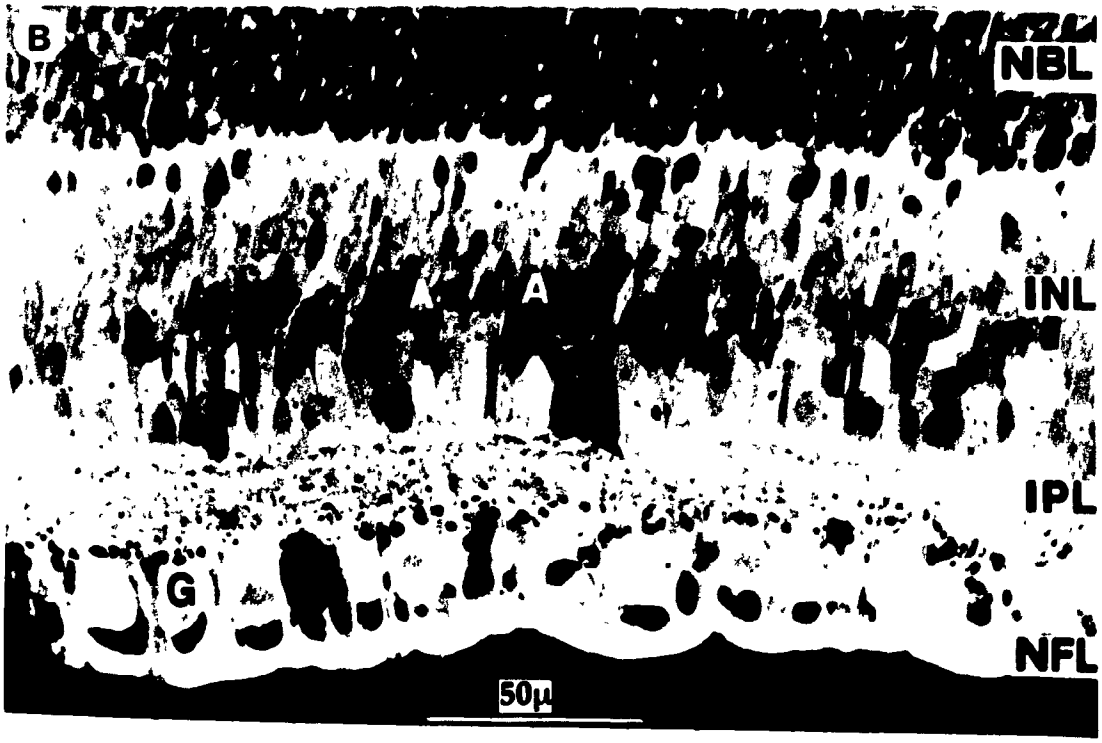

B
NBL
A
A
INL
IPL
G
NFL
50µ

Figure 11 : PN day 3 rabbit retina. Both sections are from the same pup but the top section (panel A) is from peripheral retina and the lower section (panel B) is from a more central area of the retina. A central to peripheral gradient of development is apparent : the inner nuclear layer (INL) of the central region contains more differentiated somata and has higher levels of taurine-IR than the periphery. In B note that both the ganglion cell somata (G) and their axons in the nerve fiber layer (NFL) are taurine-IR. In A, several taurine-IR cells with characteristics of the interplexiform cell are seen (i). Black arrowheads point out taurine-IR horizontal cells and their lateral processes. Close to the outer limiting membrane (OLM) taurine-IR can be seen in the developing inner and outer segments of the photoreceptors.

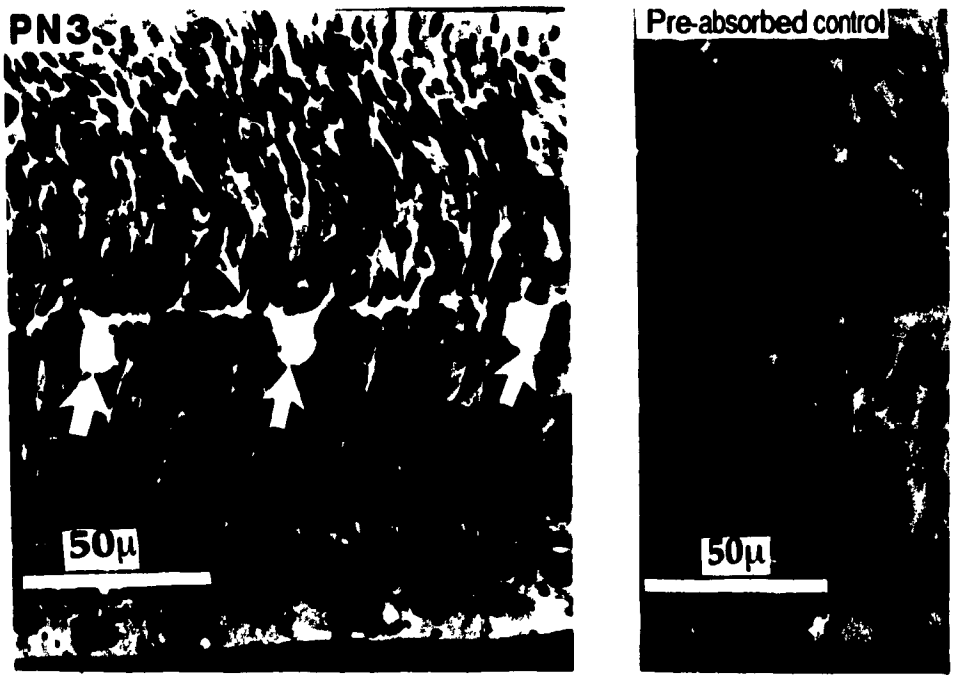


Figure 12 : Other one micron sections of PN day 3 rabbit retina. The left panel shows taurine-IR horizontal cells (large arrows) particularly clearly. The taurine-IR terminals of photoreceptors [PRs] (small arrows) are also seen at the sites where they will form synaptic junctions with horizontal and bipolar cell dendrites in the outer plexiform layer. On the right is an adjacent section processed with serum preadsorbed with taurine conjugates : it shows a lack of reactivity, ie. no bright white immunofluorescence (except for dust particles).

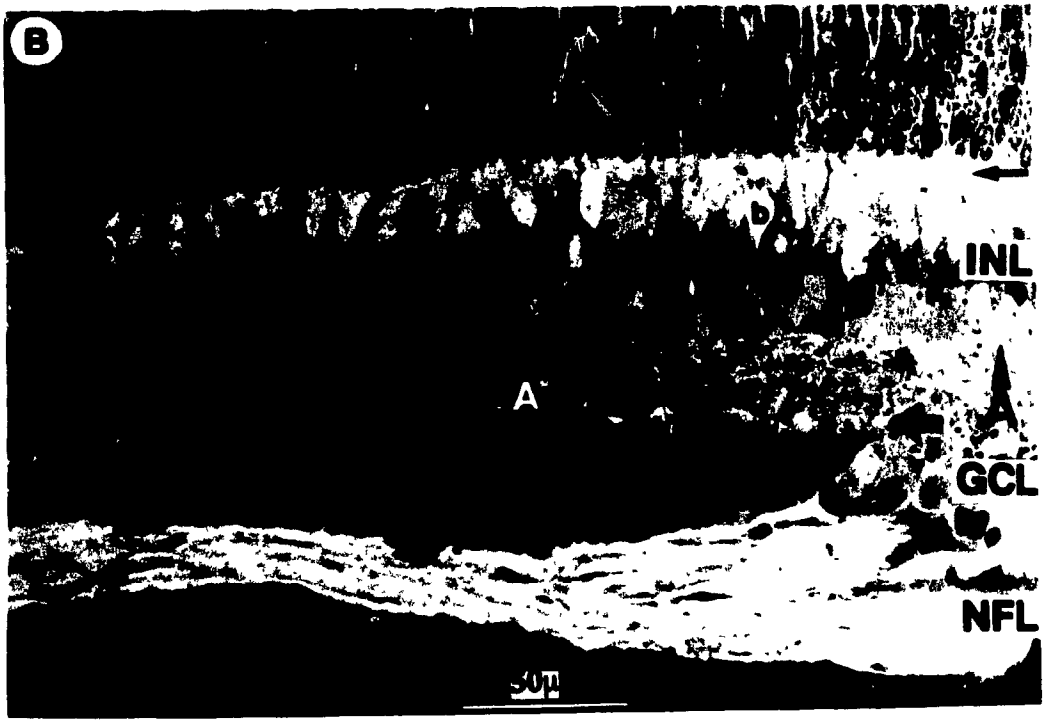


Figure 13 : The medullary ray area of PN day 7 rabbit retina. Taurine-IR is pronounced in the still unmyelinated axons of the NFL. Many unlabeled glial cell bodies (empty black arrow in B) are seen at this time in the NFL. Taurine-IR is also seen in the cytoplasm of the developing PRs in their distal expansions near the unreactive PE and in their synaptic terminals (thin black arrows). In the inner nuclear layer (INL) bipolar cells (b) are the most numerous taurine IR cells, although taurine-IR is also seen in horizontal cells (black arrowheads), and in a few amacrine cells (white A). In the inner plexiform layer (IPL) synaptic terminal-like structures (punctate taurine-IR) are seen (large black arrows), especially in the region close to the ganglion cells (G) that are mostly unreactive at this time.

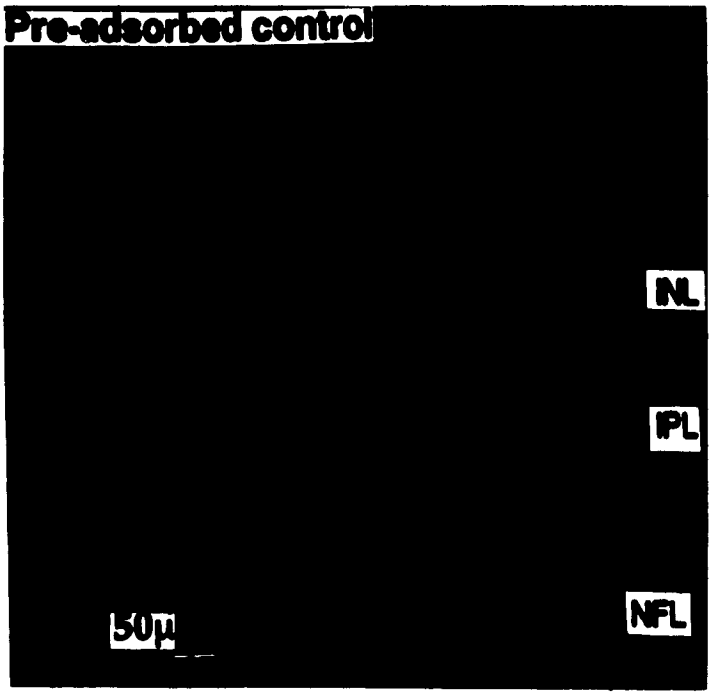


Figure 14 : PN day 7 immunocytochemical control. The primary anti-taurine serum was preadsorbed with the taurine conjugates prior to use in the immunocytochemical processing. Note the complete lack of reactivity, compared to Figures 13 and 15.

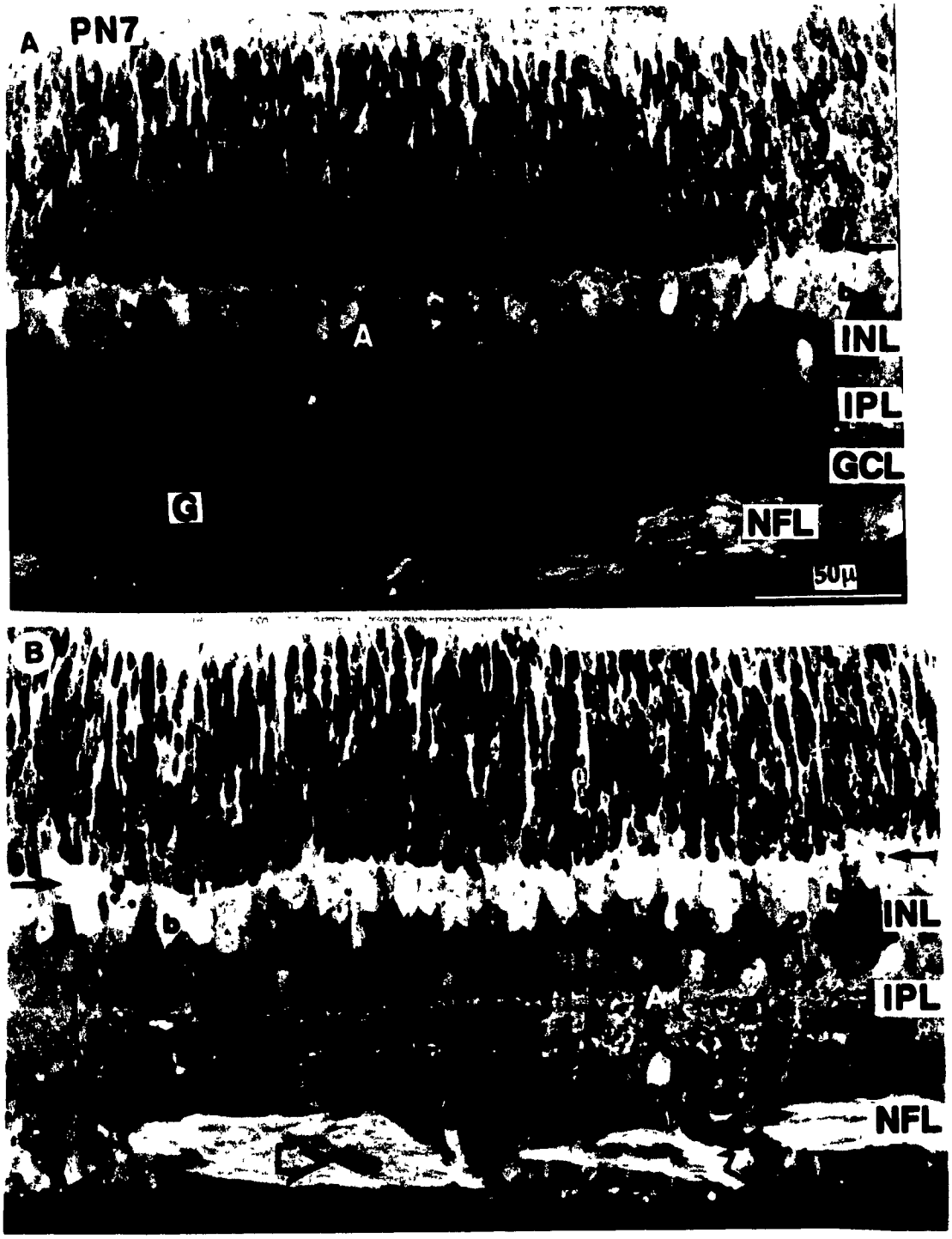

A
PN7
A
INL
IPL
GCL
G
NFL
50μ
B
INL
IPL
A
NFL

Figure 15 : Two additional one micron sections from the medullary ray region of PN day 7 rabbit retina. Scale bar is the same for A and B. There is marked expression of taurine-IR in the still unmyelinated axons of the NFL, whereas the glial cells are unlabeled (empty black arrows in B). Most bipolar cells are taurine-IR, but only a few amacrines (A) are.

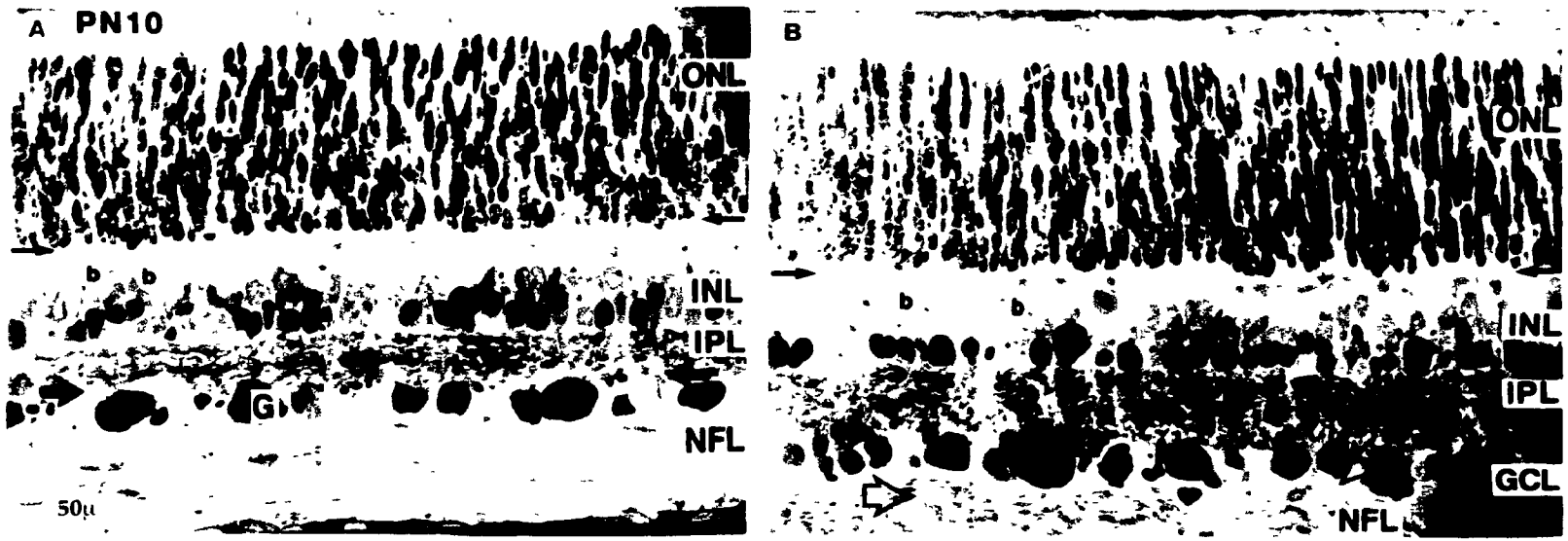


Figure 16 : PN day 10 rabbit retina. Scale bar is the same for A and B. The inner and outer segments of the PRs above the outer nuclear layer (ONL) have grown considerably and are markedly taurine-IR. Photoreceptor cell synaptic terminals (thin black arrows) have high levels of taurine-IR and form a distinct band in the outer plexiform layer. While the overall number of taurine-IR neurons in the inner nuclear layer have declined, those remaining include some horizontal cells (black arrowheads, no axons) and many bipolar cells (b) with axons projecting to the inner plexiform layer (IPL) and contributing to the strata of synaptic terminals there (large black arrows). Only a few amacrine cells remain taurine-IR including some displaced amacrines (panel A, white A). Taurine-IR in the ganglion cell axons of the nerve fiber layer (NFL) appears diminished compared to previous ages. While all glial cell somata in the NFL were unreactive at younger ages, now some show taurine-IR (open black arrows in B), though a population of unreactive glia remain.

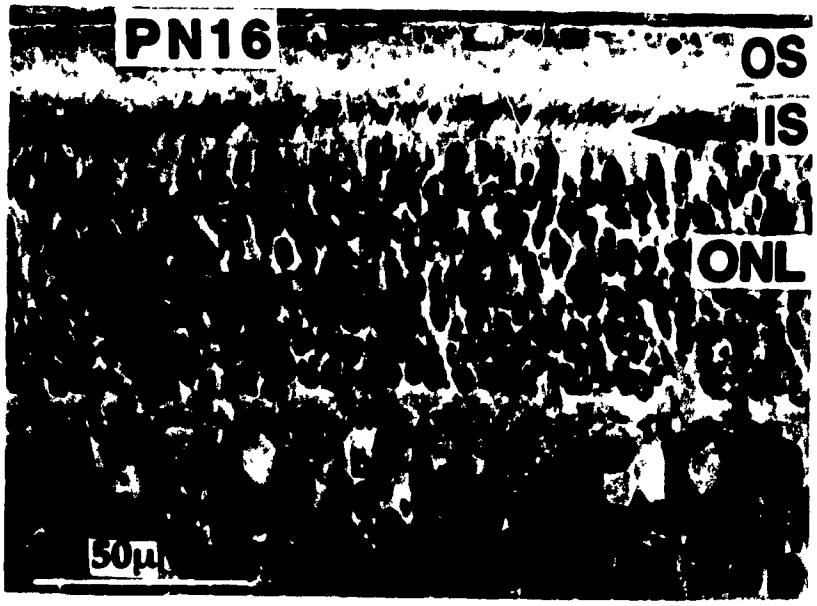


Figure 17 : PN day 16 rabbit retina. Cellular differentiation of the retina closely resembles the adult. One sees a stratification not apparent at earlier ages, in taurine-IR of the PR inner (IS) and outer segments (OS), with the myoid (black arrow) but not the ellipsoid regions being reactive.

C) THE DEVELOPING AND ADULT RABBIT OPTIC NERVE

Localization of taurine immunoreactivity (taurine-IR) in the optic nerve of the developing and adult rabbit

A general description of the changes in the optic nerve during development was presented in the introduction. Figure 18 shows histological cross sections of the optic nerves I used for my study. Early postnatal optic nerve is very delicate tissue and showed some fixation damage. The limited material I had did not allow reliable localization, so I have begun with PN day 7 results.

PN day 7 (Figure 19)

PN 7 and older tissues had better tolerance for our processing solutions. At PN day 7, one sees unreactive glial cells and highly taurine-IR optic axons. At this stage most of the axons are unmyelinated.

The right panel of Figure 19 is an immunocytochemical control, a PN day 7 section that shows lack of background immunoreactivity, which was the finding with the controls done at all the ages, when the taurine antiserum was preadsorbed with taurine conjugates (antigen) prior to use.

PN day 10 (Figure 20)

There is a substantial change in the localization of taurine-IR by PN day 10. There is still some punctate immunoreactivity in the optic axons but the overall level is less than at PN day 7. In addition, some glial somata are now reactive, and the peripheral rim of the nerve is intensely fluorescent in the location of the glia limitans – a barrier structure made up of astrocyte processes (see Figure 20).

PN day 16 (Figure 21)

The intensity and number of immunoreactive axons has declined while numerous immunoreactive glia are seen.

Adult (Figure 22)

In the adult optic nerve taurine immunofluorescence is almost exclusively in

glial perikarya and their processes, some of which group the unreactive myelinated axons into fascicles. The blood vessels are often lined with immunoreactive glial endfeet, that probably arise from astrocytes. There are at least two different glial morphologies : one type found in clusters, has more immunoreactive cytoplasm and processes while the second type has nuclei surrounded by less cytoplasm. The peripheral immunoreactive glia in the position of the glia limitans are likely to be astrocytes, however, whether the remainder correspond to astrocytes or oligodendrocytes is not known and further characterization with class-specific antibodies is required.

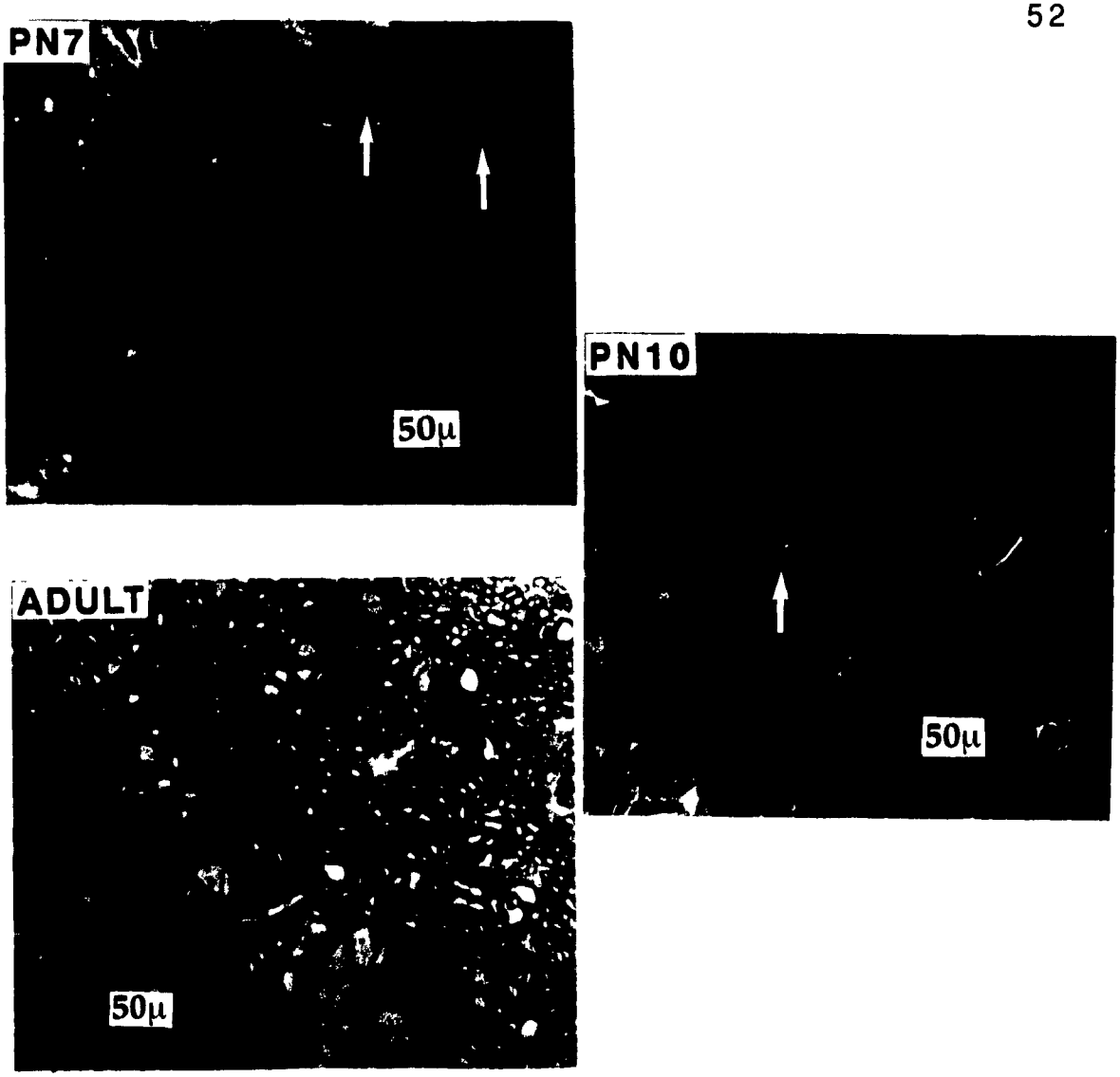


Figure 18: Cross sections of rabbit optic nerve (1 µm, toluidine blue stained). The optic nerve consists of glial cells (white arrows point to glial nuclei) and ganglion cell axons. All sections are at the same magnification. At the youngest age (PN7) glial nuclei can be seen but the unmyelinated nerve fibers cannot be individually resolved. By PN10 a percentage of fibers have become myelinated and the dark staining myelin outlines many nerve fibers but axon diameter is still small. The growth in axon diameter with postnatal development results in the adult optic nerve where many myelinated fibers are now easy to resolve (black arrowheads).

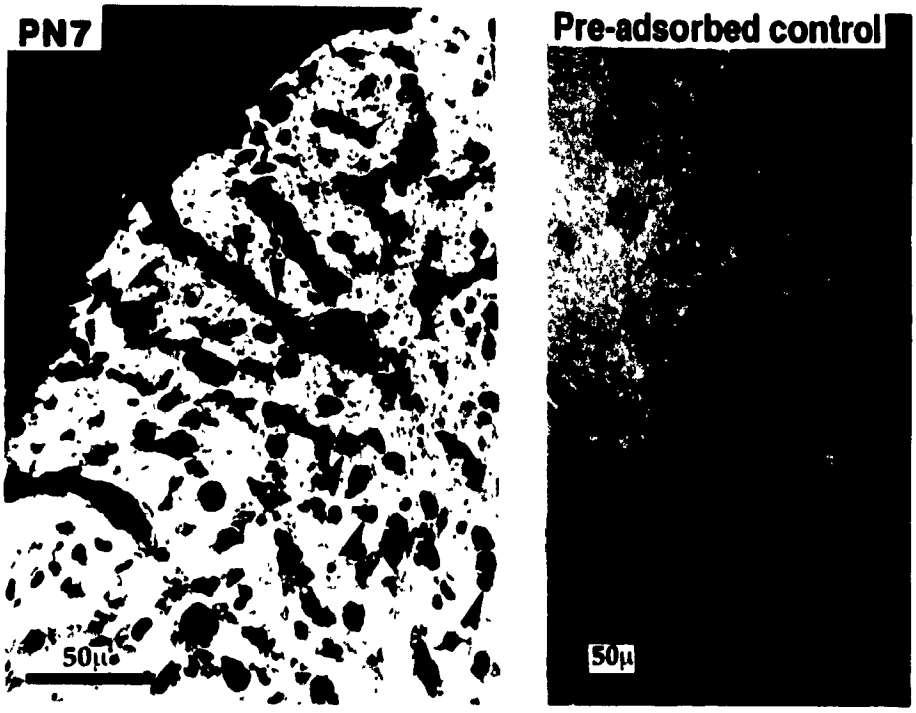


Figure 19: Taurine-IR in PN day 7 rabbit optic nerve. **Left panel** : Axons are highly taurine-IR in this cross-section of PN7 nerve. The periphery of the nerve is at the upper left. Blood vessels (bv) and glial cell nuclei (black arrowheads) are unreactive. On the right is an adjacent section processed with anti-serum preadsorbed with taurine conjugates. Note the lack of immunofluorescence, which was the finding with the controls done at all the ages.

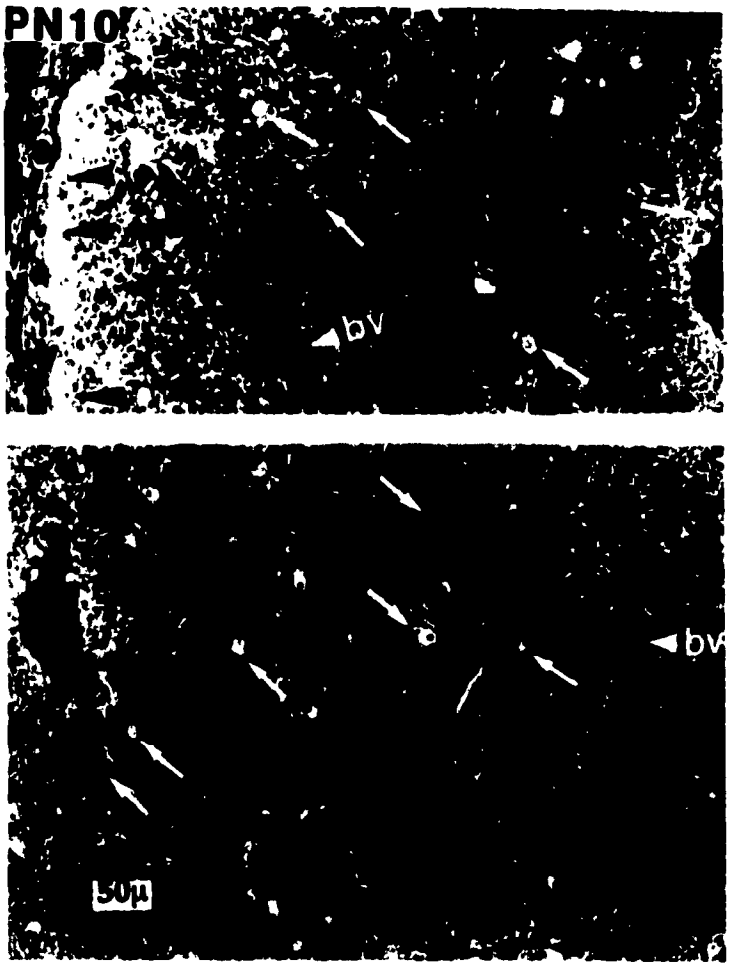


Figure 20: PN day 10 rabbit optic nerve. **Upper panel** : nerve periphery. **Lower panel** : central region. The overall level of taurine-IR in the nerve is reduced as compared to PN7. Glial cell nuclei that were unreactive at younger ages are now highly taurine-IR (white arrows). The peripheral rim of the nerve is intensely fluorescent in the location of the glia limitans (black arrowheads in upper panel) [bv=blood vessel].

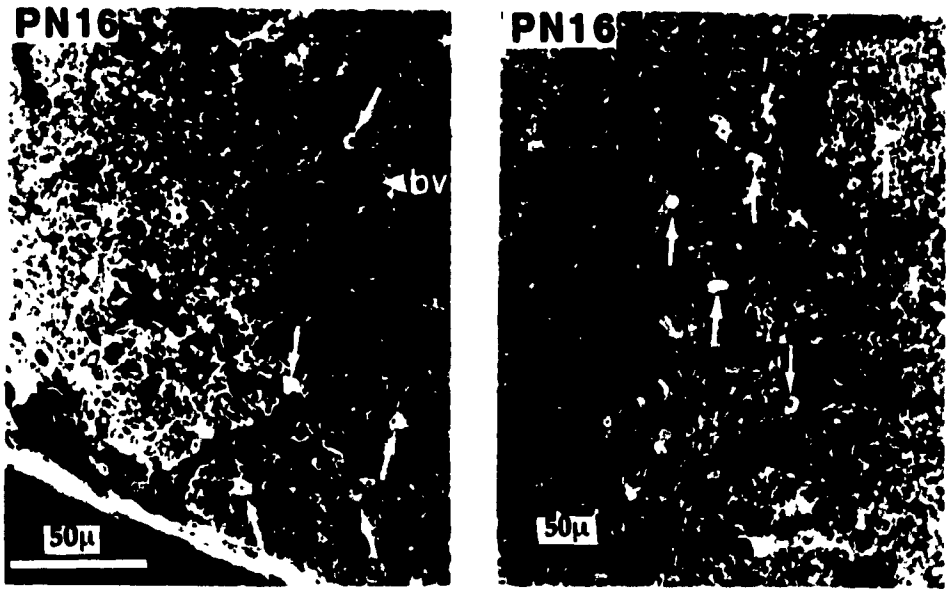


Figure 21: PN day 16 rabbit optic nerve. Many axons are unreactive. The intensity of taurine-IR within axons is further decreased. Numerous immunoreactive glia are seen (arrows) [bv=blood vessel]. **Left** : A peripheral area of the nerve. The tendency for blood vessels to be bounded by taurine-IR is beginning to be seen (compare with adult). The meninges wrapping the nerve are folded and artefactually fluorescent. **Right** : An interior region of the nerve.

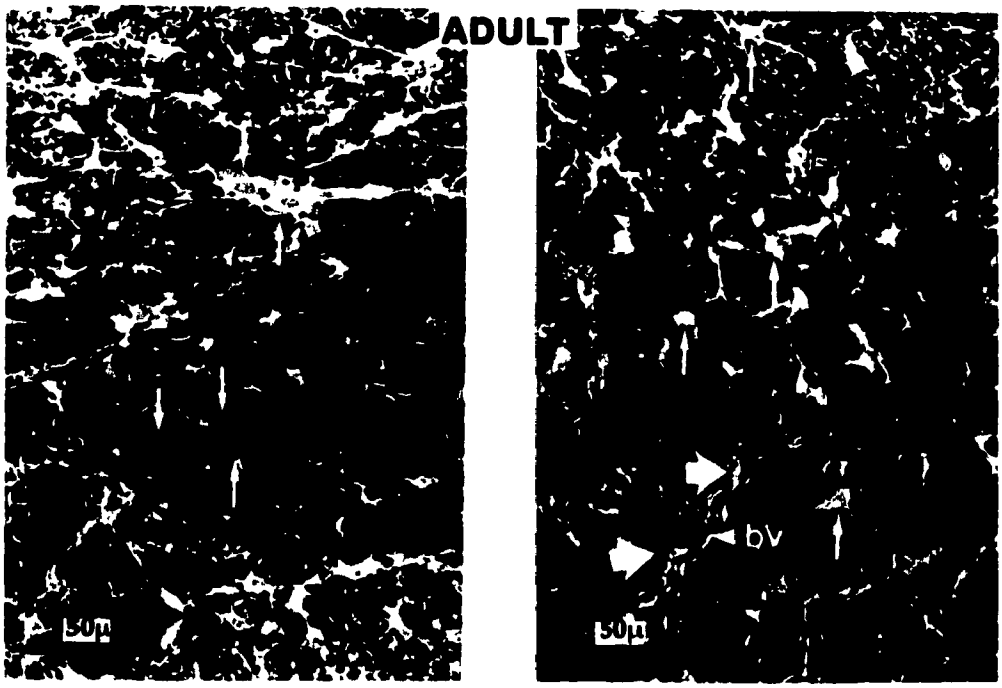


Figure 22: Adult rabbit optic nerve. Taurine-IR is now absent from axons and is most prominent in the glial perikarya (arrows) and in their radial processes that subdivide the unreactive axons into fascicles. In the right panel is seen a blood vessel (bv) bounded by taurine-IR astrocytic endfeet (thick arrows).

GENERAL DISCUSSION

A) Taurine in the Retina

Postnatal Development

Taurine is present in the rabbit retina at birth and increases in concentration four-fold during the first month to attain adult levels of about 40 to 50 µmol/g wet weight (Voaden et al, 1981). Using immunocytochemistry, I have been able to provide the first description in rabbits of the cellular location of this taurine. I have noted a general pattern of expression of taurine immunoreactivity during an early phase of neuronal differentiation and maturation when cell processes and growth cone formation begins. There is some evidence that taurine plays some role in the growth cone since it is found there (Taylor and Gordon-Weeks, 1989 ; Huxtable, 1989) and can facilitate neurite outgrowth (Spoerri et al, 1990a, 1990b ; Lima et al, 1988). In some retinal cell types this taurine-IR is a transitory feature such as in the ganglion cells ; in other cell types it remains to maturity such as in bipolar cells and the photoreceptor cells, and in some horizontal and amacrine cells.

At postnatal day 1, the earliest time I studied, taurine-IR is prominent in the type-A horizontal cells whose differentiation precedes that of other neurons in the outer neuroblast layer and whose lateral processes organize the development of the outer plexiform layer. Kainic acid destruction of these horizontal cells at birth disrupts later photoreceptor development and outer plexiform layer organization (Messersmith and Redburn, 1990). The rod/cone ratio is significantly higher and synapses fail to form although the cell processes of the rods, cones, bipolars and type-B horizontal cells are there. This is interpreted as a lack of a neurotrophic substance usually released from the horizontal cells, which is crucial for synaptic development. GABA has been implicated in these neurotrophic effects and my findings suggest that taurine may share this role.

I have found that taurine-IR remains detectable in some adult horizontal cells, unlike GABA-IR which is lost (Redburn and Madtes, 1986), suggesting that in the adult, taurine could function as a transmitter in these inhibitory cells. Redburn and Keith (1987) have previously speculated that the type-A horizontal cells change transmitter phenotype during postnatal development and utilize another inhibitory

transmitter for their involvement in mature visual pathways.

B) Taurine in Retinal Ganglion Cell Axons

Another striking localization of taurine-IR at early times is within ganglion cell axons in the retinal nerve fiber layer, at high levels from birth to postnatal day 7 and decreasing by postnatal day 10. I have also shown taurine-IR in the continuation of these axons in the optic nerve during this period. Here it is also maximal at early times and declines sharply around postnatal day 10, while its appearance within optic nerve glia increases in prominence being maximal in adults.

It has been noted by others that optic nerve axoplasmic transport to higher visual centers of labelled taurine injected into the eye is greater at early postnatal times, prior to and during the period of synaptic formation, than in mature tissues (Sturman, 1978). My studies show that this probably is reflective of the transport of endogenous taurine that has a magnitude dependent on developmental age.

Some speculations on the function of this axonal taurine include a role in [a] the stabilization of the enlarging axonal diameters (Spoerri et al, 1990a, 1990b : in vitro, taurine enhances cytoskeletal proliferation), and/or [b] in the refinement of the connections of retinal afferents within higher visual centers occurring during this time (Huxtable et al, 1989 : correlations of taurine content and membrane phospholipid composition in developing brain synaptosomes). [c] Another possible role of axonal taurine is modulation of axonal conduction properties. Work by Simmonds (1983) has shown that conduction properties of mature optic nerve axons are modulated by exogenous applications of taurine. Taurine produced depolarization that was unaffected by bicuculline and was only partly antagonized by strychnine. This indicated that taurine did not activate GABA receptors, had a minor effect on glycine receptors, but that most of its action involved a unique population of receptors. These findings have been treated as a curiosity since the lack of synapses within the nerve meant there was no obvious source of these "neurotransmitters".

My studies showed that in developing optic nerve, taurine-IR was prominent and localized specifically to the optic nerve axons. By adulthood, its predominant localization shifted to the glial perikarya and their processes. My findings suggest at early times (up to PN day 10) axons could autoregulate their conduction properties by

taurine release and that after that glial cells may be the endogenous source of this nonsynaptic modulation.

C) Taurine as a Putative Neurotransmitter in the Retina

For some of the cells in the retina, expression of taurine-IR is a transient phenomenon, but for certain sub-populations of amacrine cells and horizontal cells, taurine-IR is retained through adulthood. It is a candidate for the neurotransmitter of these cells since they are inhibitory interneurons and taurine has a potent depressant action (Krnjevic, 1974).

I found in late postnatal and adult retinas that the inner plexiform layer (IPL) has punctate deposits of taurine-IR which are most probably the synaptic terminals of taurine-IR bipolar and amacrine cells. Numerous, large taurine-IR processes resembling terminals close to the ganglion cell bodies, in sublamina b of the IPL, are also seen and these are in the position of rod bipolar terminals to the ON-center ganglion cells. In the rat, using electron microscopic immunocytochemistry similar endings have been positively identified as bipolar terminals by their characteristic synaptic ribbon content (Lake and Verdone-Smith,1989).

High levels of taurine-IR are also found in photoreceptors and rod bipolars both during development and in the adult, especially in the photoreceptor outer segments and the myoid regions of their inner segments, and the synaptic terminals of photoreceptors and bipolars. The photoreceptor and bipolar neurotransmitter is known to be excitatory and is believed to be glutamate and aspartate. Since taurine has an inhibitory glycine-like action, it is unlikely to be the neurotransmitter in these cells, although there is always the possibility of co-release. Since taurine has been implicated in actions on the cytoskeleton (discussed above) and shown to regulate intracellular high affinity calcium binding (Huxtable and Sebring, 1986) perhaps its role has more to do with the cytoskeleton and mechanisms of transmitter release, rather than it being a neurotransmitter in these two cell types.

Although the postnatal increase in retinal taurine does coincide with photoreceptor cell development, my studies have shown that expression of taurine-IR is not restricted to these cells. The expression of taurine-IR in cells and their processes at an early stage in the differentiation of most of the retinal neurons is circumstantial

evidence that taurine plays some role during development and is complementary to the evidence from other researchers of delayed or distorted development in taurine-deficient animals.

The lack of taurine-IR in retinal glia is surprising in view of their marked capacity to accumulate radioactive taurine as documented with autoradiography by Ehinger (Ehinger, 1973).

My studies are only descriptive, but they do suggest a rather generalized role for taurine in development. Precise mechanisms can only be elucidated through further studies, for example, of the effects of taurine content on the transcription and translation of cytoskeletal proteins.

For many cells expression of taurine-IR is a transient phenomenon ; presumably taurine may serve a different role in those cells in which it persists through adulthood. Functional studies will have to be made in order to establish what these roles may be.

REFERENCES

Barnstable, C.J., Blum, A.S., Devoto, S.H., Hicks, D., Morabito, M.A., Sparrow, J.R. and Treisman, J.E. (1988). Cell differentiation and pattern formation in the developing mammalian retina. Neuroscience Research, Suppl.**8**: S27-S41.

Battista, G. and Lake, N. Unpublished.

Borg, J., Balcar, V.J., Mark, J. and Mandel, P. (1979). Characterization of taurine uptake by neuronal and glial cells in culture. J. Neurochem. **32:** 1801-1805.

Campistron, G., Geffard, M. and Buijs, R.M. (1986). Immunological approach to the detection of taurine and immunocytochemical results. J. Neurochem. **46**: 862-868.

Carter-Dawson, L.D. and LaVail, M.M. (1979). Rods and cones in the mouse retina. II. Autoradiographic analysis of cell generation using tritiated thymidine. J. Comp. Neurol. **188**: 263-272.

Collins, G.G.S. (1977). On the role of taurine in the mammalian central nervous system. **In:** "Essays in neurochemistry and neuropharmacology", Vol.1 (eds. : Youdin, M.B.H., Lovenberg, W., Sharman, D.F., Lagnado, J.R.), pp. 43-72. Wiley.

Crespo, D., O'Leary, D.D.M. and Cowan, W.M. (1985). Changes in the numbers of optic nerve fibers during late prenatal and postnatal development in the albino rat. Dev. Brain Research **19**: 129-134.

Crespo, D. and Viadero, C.F. (1989). The microvascular system of the optic nerve in control and enucleated rats. Microvascular Research **38**: 237-242.

Dowling, J.E. and Boycott, B.B. (1966). Organization of the primate retina : electron microscopy. Proc. Roy .Soc. B **166**: 80.

Dowling, J.E. (1987). The retina– An approachable part of the brain. The Belknap Press of Harvard University Press, Cambridge, Massachusetts, and London, England.

Ehinger, B. (1973). Glial uptake of taurine in the rabbit retina. Brain Research **60**: 512-516.

Frederickson, R.C.A., Neuss, M., Morzorati, S.L. and McBride, W.J. (1978). A comparison of the inhibitory effects of taurine and GABA on identified Purkinje cells and other neurons in the cerebellar cortex of the rat. Brain Research **145:** 117-126.

Gaull, G.E. (1989). Taurine in pediatric nutrition : review and update. Pediatrics **83(3):** 433-442.

Haas, H.L. and Hosli, L. (1973). The depression of brainstem neurones by taurine and its interaction with strychnine and bicuculline. Brain Research **52:** 399-402.

Hamaguchi, T., Azuma, J. and Schaffer, S. (1991). Interaction of taurine with methionine : inhibition of myocardial phospholipid methyltransferase. J. Cardiovasc. Pharmacology **18:** 224-230.

Hayes, K.C., Carey, R.E. and Schmidt, S.Y. (1975). Retinal degeneration associated with taurine deficiency in the cat. Science **188:** 949-950.

Hayes, K.C. (1976). A review on the biological function of taurine. Nutrition Reviews **34(6):** 161-165.

Hayes, K.C., Stephan, Z.F. and Sturman, J.A. (1980). Growth depression in taurine-depleted infant monkeys. Nutrition **110**: 2058-2064.

Horsburgh, G.M. and Sefton, A.J. (1986). The early development of the optic nerve and chiasm in embryonic rat. J. Comp. Neurol. **243**: 547-560.

Hruska, R.E., Padjen, A., Bressler, R. and Yamamura, H.I. (1978). Taurine : Sodium dependent, high affinity transport into rat brain synaptosomes. Molecular Pharmacology **14:** 77-85.

Hughes, A. (1971). Topographical relationships between the anatomy and physiology of the rabbit visual system. Documenta Ophth. **30**: 33-159.

Huxtable, R.J. (1981). Insights on function : metabolism and pharmacology of taurine in the brain. **In:** "The role of peptides and amino acids as neurotransmitters", pp. 53-97. Alan R.Liss Inc.

Huxtable, R.J. and Sebring, L.A. (1983). Cardiovascular actions of taurine. **In:** "Sulfur amino acids : biochemical and clinical aspects", pp.5-34. Alan R.Liss.

Huxtable, R.J. (1989). Taurine in the central nervous system and the mammalian actions of taurine. **In:** Progress in Neurobiology **32:** 471-533.

Huxtable, R.J., Crosswell, S. and Parker, D. (1989). Phospholipid composition and taurine content of synaptosomes in developing rat brain. Neurochem. Int. **15(2):** 233-238.

Huxtable, R.J. (1992). Physiological actions of taurine. Physiol. Rev. **72(1):** 101-163.

Jacobsen, J.G. and Smith, L.L.H. (1968). Biochemistry and physiology of taurine and taurine derivatives. Physiol. Rev. 48: 424-511.

Jacobsen, J.G. (1980). Possible physiological functions of taurine in mammalian systems. **In:** "Natural Sulfur Compounds", (eds. : Cavallini, D., Gaull, G.E. and Zappia, V.), pp.163-173. Plenum Press, New York and London.

Krnjevic, K. (1974). Chemical nature of synaptic transmission in vertebrates. Physiol. Rev. **54(2)**: 418-540.

Lake, N. (1983). Taurine depletion of lactating rats : Effects on developing pups. Neurochemical Research **8(7):** 881-887.

Lake, N. (1986). Electroretinographic deficits in rats treated with guanidinoethyl sulfonate, a depletor of taurine. Exp. Eye Res. **42:** 87-91.

Lake, N. and Malik, N. (1987). Retinal morphology in rats treated with a taurine transport antagonist. Exp. Eye Res. **44:** 331-346.

Lake, N. and Verdone-Smith, C. (1989). Immunocytochemical localization of taurine in the mammalian retina. Current Eye Research **8(2)**: 163-173.

Larsen, B.R., Grosso, D.S. and Chang, S.Y. (1980). A rapid method for taurine quantitation using high performance liquid chromatography. J. Chromatogr. Sci. **18**: 233-236.

Lewis, G.P., Erickson, P.A., Kaska, D.D. and Fisher, S.K. (1988). An immunocytochemical comparison of Muller cells and astrocytes in the cat retina. Exp. Eye Res. **47**: 839-853.

Lima, L., Matus, P. and Drujan, B. (1988). Taurine effect on neuritic growth from goldfish retinal explants. Int. J. Devl. Neuroscience **6(5)**: 417-424.

Madsen, S. (1990). Immunocytochemical visualization of taurine-containing and taurine-synthesizing cells. **In:** "Taurine : Functional Neurochemistry, Physiology and Cardiology", (eds. : Pasantes-Morales, H., Martin, D L., Shain, W. and Martin del Rio, R),.pp.21-28. Wiley-Liss Inc.

Marc, R.E. (1986). The development of retinal networks. **In**: "The Retina--A model for cell biology studies, Part 1", (eds. · Adler, R. and Farber, D), pp.17-65. Academic Press, Inc.

Messersmith, E.K. and Redburn, D.A. (1990). Kainic acid lesioning alters development of the outer plexiform layer in neonatal rabbit retina. Int. J. Devl.

Neuroscience **8(4)**: 447-461.

Messersmith, E.K. and Redburn, D.A. (1992). Gamma-aminobutyric acid immunoreactivity in multiple cell types of the developing rabbit retina. Visual Neuroscience **8**: 201-211.

Neuringer, M. and Sturman, J.A. (1987). Visual acuity loss in rhesus monkey infants fed a taurine-free human infant formula. J. Neuroscience Research **18**: 597-601.

O'Leary, D.D.M., Stanfield, B.B. and Cowan, W.M. (1981). Evidence that the early postnatal restriction of the cells of origin of the callosal projection is due to the elimination of axonal collaterals rather than to the death of neurons. Dev. Brain Research **1**: 607-617.

Ottersen, O.P. and Storm-Mathisen, J. (1985). Different neuronal localization of aspartate-like and glutamate-like immunoreactivities in the hippocampus of rat, guinea pig and Senegalese baboon (Papio papio), with a note on the distribution of gamma-aminobutyrate. Neuroscience **16**: 589-606.

Ottersen, O.P. (1988). Quantitative assessment of taurine-like immunoreactivity in different cell types and processes in rat cerebellum : an electron microscopic study based on a postembedding immunogold labelling procedure. Anat. Embryol. **178**: 407-421.

Palackal, T., Moretz, R.C., Wisniewski, H.M. and Sturman, J.A. (1988). Ultrastructural abnormalities in the visual cortex of kittens from taurine-deficient mothers. Brain Dysfunction **1**: 71-89.

Politis, M.J. and Ingoglia, N.A. (1979). Axonal transport of taurine along neonatal and young adult rat optic axons. Brain Research **166**: 221-231.

Polley, E.H., Zimmerman, R.P. and Fortney, R.L. (1989). Neurogenesis and maturation of cell morphology in the development of the mammalian retina. **In**: "Development of the vertebrate retina", (eds. : Finlay, B.L. and Sengelaub, D.R),.pp.3-29. Plenum Press, New York and London.

Provis, J.M. and Penfold, P.L. (1988). Cell death and the elimination of retinal axons during development. Progress in Neurobiology **31**: 331-347.

Raff, M.C. (1989). Glial cell diversification in the rat optic nerve. Science **243**: 1450-1455.

Rapaport, D.H. and Stone, J. (1983). Time course of the morphological differentiation

of cat retinal ganglion cells : influences on cell size. J. Comp. Neurol. **221**: 42-52.

Rassin, D.K. (1981). The function of taurine in the central nervous system. Adv. Biochem. Psycho. Pharmacol. **29:** 127-134.

Raviola, G. and Raviola, E. (1967). Light and electron microscopic observations on the IPL of the rabbit retina. Amer. J.Anat. **120**: 403-426.

Redburn, D.A. and Madtes, P. (1986). Postnatal development of ^{3}H-GABA accumulating cells in rabbit retina. J. Comp. Neurol. **243**: 41-57.

Redburn, D.A. and Keith, M.E. (1987). Developmental alterations in retinal Gabaergic neurons. **In:** "Neurotrophic activity of GABA during development", pp.79-108.

Reichenbach, A., Schnitzer, J , Friedrich, A., Ziegert, W., Bruckner, G and Schober, W. (1991). Development of the rabbit retina I. Size of eye and retina, and postnatal cell proliferation. Anat. Embryol. **183**: 287-297.

Robinson, S.R., Horsburgh, G.M., Dreher, B. and McCall, M.J (1987). Changes in the numbers of retinal ganglion cells and optic nerve axons in the developing albino rabbit. Dev. Brain Research **35**: 161-174.

Robinson, S.R. and Dreher, Z. (1989). Evidence for three morphological classes of astrocyte in the adult rabbit retina : functional and developmental implications Neurosci. Lett. **106(3)**: 261-268.

Schnitzer, M. and Rusoff, A.C. (1984). Horizontal cells of the mouse retina contain glutamic acid decarboxylase-like immunoreactivity during early development stages. J. Neurosci. **4**: 2948-2955.

Schnitzer, J. (1985). Distribution and immunoreactivity of glia in the retina of the rabbit. J. Comp. Neurol. **240**: 128-142.

Schnitzer, J. (1988a). The development of astrocytes and blood vessels in the postnatal rabbit retina. J. Neurocytol. **17**: 433-449.

Schnitzer, J. (1990). Postnatal gliogenesis in the nerve fiber layer of the rabbit retina : an autoradiographic study. J. Comp. Neurol. **292**: 551-562.

Sefton, A.J. and Lam, K. (1984). Quantitative and morphological studies on developing optic axons in normal and enucleated albino rats. Exp. Brain Res. **57**: 107-117.

Simmonds, M.A. (1983). Neuronal responses to taurine are distinct from those to GABA and glycine in rat cuneate nucleus and optic nerve. British J. Pharmacol. **78**:

19P.

Spoerri, P.E., Caple, C.G. and Roisen, F.J. (1990a). Taurine-induced neuronal differentiation : the influence of calcium and the ganglioside GM1. Int. J. Devl. Neuroscience **8(4)**: 491-503.

Spoerri, P.E., Dozier, A.K. and Roisen, F.J. (1990b). Calcium regulation of neuronal differentiation : the role of calcium in GM1-mediated neuritogenesis. Dev. Brain Research **56**: 177-188.

Sternberger, L.A., Hardy, P.H., Cuculis, J.J. and Meyer, M.G. (1970). The unlabelled antibody-enzyme method of immunohistochemistry. J. Histochem. Cytochem. **18**: 315-333.

Stone, J., Egan, M., Rapaport, D.H. (1985). The site of commencement of retinal maturation in the rabbit. Vision Research **25**: 309-317.

Strettoi, E., Dacheux, R.F. and Raviola, E. (1990). Synaptic connections of rod bipolar cells in the inner plexiform layer of the rabbit retina. J. Comp. Neurol. **295**: 449-466.

Sturman, J.A. (1978). Taurine in the developing rabbit visual system : changes in concentration and axonal transport including a comparison with axonally transported proteins J. Neurobiology **10(3)**: 221-237.

Sturman, J.A. (1979). Taurine in developing rat brain : changes in blood-brain barrier. J. Neurochem. **32:** 811-816.

Sturman, J.A., Moretz, R.C., French, J.H. and Wisniewski, H.M. (1985). Postnatal taurine deficiency in the kitten results in a persistence of the cerebellar external granule cell layer : correction by taurine feeding. J. Neuroscience Research **13**: 521-528.

Sturman, J.A., Palackal, T., Imaki, H., Moretz, R.C., French, J. and Wisniewski, H.M. (1987). Nutritional taurine deficiency and feline pregnancy and outcome. **In**: "The biology of taurine", (eds. : Huxtable, R.J., Franconi, F. and Giotti, A.), pp.113-124. Plenum Press, New York and London.

Sturrock, R.R. (1975). A light and electron microscopic study of proliferation and maturation of fibrous astrocytes in the optic nerve of the human embryo. J. Anat. **119**: 223-234.

Taylor, J. and Gordon-Weeks, P.R. (1989). Developmental changes in the calcium

dependency of ∂-Aminobutyric acid release from isolated growth cones : correlation with growth cone morphology. J. Neurochem. **53:** 834-843.

Taylor, J., Docherty, M. and Gordon-Weeks, P.R. (1990). GABAergic growth cones : release of endogenous gamma-Aminobutyric acid precedes the expression of synaptic vesicle antigens. J. Neurochem. **54(5)**: 1689-1699.

Thurston, J.H., Hauhart, R.E. and Dirgo, J.A. (1980). Taurine : a role in osmotic regulation of mammalian brain and possible clinical significance. Life Sciences **26:** 1561-1568.

Trachtman, H., Del Pizzo, R., Sturman, J.A., Huxtable, R.J. and Finberg, L. (1988). Taurine and osmoregulation II. Administration of taurine analogues affords cerebral osmoprotection during chronic hypernatremic dehydration AJDC **142:** 1194-1198.

Trenkner, E. (1990). Possible role of glutamate with taurine in neuron-glia interaction during cerebellar development. **In:** "Taurine : Functional Neurochemistry, Physiology and Cardiology", (eds. : Pasantes-Morales, H., Martin, D.L., Shain, W. and Martin del Rio, R.), pp.133-140. Wiley-Liss Inc

Troll, W. and Cannan, R. (1953). A modified photometric ninhydrin method for the analysis of amino and imino acids. J. Biol. Chem. **200**: 803-811.

Vaney, D.I. and Hughes, A. (1976). The rabbit optic nerve : Fibre diameter

spectrum, fibre count and comparison with a retinal ganglion cell count. J. Comp Neurol. **170**: 241-252.

Vaney, D.I., Young, H.M. and Gynther, I.C. (1991). The rod circuit in the rabbit retina. Visual Neuroscience **7**: 141-154.

Voaden, M.J., Oraedu, A.C.I., Marshall, J. and Lake, N. (1981). Taurine in the retina. **In**: "The effects of taurine on excitable tissues", (eds. : Schaffer, S.W., Baskin, S.I. and Kocsis, J.J), pp.145-160. Spectrum Publications Inc., New York.

Walsh, C. and Polley, E.H. (1985). The topography of ganglion cell production in the cat's retina. J. Neurosci. **5**: 741-750.

Wright, C.E., Tallan, H.H. and Lin, Y.Y. (1986). Taurine : biological update. Ann. Rev. Biochem. **55**: 427-53.

Wu, J.-Y., Liao, C., Lin, C.J., Lee, Y.H., Ho, J.-Y. and Tsai, W.H. (1990). Taurine receptor in the mammalian brain. **In:** "Taurine : Functional Neurochemistry,

Physiology, and Cardiology", (eds. : Pasantes-Morales, H., Martin, D.L., Shain, W. and Martin del Rio, R.), pp.147-156.

Yates, C., Dewar, A., Wilson, H., Winterburn, A. and Reading, H. (1974). Histological and biochemical studies on the retina of a new strain of dystrophic rat. Exp. Eye Res. **18**: 119-133.