nine sulfoximine (*24*), whose primary biochemical effect appears to be the inhibition of glutamine synthetase (*25*).

Few definitive data are available regarding glial physiology. The finding that glutamine synthetase is restricted to glia defines a metabolic function for these cells and may clarify their role in certain neurologic disorders of man.

ANTONIO MARTINEZ-HERNANDEZ
Department of Pathology, University of Colorado Medical Center, Denver 80220
KATHERINE P. BELL
MICHAEL D. NORENBERG
Laboratory Service, Denver Veterans Administration Hospital, Denver 80220 and *Department of Pathology, University of Colorado Medical Center*

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Visual Pigment Changes in Rainbow Trout in Response to Temperature

Abstract. *Lower water temperature (6°C in comparison to 16°C) favors a higher proportion of porphyropsin in the retina of rainbow trout (*Salmo gairdneri*), regardless of the light conditions (constant darkness or 12 hours of light and 12 of darkness). This response to temperature does not follow a* Q_{10} *relation, namely an increase in the rate of a reaction produced by raising the temperature 10°C.*

Many species of fishes have paired visual pigments in their retinas (*1, 2*). The visual pigment composition in several of the paired-pigment species changes seasonally or during the spawning migration (*2*).

Both light (quality, intensity, and photoperiod) and temperature have been implicated as factors involved in changes in visual pigment composition (*2–8*). Light (as compared with darkness) is associated with decreases in the percentage of porphyropsin in some of these species [rudd, *Scardinius erythrophthalmus*; golden shiner, *Notemigonus crysoleucas*; and common shiner, *Notropis cornutus* (*3, 4, 6, 8*)] and increases in others [redside shiner, *Richardsonius balteatus*; rainbow trout, *Salmo gairdneri*; and brook char, *Salvelinus fontinalis* (*5, 7*)]. On the other hand, higher temperatures favor decreases in the proportion of porphyropsin in golden shiner (*6*), rainbow trout, and common shiner (*8*). From this, it has been suggested that temperature is an important factor in the similar seasonal alteration of visual pigment composition (higher porphyropsin in winter) in species that do not respond in the same way to light and darkness (*7*).

A question with respect to the effect of temperature is whether temperature directly influences a change in visual pigment composition or only modifies the increasing rate of a chemical reaction with each 10°C increase in temperature (a Q_{10} effect) irrespective of the direction of the change (relative increase or decrease in porphyropsin). We now report evidence that temperature is directly associated with a change in the molar percentage of porphyropsin in rainbow trout.

Juvenile rainbow trout (35 to 140 g) were obtained from the Alberta Provincial Government Rearing Station at Crammond, Alberta, in January 1975. The water temperature at the rearing station was 5°C. The fish were divided into two groups; one group was used immediately in the first set of temperature experiments, and the other group was exposed to constant darkness at 16°C for 30 days. During the experiments, the fish were held in 120-liter aquamarine, fiber glass tanks covered with recessed, lightproof lids painted white on the inside. For fish exposed to light, two 20-watt fluorescent tubes (General Electric Cool White) were placed in the top of the lid, and the photoperiod, 12 hours of light and 12 hours of darkness (LD 12 : 12), was controlled by an automatic timer (Tork). A 7.5-watt red bulb was used in the continuously dark tanks for daily (1- to 2-minute) inspection of the fish. The total radiant energy at the water surface in the illuminated tanks, recorded from the remote probe of a spectroradiometer (Isco model SR), was from 50 to 87 μw/cm^2 over the range of 380 to 750 nm. For the red bulb it was very low (0.6 to 0.9 μw/cm^2 from 380 to 750 nm). Water temperature was controlled by a thermoregulator (CanLab C-Series, T3345) in combination with a relay connected to a solenoid valve on a warm (18°C) water line. Cold (5°C) water constantly flowed into the tank, and the desired temperature (6° or 16°C) was attained by adding warm water from the controlled solenoid valve. Total flow was adjusted to provide about 150 liter/hour. Fish were not fed during any experiment.

At the beginning of each set of experiments, the trout were dark-adapted for 2 hours and anesthetized with tricaine

methanesulfonate; the left eyes were removed (*9*) under dim red light (Kodak Wratten Series 2 filter). At the end of an experiment, the fish were again dark-adapted; they were killed and their right eyes were removed. Preparation and analysis of the visual pigment extracts followed a standard procedure (*10*).

When the trout arrived from the rearing station, porphyropsin predominated in their retinas (Fig. 1A, mean molar percentage = 85 percent). After 28 days, the trout held at 6°C (groups Y and W) had higher mean molar percentages of porphyropsin than those held at 16°C (groups Z and X) regardless of the light period. The largest change occurred in group X (16°C, LD 0 : 24) in which rhodopsin came to predominate. Trout in a light-dark cycle (LD 12 : 12) had, on the average, higher percentages of porphyropsin than those in constant darkness. These results agree with the conclusions of previous investigators (*7, 8, 11*), that light and low temperature, compared with darkness and high temperature, favor higher molar percentages of porphyropsin in rainbow trout.

A second set of experiments, with trout previously held for 30 days in constant darkness at 16°C, was performed in order to determine whether low temperature (6°C) would increase the porphyropsin in fish in which rhodopsin was predominant. Initially these fish had a mean molar percentage of porphyropsin of 35 percent (Fig. 1B), which agrees with that of group X (16°C, LD 0 : 24) (Fig. 1A). After 28 days in the specified experimental conditions, group C (6°C, LD 12 : 12) showed the greatest change in visual pigment composition, increasing to a mean molar percentage of porphyropsin of 85 percent. This agrees with the mean value (80 percent) for group Y (6°C, LD 12 : 12) in the first experiment (Fig. 1A). It is not likely that the response to temperature is a Q_{10} effect because, although trout of group A (16°C, LD 12 : 12) had a small increase in the mean molar percentage of porphyropsin, it did not compare to that of group C. Regardless of the light conditions, the two groups held at a lower temperature had higher mean molar percentages of porphyropsin than their counterparts held at a higher temperature.

As in the first experiment, a group held in the light-dark cycle had more porphyropsin than a group held in darkness at the same temperature (Fig. 1B). Although the mean molar percentages of porphyropsin in groups Y and C are similar, those of groups Z and A, W and D, and X and B are not. This is not unexpected because, in the first experiment

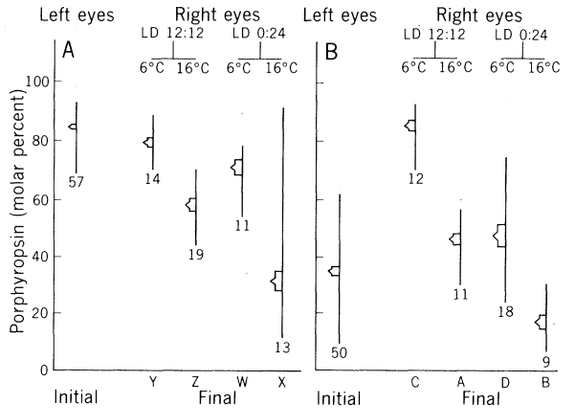

Fig. 1. Composition of visual pigment (*1*) in rainbow trout at the beginning (left eyes) and at the end (right eyes) of the first (A) and second (B) experiments. In each experiment, the left eyes were removed before the fish were separated into four groups. Experimental treatments entailed subjecting the fish to light and temperature regimes for 28 days. The experiments were identical except that before experiment B began, the fish were held at 16°C in constant darkness for 30 days. Each vertical line is the range, the vertical bar is one standard error from the mean, and the sample size appears beneath each range.

(Fig. 1A), the initial percentage of porphyropsin was high and the changes were toward decreases, whereas in the second experiment (Fig. 1B), the initial percentage of porphyropsin was low and, except for group B, the changes were toward increases in porphyropsin. That group B had been held at 16°C in complete darkness for an additional 30 days compared to group X may account for the further decrease in porphyropsin. It seems evident that the lower temperature favors the maintenance of higher proportions of porphyropsin (Fig. 1A) or an increase in porphyropsin (Fig. 1B); in the latter case the change in the composition of visual pigment cannot be attributed to a simple Q_{10} effect. Moreover, the results indicate that both light (or its absence) and temperature significantly (*12*) influenced the rhodopsin-porphyropsin ratios in rainbow trout.

The report of a seasonal change in the rhodopsin-porphyropsin ratio in the rudd some 15 years ago (*3*), as well as earlier studies (*13*) on visual pigment changes in other fishes during their life cycle, has led to many subsequent investigations of the rhodopsin-porphyropsin ratios in several teleost species [for reviews, see (*2*)]. Our study demonstrates that seasonal changes in the composition of visual pigment in certain paired-pigment species may be related to seasonal temperature changes. Certain hormones (thyroxine, bovine thyroid stimulating hormone, and ovine prolactin) can change the visual pigment ratios in several species (*5, 9, 14–16*). Consequently, a possible explanation of the effect of temperature is that it acts through a neuroendocrine system. Although thyroid hormones effectively increase the proportion of porphyropsin in several salmonid species [rainbow trout (*9, 14*); kokanee salmon, *Oncorhynchus nerka* (*15, 16*), and coho salmon, *Oncorhynchus kisutch* (*16*)], the plasma levels of thyroxine did not differ significantly among any of the groups of rainbow trout in this study (*17*).

The ability to predictably manipulate the visual pigment composition of rainbow trout has important implications for further studies on the intrinsic mechanisms controlling rhodopsin-porphyropsin ratio changes in paired-pigment species. In addition, because the light environment as well as temperature affects the composition of visual pigment in certain paired-pigment teleosts (*2–8*), further investigations on seasonal changes of the light and temperature environments in relation to visual pigment changes are necessary in order to elucidate the biological significance of these events.

ANDREW T. C. TSIN
DAVID D. BEATTY
Department of Zoology,
University of Alberta,
Edmonton, Alberta, Canada T6G 2E9

References and Notes

1. "Paired visual pigments" refers to the presence of rhodopsin and porphyropsin, either simultaneously or sequentially, in the retina of a species. The rhodopsin and the porphyropsin of a species result from the respective conjugation of 11-*cis* retinal and 11-*cis* 3-dehydroretinal to the same opsin (*2*). The composition of the visual pigment in the retina is expressed by the relative proportion (molar percentage) of the two visual pigments. Absorbance percentages (*10*) were converted to molar percentages using 40,600 and 30,000 as the molar extinction values of rhodopsin and porphyropsin, respectively.
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Identification of Retinoyl Complexes as the Autofluorescent Component of the Neuronal Storage Material in Batten Disease

Abstract. *Cytosomes filled with intensely fluorescent material in the form of curvilinear bodies were isolated by density gradient centrifugation followed by pronase digestion from the cerebral cortex of a child who had died at age 7 from the late infantile form of Batten disease. Forty-three percent of the dry weight of the storage material was extracted by a mixture of chloroform and methanol, leaving a water-insoluble amorphous fluorescent residue. Infrared spectroscopy, proton magnetic resonance spectroscopy, and mass spectrometry of this residue strongly suggested the presence of retinoyl polyenes linked to a small peptide. Base hydrolysis and methanolysis yielded retinoic acid and methyl retinoate, respectively. Ozonolysis yielded a product derived from the substituted cyclohexenyl ring of vitamin A. The results indicate that the fluorescent component of the neuronal storage material is a retinoyl complex and is not derived from peroxidized polyunsaturated fatty acids as previously thought.*

The late infantile type of Batten disease is an inherited neurological disorder of children which starts between 2 and 4 years of age with seizures and visual impairment, followed by progressive intellectual retrogression, blindness, and cerebellar dysfunction, and leads to death between 5 and 9 years. Pathologically, neurons and cells in many other tissues are filled with cytosomes that contain an autofluorescent lipopigment often referred to as ceroid (*1*). By electron microscopy the storage material appears in the form of masses of small crescentic stacks of lamellae referred to as curvilinear bodies (CLB's) (*2*). The term neuronal-ceroid-lipofuscinosis is often used for both the late infantile and the juvenile form of this disease (*3*). The chemical nature of the storage material and biochemical defect in this disease are unknown. A widely held hypothesis is that the fluorophore of both ceroid and lipofuscin is derived from the peroxidation of polyunsaturated fatty acids (*4*). The released malonyldialdehyde could then form Schiff-base complexes with amino groups to form the fluorescent iminopropene chromophores, R−N=CH−CH=CH−NH−R, postulated as the fluorescent products of lipid peroxidation of liver mitochondria and microsomes (*5*). Reports of a *p*-phenylenediamine-dependent myeloperoxidase deficiency in leukocytes in Batten disease (*6*) have not been confirmed by several groups (*7*). We report here chemical studies on isolated cytosomes with curvilinear bodies which identify the fluorescent component as a derivative of retinoic acid.

A 7-year-old child was diagnosed before death as having Batten disease by appendiceal biopsy. Eight hours after death cerebral cortex was dissected free of white matter from the brain of this child. The cerebral tissue, homogenized in a Teflon glass homogenizer in 0.32*M* sucrose, was subfractionated by differential and discontinuous sucrose gradient centrifugation (*8*), and the fractions were monitored for the presence of CLB's by fluorescence and electron microscopy. The bulk of the autofluorescent granules was found in the P_1 or crude nuclear fraction. Vigorous rehomogenization of the P_1 fraction and centrifugation at 53,500*g* for 2 hours on a gradient of 0.8 to 1.8*M* sucrose revealed an intensely fluorescent interface between 1.4 and 1.6*M* sucrose (P_1D fraction), which was found by electron microscopy to be very rich in CLB's. This fraction was diluted with water and sedimented at high speed to give a pellet, which was incubated twice with pronase (*9*). The residue after the pronase digestions was suspended in distilled water and sedimented several times by centrifugation (100,000*g* for 1 hour). The final washed pellet was fixed in glutaraldehyde and osmium tetroxide and embedded. Sections revealed by electron microscopy a fraction consisting almost entirely of CLB's identical in morphology to those seen in intact brain tissue of patients (Fig. 1, A and B). Many repetitions of this procedure yielded 80 mg of the CLB's, which was sufficient for chemical analyses.

Extraction with a mixture of chloroform and methanol (2:1 by volume) removed 43 percent of the dry weight but none of the fluorescent component of CLB's. Standard lipid fractionation procedures (*10*) showed that phospholipids, cholesterol, and free fatty acids were the major lipid classes; neutral glycolipids and gangliosides were not found. The molar ratio of cholesterol to phospholipid was 0.45. Phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and sphingomyelin accounted for 43, 23, 11, and 10 percent, respectively, of the phospholipid phosphorus, with small amounts of phosphatidylinositol and lysolectithin and 6 percent unidentified. The major fatty acids of the total phospholipids were palmitic, stearic, and oleic acids, with 10 percent arachidonic and 6 percent docosahexenoic acids. However, electron microscopy of the fluorescent lipid-free residue revealed only an electron-opaque amorphous material, with complete loss of the lamellated curvilinear profiles.

The fluorescent component of lipid-free CLB's was not solubilized by 8*M* urea, 10 m*M* EDTA, or any common organic solvent except a mixture of dimethyl sulfoxide and water (2:1 by volume). The fluorescence emission maximum in the latter solvent system was at 425 to 430 nm, excited at 355 to 360 nm. Base hydrolysis (4*N* NaOH for 12 hours at 100°C) solubilized the CLB's without destroying the fluorescence, part of which could be extracted with dichloromethane. Acidification of the dichloromethane quenched the fluorescence. Ultraviolet spectra of the unhydrolyzed CLB's in dimethyl sulfoxide–water showed a maximum at 280 nm with a broad shoulder extending to 340 nm. After base hydrolysis the 280-nm peak was not present, but in ethanol a peak between 340 and 350 nm became apparent. Infrared spectra of the lipid-free CLB's in KBr pellets were informative. A broad peak at 3300 to 3400 cm^{-1} was assigned to N–H and O–H stretching, small absorptions at 2860 to 2960 cm^{-1} to C–H stretchings of CH_3- and CH_2- groups, strong peaks at 1650 and 1520 cm^{-1} to amide I (C=O stretching) and amide II (N–H bending), and a small peak at 1380 cm^{-1} to $-CH_3$ bending. Deuterium exchange shifted the amide II peak from 1520 to 1400 cm^{-1} and revealed a small shoulder at 1600 cm^{-1} due to C=C stretching. The 1650-