Effects of Prolonged Light Deprivation on the Development of Retinal Enzymes in the Rabbit*

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Whereas sensory input and reflex discharge are constant phenomena in brain, the retina receives its functional stimuli from a specific, controllable source (1). Brattgard (2) has investigated effects of light deprivation on total mass, percent nucleoprotein, and protein in ganglion cells of rabbit retina. Zetterstrom (3) determined electroretinographic responses of kittens grown in total darkness, and with Hellstrom (4) examined —SH group-staining properties of such retinas. This investigation was undertaken to determine the effects of total light deprivation on retinal enzyme activities. Rabbits were grown from birth either in total darkness or in normal diurnal light variation. At various ages their retinas and brains were assayed for dry weight, lipid, and protein content, and activities of several different enzymes (alkaline and acid phosphatases, isocitric, glutamic, lactic, malic, and glucose 6-P dehydrogenases). Histological and functional properties of the retinas were also examined.

Significant decreases were found in malic and lactic dehydrogenases, and acid phosphatase, and an increase in glucose 6-P dehydrogenase in the retinas of animals grown in total darkness from birth. These changes were not found in brain, nor did they occur in adult animals deprived of light for a similar period of time. These effects were not a result of changes in total protein content, per cent dry weight, or lipid. Histological investigations did not reveal any tissue changes that could be related to the alterations of enzyme patterns.

EXPERIMENTAL

General—The albino rabbits used in these experiments were obtained from the same inbred stock. All animals designated as adults were 10 to 12 months of age. Pregnant rabbits were obtained 7 to 10 days before delivery. Each was placed in a single cage where the litters were born and housed throughout the duration of the experiment. Two litters were grown in ordinary cages; two others were grown in cages from which all light was excluded. The two pairs of "light" and "dark" litters were separated in age by 14 days. At 26, 60, and 120 days of age several animals were removed from each cage and the determinations performed as described below. Cages from which all light was excluded were constructed of plywood and painted dull black on all inside surfaces. Ventilation, feeding, and cleaning were performed without introduction of light by means of suitable light-excluding devices. Frequent exposure of photographic film inside the cages revealed no demonstrable light except when animals were being removed.

Because of the large number of assays performed on each retina and brain, at most 5 or 6 animals could be used on any given day. Therefore all animals of a given age, both light and dark, were randomized into two groups, and the assays repeated on two consecutive days. The order of preparation of the animals in each day's experiment was determined by random lot, and this order was subsequently followed in the specific assays performed.

The animals were briefly tested for direct and consensual pupillary light reflexes in a dimly lit room. Newborn and 26-day-old animals were killed by decapitation; older animals were killed by air injection. The eyeballs and brain were rapidly removed in a cold room. The retinas were prepared first. The sclera was incised along the limbus and the cornea, lens, and vitreous were removed. The retina and sclera were flatly on filter paper and blotted dry. The retina was scraped free of the sclera with a small spatula, and the choroid with its large blood vessels was then lifted free from the retinal tissue. The retina was divided into two equal portions; one was placed in a weighed vial, the other weighed on a torsion balance and made into a 1:200 homogenate with glass-distilled water and with use of a hand-driven glass homogenizer. The entire brain was excised and divided longitudinally into two equal samples and treated essentially as described for retina. The time consumed from the death of the animal until completion of the tissue preparation was never longer than 20 minutes. This routine was altered in the case of newborn animals where an entire retina was used for weight determinations and another for enzyme assays because of the small amount of tissue. One or two adult rabbits were used in each experiment as controls.

Microscopic Sections—Samples from the macular area and the periphery of formalin-fixed retinas were stained with hematoxylin and eosin. Sections were made of a single retina of each of the following: adult, newborn, 60-day light, 60-day dark, 120-day dark, and 120-day light.

Wet Weight, Dry Weight, Fat-Free Weight, Total Protein—The tissues were dried under vacuum until weight was constant. Lipides were extracted with absolute alcohol at room temperature for two 12-hour periods followed by washing with n-hexane. Total protein was determined on the homogenate by the method of Lowry et al. (5).

Enzyme Assays—All assays of enzyme activity were performed on the 1:200 water homogenates. Enzyme activities were assayed in the following sequence in each day's experiment: alkaline phosphatase, acid phosphatase, and isocitric, glucose-6-P, glutamic, lactic, and malic dehydrogenases. All assays were performed on 1.0 to 3.0 µg. wet weight of tissue, with the procedures...
for handling samples, making and calibrating Lang-Levy pipettes, and cleaning the micro test tubes as described by Lowry et al. (6). Alkaline and acid phosphatases were assayed by the methods of Lowry et al. (i). The p-nitrophenyl phosphate was obtained from the Sigma Chemical Company, St. Louis, Missouri. The incubation tubes were sometimes frozen directly after addition of the 0.2 N NaOH and stored for 1 to 2 days without increase in blank readings or changes in enzyme activity.

All dehydrogenases studied were pyridine nucleotide-linked and their assays depend on the measurement of DPN, DPNH, or TPNH formed. The fluorometric method of Lowry et al. (5) was used for these measurements. Lactic and malic dehydrogenases were assayed by modifications of the methods of Strominger and Lowry (9). 2-Amino-2-methyl-1-propanol (Eastman Chemical Works, Rochester, New York) and n-malic acid (Nutritional Biochemicals Corporation, Cleveland, Ohio) were used as substrates. Glutamic dehydrogenase was assayed by the method of Lowry et al. (10). Glucose 6-P dehydrogenase was assayed by the method described by Kuhlman and Lowry (11). Isocitric dehydrogenase was assayed by the method developed by Lowry and Lewis1 in which fluorescence of TPNH is measured. a-ketogluutaric acid, glucose 6-phosphate, isocitric acid, and all pyridine nucleotides were obtained from Sigma Chemical Company.

All enzyme assays and protein determinations were done in quadruplicate on each sample. Enzyme activity is expressed as moles of product formed per kg. wet weight of tissue per hour of incubation, and is expressed as moles/kg/hr.1. In enzyme assays on adult retinas the variance between animals or between experiments was not greater than that between pairs of retinas from individual animals. Consequently each retina was routinely considered as an independent sample rather than the mean of both retinas from an animal.

Results

The animals grown in total darkness showed no ill effects; their weights at all ages were in the same range as animals grown in daylight. At birth sluggish pupillary responses were present. The 26-day-old animals grown in total darkness showed good responses to direct light but a consistent absence of consensual pupillary light reflex. The 26-day-old animals and all animals of older ages had normal, brisk light reflexes.

Histology—There were no differences in staining properties of retinas of animals grown in total darkness at either 60 or 120 days, nor were there any structural changes compared with control animals of similar age. At birth the inner and outer rod and cone segments are poorly developed and are represented by short, undifferentiated extensions of the rod and cone cells. The rod and cone and bipolar cell nuclear layers have not become distinct layers, and the ganglion cells are much smaller than in adults. By 60 days the retina shows a histological pattern similar to the adults.

Total Weight, Dry Weight, Lipide-free Weight, Total Protein—The results are summarized in Table I. There were no differences between animals grown in total darkness and those grown in daylight. It can be seen that retina contains more protein, more water, and much less total lipid than does brain at each age. Furthermore the increase of weight, protein, and lipide in the retina is not complete until 120 days, whereas brain attains its weights at all ages in the same range as animals grown in daylight. The animals grown in total darkness showed good responses to direct light but a consistent absence of consensual pupillary light reflex.

<table>
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<tr>
<th>Age</th>
<th>No. of samples</th>
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<th>Lipide-free</th>
<th>Total protein</th>
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</thead>
<tbody>
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<td></td>
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<td>D</td>
<td>L</td>
<td>D</td>
<td>L</td>
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<tr>
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<td>8.70</td>
<td>8.70</td>
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</tr>
</tbody>
</table>

TABLE I

Total weight, per cent dry weight and lipide content, and total protein of newborn, 26-, 60-, 120-day, and adult rabbits grown in total darkness or in daylight.

All the data are mean values for the number of samples indicated at each time. Directly under each mean is recorded the standard deviation of the individual observations. The per cent dry weight and lipide-free weight are based on the wet weight as 100 per cent. Total protein is expressed in gm. per kg. wet weight. L and D denote animals grown in total darkness or in daylight.
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Enzyme activities in brain and retina of rabbits at different ages grown in daylight or total darkness

Activities are expressed as moles of product formed per kg. wet weight of tissue per hour at 37°C. Values are expressed as means ± standard errors. N for brain and retina are 4 and 6, respectively, except for adult rabbits grown in total darkness in which cases N is 2 and 4, respectively. L and D denote animals grown in daylight and total darkness, respectively. Data for adult controls under D represent fully grown animals placed in total darkness for 120 days.

<table>
<thead>
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<th>Age (days)</th>
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<th>Phosphatases</th>
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<td>D</td>
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<td>120</td>
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<tr>
<td>Adult controls</td>
<td>1.07±0.02</td>
<td>0.185±0.006</td>
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</table>

*Significant differences (P < .01) between light and dark groups.

1.3 for isocitric dehydrogenase to 5.3 and 12.8 for acid phosphatase and glucose 6-P dehydrogenase, respectively; the other ratios were all approximately 2.0. In brain only lactic, glutamic, and malic dehydrogenases showed increasing activity with age, and this increase was largely confined to the first 26 days. On the other hand in retina all but alkaline phosphatase showed increasing activity with age, and in most cases this took place over the entire first 60 days. The pattern of alkaline phosphatase was unusual in that peaks of activity were found at 26 and 42 days only.

No significant differences in enzyme activity could be demonstrated in brains of the animals deprived of light from birth compared with those grown in normal daylight. In retinas, on the other hand, certain differences were found in the animals grown in total darkness. In the case of both enzymes found to be much richer in retina than in brain (acid phosphatase and glucose 6-P dehydrogenase), significant differences are present at most ages. Changes were also noted in lactic and malic dehydrogenases. Except for glucose 6-P dehydrogenase which showed increased activity, all changes were decreases in activity in retinas deprived of light stimulation. In the cases of malic dehydrogenase and acid phosphatase the change was present at every age and amounted, respectively, to 10 and 15 per cent decreases from normal. With lactic dehydrogenase the difference was not found until 60 days. In the case of glucose 6-P dehydrogenase by the end of 120 days the higher level in dark animals was no longer significant. There did not appear to be any consistent pattern in the development of enzyme activity either in brain or retina that would differentiate those enzymes which showed effects from light deprivation from those that did not.

Two adult animals were placed in total darkness for 120 days. At the end of this time they showed no differences from the adult controls in enzyme activity of either brain or retina, nor were there any differences in total weight, per cent dry weight or lipide, or total protein. Thus adult rabbits subjected to a similar period of light deprivation do not show the alterations of enzyme activity displayed by the growing animals.

**DISCUSSION**

These results show that specific enzyme alterations can be induced in a particular nervous tissue by changing the functional status of that tissue. Although the changes in enzyme activities observed here in the retinas of animals raised in the dark ranged only from 4 to 18 per cent of the normal values, they were...
highly significant statistically. The reality of the changes noted for certain enzymes is emphasized by the fact that in the same retina the activities of other enzymes showed no light-dark differences whatsoever at any age, and also by the complete absence of light-dark differences in brains of the same animals.

Unfortunately too little is yet known about the roles of the enzymes studied here in the visual process to permit interpretation of the specific changes induced by light deprivation. If any of these changes were confined to certain retinal layers, their magnitudes could obviously be greater than appears in the analysis of whole retina performed here. Such a possibility is suggested by the findings of Strominger and Lowry (9) of markedly different distributions of lactic, malic, and glutamic dehydrogenases in the layers of adult rabbit retina. Malic dehydrogenase, for example, was richest in the inner rod and cone segments, and lactic dehydrogenase was likewise richer in the inner retinal layers. Kuhlman and Lowry (11) have shown that enzyme activity may increase in one layer while decreasing in other layers during the postnatal development of the rat cerebral cortex. Extension of our findings to the effects of light deprivation on enzymes of the individual retinal layers would be useful.

In an investigation of biochemical changes in retinal ganglion cells under various physiological conditions, including prolonged light deprivation, Brattgard (2) showed that prolonged light deprivation for periods up to 4 months was required to produce a 50 per cent decrease in cell protein content, although pentose nucleoprotein was rapidly depleted in several days. He also found that although light deprivation was without effect on ganglion cell protein in the adult rabbit, decreased protein content could be shown in growing animals deprived of light from birth. The results reported here indicate that the decrease in enzyme protein does not involve all enzyme activities, but rather that only certain ones are altered. The results on total protein in retinal homogenates do not confirm such large decreases in protein content as found by Brattgard (2) for ganglion cells, and hence his finding cannot be assumed for all cellular elements in retina. It might be, however, that enzymes more specifically involved in vision, e.g. the alcohol dehydrogenase and retinene isomerase of Hubbard and Wald (12) and Hubbard (13), would have shown much larger differences.

Light deprivation does appear to have effects on certain electrophysiological properties of retina. Zetterstrom (3) found that the onset of the electroretinogram of kittens reared in total darkness could be delayed at most for 28 days, after which time it rapidly became indistinguishable from that of control animals. Hellstrom and Zetterstrom (4) subsequently showed that the extent of —SH group-staining in retina, as demonstrated by histochemical techniques, was diminished, particularly in the nuclear layers of the retinas of kittens deprived of light from birth. Upon exposure to light, the —SH group-staining increased to normal intensity within several hours. Boell and Shen (14) have shown that the extirpation of the eye of Amblystoma before the optic nerve develops results in hypoplasia of the midbrain on the involved side with failure of development of cholinesterase activity.

The results presented here together with those of the workers cited above suggest a role of normal function in the proper biochemical development of nervous tissue. In rabbit retina continued stimulation appears to be necessary for normal development of some, but not all, enzyme activities. Absence of stimulation may result in either increases or decreases of enzyme activity.

**SUMMARY**

1. The effects of light deprivation on retinal enzyme levels of alkaline and acid phosphatase, isocitric dehydrogenase, glucose 6-phosphate dehydrogenase, lactic dehydrogenase, and malic dehydrogenase, total weight, per cent dry weight, and lipide and protein content were investigated in rabbits born and reared in total darkness for periods up to 120 days.

2. Small but significant decreases in activity of malic dehydrogenase, lactic dehydrogenase, and acid phosphatase and an increase in glucose 6-phosphate dehydrogenase were found in the animals reared in total darkness. These effects were present only in retina and did not occur in brain, nor in retina or brain of adult animals deprived of light. The changes in enzyme activities did not result from alterations in water, total protein or lipide content. Histological examinations revealed no structural abnormalities in the retinas of rabbits deprived of light.

3. It is proposed that normal biochemical development of a nervous tissue may be dependent upon continued functional stimulation.

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**REFERENCES**

Effects of Prolonged Light Deprivation on the Development of Retinal Enzymes in the Rabbit
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