Further studies on the effect of red and far red light on rat retinal development

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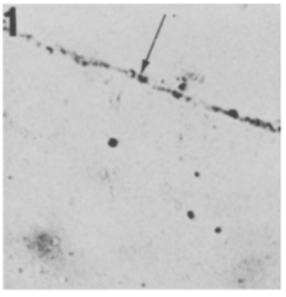
Summary. The influence on the development of the outer segments of the rat retina of far red and red light in different sequences was investigated. The far red treatment appeared to be dominating, and for animals treated with far red light first, further treatment of red light could not bring the outer segment growth back to normal. The treatments also initiated different dopamine uptakes in the retinas.

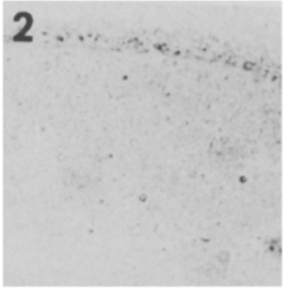
The importance of light stimulation in the visual system has received much attention, and dark or light environments in development plays an important role on the growth and differentiation of the retina[1,2]. However it is also true that different spectrum of light may have different effects e.g. in our laboratory, we have found that red light stimulates outer segment growth and far red inhibits[3]. This paper is to clarify the problem one step further: 1. to evaluate what effect will be obtained if we rotate the red-far red treatment; 2. what effect do the red and far red light have on the dopamine uptake of the retina.

Materials and methods. 7-day-old neonatal rats (strain: Simonsen) were selected for the experiment. The rats were divided into 7 groups, i.e. F, FR, FRF, R, RF, RFR and control. Each group had 6 animals. R and F groups were only treated with red ($\lambda = 660$ nm) or far red ($\lambda = 730$ nm) lights respectively, while the other groups were treated with alternation of red and far red lights. FR group was treated with far red and then red lights; FRF group, far red, red and far red lights; RF group, red and far red lights; RFR group, red, far red and red lights. The intensity of red light illumination was 200 $\mu W\ cm^{-2}$, and the intensity of far red light was 50 $\mu W\ cm^{-2}$. The duration of red light treatment was 20 min and the duration of far red light was 5 min (the duration for maximum effect as reported previously[3]). The control group was in ordinary daylight-dark cycle. The experimental animals were given their red or far red light treatments every 2 days and were otherwise in the usual daylight-dark cycle. After the treatment, both experimental and control animals (6 animals for each of the 7 groups) were killed and their eyeballs taken out. These were then fixed in Bouin, dehydrated, cleared and embedded in paraffin. The eyes were then cross sectioned at right angle to the longitudinal axis at 6 µm thickness and the 20th sections passed the lens were selected for observation, thus ensuring that almost identical regions were used. The sections were then stained with hematoxylin and eosin. The thickness of the outer segments and the thickness of the retinas were measured with an ocular and stage micrometer under the same magnifications (magnification = × 1500). The ratios of the thickness of the outer segments to the thickness of the whole retinas were then calculated. The retinas of R, F and control groups were also taken out for H^3 - dopamine incubation (80 µCi/1 ml of TC199) with 95% CO_2 and 50% O_2. After 5 min, the retina was then fixed in Bouin, dehydrated, cleared and embedded in paraffin. The retina was then cross-sectioned at 6 µm thickness and processed for autoradiography.

Results. The morphometric results of outer segment growth in different groups are indicated in the table. For each group, the mean and the SD are given and the results compared with control by using the t-test for significance. For the dopamine autoradiography, a limited amount of labelled dopamine was observed in the visual cell layer (figure 1) (bordering the outer nuclear layer) in the control retinas. In the retinas exposed to red light, conspicuous amounts of labelled sites were observed in the visual cell layer (figure 2). They were located into 2 regions, 1 proximal (near to the outer nuclear layer) and 1 distal in the visual cell layer. Small granules of activity were also detected in the nuclear layers and plexiform layers (particularly the outer nuclear layer). In the retinas exposed to far red light, very little labelled activity was observed.

Conclusion. Our results indicate that far red light appears to be more dominating when compared with red light treat-





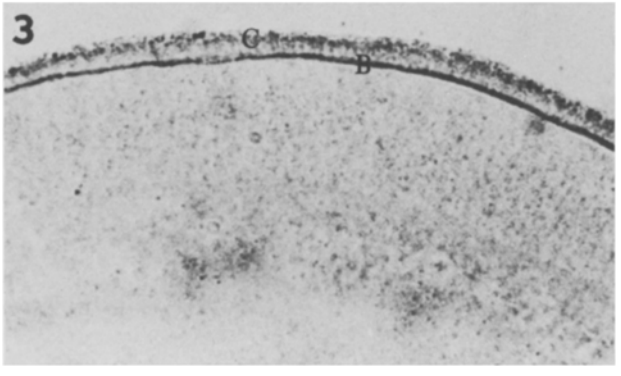


Fig. 1. Control retina. Note presence of some labelled dopamine particles (arrow). × 1400. Fig. 2. Retina after red light treatment. Note a large number of labelled particles mainly in visual cells (C) and base of visual cells (B). × 1400. Fig. 3. Retina after far red light treatment. Note very few labelled particles. × 1400.

Table

Treatment	$\bar{x} \pm SD$	Decrease growth or increase growth of outer segment when compared with control
Far red	0.0107* ± 0.0031**	Decrease
Far red - red	0.0118* ± 0.0036**	Decrease
Far red - red - far red	0.0100* ± 0.0034**	Decrease
Control	0.0220* ± 0.0031	
Red	0.0340* ± 0.0056**	Increase
Red - far red	0.0210* ± 0.0019	Equivalent
Red - far red - red	0.0200* ± 0.0017	Equivalent

* Ratio of outer segment/retinal thickness. ** Significant differences when compared with control by using the t-test ($p < 0.05$).

ment. If the animals are treated with far red light first, further treatment of red light will not be able to bring the outer segment growth back to that equivalent to control. On the other hand, treatment of red light first followed by far red light will tend to lower the stimulating effect of red light to a very low level, so that the outer segment growth is about the same as that of control. Further additional treatment of red light (e.g. red - far red - red) will give no further promoting effect. The red and far red effect also reflect on the dopamine uptake of the retinas, and it is possible that they act through the neurotransmitter system.

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On the formation of the myotomes in avian embryos. An experimental and scanning electron microscope study[1]

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Summary. The formation of the myotomes of chick embryos was studied by using experimental and scanning electron microscope techniques. With the aid of the quail-chick marker system, it was demonstrated that the dermatome gives rise to the myotome plate. SEM studies support the conclusion that the myotome cells originate only from the edges of the dermatome.

Since the work of Remak[2], it is well known that during early development of the chick embryo each primary spherical somite divides into a ventral mesenchymal part (sclerotome) and an epithelial upper wall (dermatomyotome)[3]. The dorsal lamella then becomes a 2-layered plate consisting of an outer layer (dermatome) and an inner layer (myotome) which is considered to be the source of voluntary musculature[4,5]. The origin of the myotome layer has been a controversial issue. According to earlier investigations[4,6-8], the myotome is mainly formed by proliferation from the dorso-medial edge of the dorsal lamella and possibly completed by cells arising from the ventrolateral edge[6,9,10]. Langman and Nelson[11], however, concluded from their labelling experiments with tritiated thymidine that the cells of the myotome directly originate throughout the surface of the overlying dermatome. On the basis of ultrastructural studies, Mestres and Hinrichsen[12] believe that cells of the sclerotome reaggregate on the apical surface of the dermatome layer and constitute the myotome.

The aim of the present paper is to examine the validity of the various theories on myotome formation by using the quail-chick marker system and the scanning electron microscope. Since the interphase nuclei of Japanese quail cells exhibit a large mass of nucleolus-associated heterochromatic DNA which does not exist in chick cell nuclei, it is possible to identify individual cells in Feulgen-stained sections after experimental intermixture of quail and chick cells[13-15].

White Leghorn chick and Japanese quail *(Coturnix coturnix japonica)* embryos were used for the grafting experiments. From the somites 13-16 of chick embryos at stage 14, according to the criteria of Hamburger and Hamilton[16], the dorsal walls together with the overlying ectoderm were removed unilaterally and corresponding parts of somites and adjacent ectoderm isolated from quail embryos at the same stage were grafted into the defect (figure 1). After subsequent incubation (1 or 2 days), the host embryos were fixed in Serra's fluid, dehydrated with graded propanol solutions and embedded in paraplast. The 7 µm serial sections were treated according to the Feulgen and Rossenbeck technique[17] and post-stained with light-green. For the scanning electron microscope studies, chick embryos at stages 13-17 were fixed in a buffered (pH 7.4) paraformaldehyde and glutaraldehyde fixative[18], washed in Hank's solution and postfixed in osmium tetroxide[19]. After dehydrating with graded propanol solutions, specimens were dried by the critical-point method[20,21] and subsequently sputtered with gold. Pictures were produced utilizing a scanning electron microscope JSM 35.

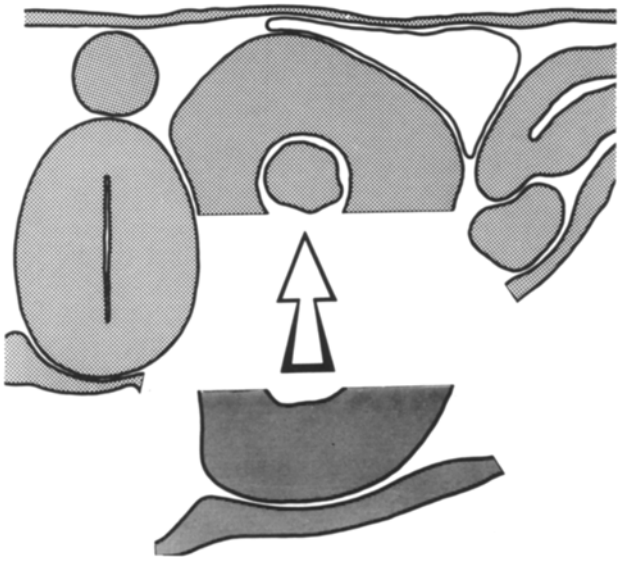

Fig. 1. Diagram showing the microsurgical procedure. After removal of the upper walls of somites 13-16 together with the overlying ectoderm of a chick embryo at stage 14 (H.H.) the corresponding parts of a quail embryo were implanted.

After 1 day of post-operative reincubation, the grafted somites are normally developed. On top of the mesenchymal sclerotome, which consists of cells containing nuclei of the chick type, the dermatome and myotome layer exhibit quail nuclei (figure 2, *A*). In the course of further development, the cells of the dermatome lose their epithelial arrangement and move towards the ectoderm, later to form the dermis. The most dorsal and ventral parts of the dermatome retain their epithelial structure. There seems to