Suppression by Actidione of Development of Rat Liver L-Tyrosine : 2-Oxoglutarate Aminotransferase Activity

Abstract. *The development of* L-*tyrosine : 2-oxoglutarate aminotransferase in newborn rats is suppressed by actidione (cycloheximide), an inhibitor of protein biosynthesis, administered immediately after birth.*

Actidione (cycloheximide) inhibits protein biosynthesis in cultured mammalian cells (*1*) and human lymphocytes (*2*). It also inhibits the inducible enzymes bacterial β-galactosidase (*3*) and rat liver tryptophan pyrrolase (*4*), but there is conflicting evidence on its effect on the inducible enzyme rat liver L-tyrosine : 2-oxoglutarate aminotransferase (EC 2.6.1.5) (TT). Induction (*4*), lack of induction, and partial inhibition of hydrocortisone induction (*5*) have been reported. We now report the effect of actidione on the developmental increase in rat liver TT which occurs during the first few hours after birth (*6, 7*).

Albino Wistar full-term rats were delivered by cesarean section under ether anesthesia. Immediately after delivery, newborn rats in the test group received 1 mg of actidione per kilogram of body weight in 0.05 ml of normal saline intraperitoneally, whereas controls received the same volume of normal saline alone. The animals were killed at timed intervals after birth. For each measurement, two livers were pooled and quickly cooled to 0°C. Assays for TT activity were carried out by the method of Lin and Knox (*8*).

Actidione consistently produced partial inhibition of developmental increase of TT activity (Table 1) from 2 to 12 hours after birth. Observations at 18 and 24 hours were precluded by heavy mortality in the animals injected with actidione.

The development of TT may be induced prematurely by glucagon (*9*) and inhibited by adrenalectomy (*7*), but the mechanism of this inhibition is unclear. Inhibition by actinomycin of TT development (*10*) suggests dependence on RNA synthesis and introduces the possibility that development results from *de novo* synthesis of enzyme protein. Indeed, in adult rats the hydrocortisone-induced increase in TT activity has been shown to be due to synthesis of new enzyme molecules (*11*), and its inhibition by actinomycin (*10*) suggests that, like the developmental increase, it is RNA-dependent.

Actidione appears to have a variety of effects on RNA synthesis. Thus it inhibits synthesis of ribosomal RNA in yeast (*12, 13*) and neurospora (*14*), but apparently not in cultured mammalian cells (*1, 15*). It suppresses RNA synthesis in cultured human lymphocytes after phytohemagglutinin stimulation, but it enhances RNA synthesis in unstimulated lymphocytes (*2*). However, in these systems protein synthesis is inhibited. The inhibitory effect by actidione on TT development is consistent with the concept that the latter results from an increase in the *de novo* synthesis of enzyme molecules.

Table 1. Effect of actidione on developmental increase of rat liver L-tyrosine : 2-oxoglutarate aminotransferase. Enzymatic activity is expressed as micromoles of *p*-hydroxyphenylpyruvate formed per gram of liver per hour at 25°C. Each value represents a measurement on two pooled livers.

Controls		Actidione injected
	Hour 0	
0.9, 1.5, 0.5, 0.9		
	Hour 2	
3.8, 4.1, 3.8, 4.9		1.9, 2.4, 1.1, 1.7
	Hour 4	
21, 16, 15, 36		2.4, 8.2, 1.5, 3.5
	Hour 6	
17, 18, 35, 62		7.5, 7.5, 3.0, 5.1
	Hour 12	
110, 150, 55, 171		3.8, 23, 18, 4.5

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16. This work was supported by the Spastics Society.

15 September 1967

Origin of Iridescent Colors on the Indigo Snake

Abstract. *In the indigo snake, a pattern of undulating lines on the surface of the skin, formed by the junction of rows of cells, acts as a two-dimensional optical diffraction grating to produce the play of colors. The distance between repetitive units of the pattern measured from observations made with an electron microscope are in agreement with those found by spectrometric analysis.*

The indigo or gopher snake, *Drymarchon corais*, variety *couperi* (Holbrook) is the largest North American snake, attaining a length of about 2.5 m (8 feet). The variety we studied inhabits the Gulf states. The specimen is jet black except for a white-to-coral strip located under the chin and extending posteriorly several centimeters. The entire scalation is highly polished and, in bright light, displays iridescent colors. Iridescence is enhanced by black pigmentation of the chromatophores located in the dermis and in the nonventral portions of the stratum corneum layer. However, rainbow colors may also be observed in the region of white or coral pigmentation.

These iridescent colors were recognized as structural colors, in which colors are produced by interference effects of the light, in contrast to color due to pigmentation. Other occurrences of structural colors in natural materials have been reported in the literature, for example, precious opal (*1*), certain feldspar minerals (*2*), crystals of potassium chlorate (*3*), fossil brachiopod shells (*4*), mollusk shells (*5*), viruses (*6*), and insects (*7*). Structural elements giving rise to interference effects are in every case arranged in a periodic array. The focus of our study was on the exact nature of the interference phenomenon and structure of the skin which cause this effect.

A skin from an indigo snake that had recently undergone ecdysis was obtained for study. The outside surface of the scales was highly polished, whereas the inside surface had a dull luster. Examination by light microscopy was made on ventral and lateral-dorsal scales. Transmitted light showed poorly resolved cellular units; reflected light revealed a pattern of uniformly spaced undulating lines extending perpendicularly to the medial axis of the specimen. This pattern was observed on both external and internal surfaces.

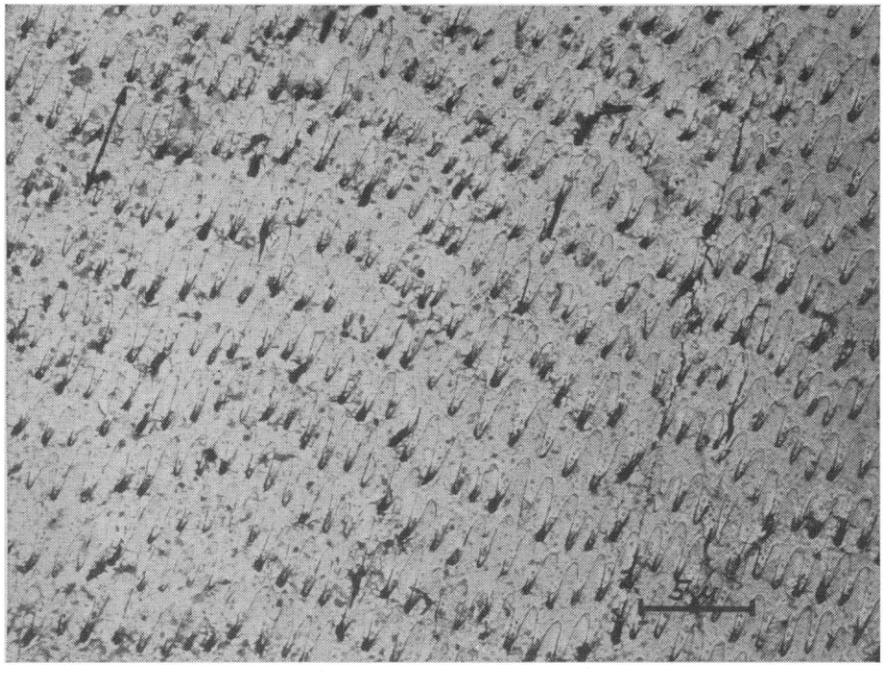

Fig. 1. External surface of a ventral scale showing the undulating line pattern that acts as an optical diffraction grating. Arrow indicates the medial axis of the specimen. (Replication electron micrograph, × 3000)

of all scales. These suture-like lines delineated the junction of rows of elongate cells.

Plastic-carbon replicas (*8*) were prepared of both inside and outside surfaces of the central and lateral-dorsal scales for examination with the electron microscope. Figure 1 shows the pattern that was typical of all the surfaces inspected and was in agreement with observations made by means of the light microscope.

This line pattern, which had topographic expression, as demonstrated by successful application of the replication technique, was believed capable of acting as a two-dimensional diffraction grating. Light may be reflected from the spaces between the lines while being scattered by the lines, and the resulting periodic variations of amplitude of the light being reflected may cause the observable diffraction effects. However, for a grating to appreciably deviate white light into its spectrum, repetition of the pattern must be of the same order of magnitude as the wavelength of light. In this respect the pattern of lines shown in Fig. 1 compares favorably to the wavelengths of white light, which may be regarded to range from 0.4 to 0.7 μ.

A qualitative test for the two-dimensional diffraction grating as an origin for the iridescence was made by preparing a plastic replica of the scales that displayed the structural colors. When this replica was viewed with the aid of an optical goniometer, a spectrum of colors was seen, which proved the ability to transfer the colors by replication and supported the premise that the line pattern on the scales may act as a diffraction grating.

The pattern on the scales was studied on a two-circle optical goniometer, in order to test quantitatively whether it was a natural diffraction grating. A small portion of a ventral scale was stiffened into a planar sheet by applying a thin coating of collodion plastic to one side and then pressing the scale between glass plates. After the skin was dried, it was removed and mounted on the goniometer. The external surface of the scale that has a glossy luster was selected for the reflecting surface so as to enhance reflection. The azimuthal angle between the incident beam of white light and the lines of the pattern was varied so that various pattern orientations could be tested for their ability to diffract light.

Two different pattern orientations gave first-order spectrums on either side of the zero order, thus proving that the scale pattern acts as a diffraction grating. The best spectrum was obtained when the incident light was directed parallel to the undulating lines, that is, normal to the medial axis of the specimen. All the spectral colors of the first order were clearly discernible. Using the violet line, we calculated the grating spacing, d, from the diffraction formula (*9*)

$$d = \pm n\lambda/(\sin\theta + \sin\varphi)$$

where n is the order of diffraction; λ, the wavelength of the light; θ, the angle of diffraction; and φ, the angle of incidence. With an angle of incidence (φ) of 10° as measured from the normal to the grating surface, the resulting angle of diffraction (θ) was observed to be approximately 40° from the normal. Since the diffraction maxima had some angular spread, the angle could be measured only to an accuracy of about ± 30′ of arc. The angle of 40° was arrived at by taking the average of six measurements. Then substituting into the above equation and using the negative sign convention for the angle of incidence, we calculated the grating spacing to be approximately 1.0 μ. This distance corresponds closely to the repetition of successive undulations of the pattern lines in Fig. 1.

The second diffraction spectrum was obtained when the incident light was directed normal to the lines, that is, parallel to the axis of the specimen. In this case the angle of diffraction was approximately 30°, with an angle of incidence of 20°. The resulting grating spacing was calculated to be nearly 2.5 μ, which compares favorably with the separation distance between adjacent lines of the pattern.

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4 August 1967