

Second, the skeletal pigment was found to react with benzidine and hydrogen peroxide, producing a pea-green colour (cf. WILLSTAEDT¹). The reaction was feeble and was shown by solutions of the pigment in water as well as those in ethanol. In view of this, and of the fact that such a reaction is characteristic of tetrapyrroles with a closed ring, it would appear that the reaction is not due to the main mass of pigment, but to accompanying traces of the porphyrin type.

The skeletal pigment thus shows the characteristics of an open chain tetrapyrrole of the bilatriene type, resembling biliverdin. Nothing is known of its metabolism.

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Zusammenfassung

Im Skelett von *Katsuwonus pelamis* kommt vereinzelt ein blaugrünes Pigment vor, dessen Verteilung im Endoskelett beschrieben wird. Das Pigment kann mit saurem Methanol extrahiert und in Chloroform übergeführt werden. Die saure Methanollösung des Pigments zeigt Absorptionsmaxima bei 680–690 und 370 m μ , die Chloroformlösung bei 660 und 380 m μ . Sowohl trockene Filme des Pigments als auch die Chloroformlösung geben eine positive Gmelinsche Reaktion, und der oxydierte, in ammoniakalischem Äthanol gelöste Zinkkomplex des Pigments zeigt im ultravioletten Licht eine intensive rote Fluoreszenz und Absorptionsmaxima bei 590 und 640 m μ . Das Pigment besitzt somit die Eigenschaften eines Bilirubin-tetrapyrrol und ähnelt weitgehend dem Biliverdin.

¹ H. WILLSTAEDT, *Enzymologia* 9, 260 (1941) (Chem. Abstr. 36, 842).

² Guggenheim Research Fellow from University College of the West Indies, Jamaica.

Effects of Iodoacetate on the Histology of the Chick Retina

In the destructive effects of sodium monoiodoacetate reported on the retina of the rabbit¹, cat and monkey², it has been pointed out that the visual cells are primarily affected. So far few reports have been published on the early effects (less than 24 h) after injections of the drug.

The present paper is intended to provide data on the striking sensitivity of the chick (*Gallus gallus domesticus*, L.) retina to iodoacetate, leading to early cellular lesions in all cell layers.

Male chicks (Leghorn), weighing 200–400 g were used. The animals received the following diet: cornmeal, 45%; rolled oats, 19%; meat meal, 14%; casein, 15%; yeast, 2%; peanuts, 3%; cod liver oil, 1%; and commercial sodium chloride, 1%, besides corn and water *ad libitum*. Sodium monoiodoacetate (40 mg/kg body weight) was administered intravenously at day light. Control animals were injected with distilled water. A preliminary experiment showed no difference in the lesions 16 h after drug administration to either light-

adapted chicks or to chicks dark-adapted for 4 h, injected under a faint red light and kept in the dark until death.

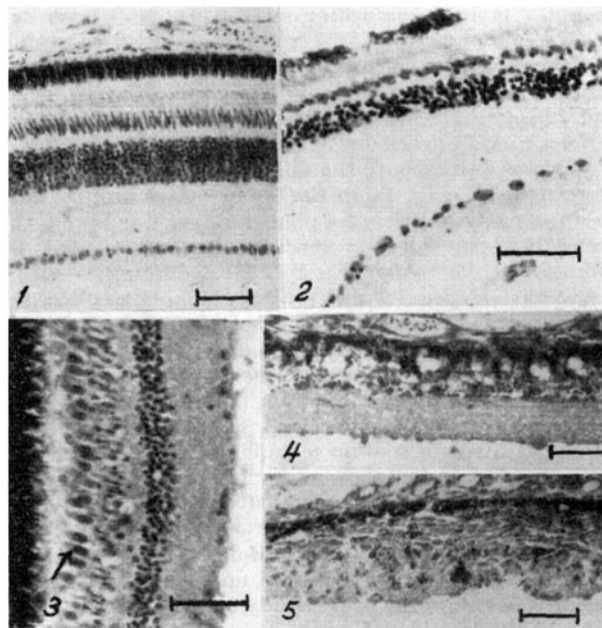


Fig. 1.—Retina of control chick; galocyanin-chromalum stain.

Fig. 2.—Chick retina 2 h after iodoacetate injection. Pycnosis in the ganglionic and inner and outer granular layers; galocyanin-chromalum stain.

Fig. 3.—24 h after iodoacetate injection. Period acid-Schiff hematoxylin stain. Glycogen still present in accessory cones (arrow).

Fig. 4.—Four days after iodoacetate injection. Profound disorganization particularly of the granular layers. Rods and cones no longer distinguishable. Migration and conglomeration of pigment cells. Periodic acid-Schiff, hematoxylin.

Fig. 5.—Six days after iodoacetate administration. Only the pigment cell layer is recognisable. Other layers have been substituted by a proliferation of "fibroblast like" cells.

After decapitation, the posterior poles of the eyes were fixed in HELLY's or in chilled GENDRE's fixative. Paraffin sections (4 and 7 micra) were stained by one of the following methods: phosphotungstic acid hematoxylin; galocyanin-chromalum; periodic acid-Schiff with hematoxylin counter-staining, with and without preliminary digestion with saliva. Three to six animals were sacrificed after each of the following periods of injection: 1, 2, 4, 6, 8, 10, 11, 12, and 24 h, and 2, 4, 6, and 7 days. Fifteen controls were studied.

Results. Of a total of 59 iodoacetate injected chicks, no difference from the control retinas (Fig. 1) was found in two of them (one in the 12 and the other in the 24 h group). Lesions showed marked variations in intensity when different parts of the same retina were compared. No special care, however, was taken to note their distribution. This may explain the fact that no strict correlation could be found between the intensity of the lesions and the periods after iodoacetate administration. In about $\frac{3}{4}$ th of the treated animals, a distinct pigment migration was found to the plane of the external limiting membrane. 8 h after injection, conglomeration of the pigment was observed. Folding of the retina, reduction in its thickness and of its inner granular layer (Figs. 2, 3) were also found in about $\frac{3}{4}$ th of the treated animals. Pycnosis of the cells of the ganglionic layer was seen 30 min after the injection of the drug, increasing

¹ G. SCHUBERT und H. BORNSCHNEIN, *Exper.* 7, 461 (1951). — W. K. NOELL, *J. Cell. Comp. Physiol.* 40, 25 (1952). — P. KARL, *Arch. Anat., Histol. Embryol.* 35, 1 (1952).

² W. K. NOELL, *J. Cell. Comp. Physiol.* 40, 25 (1952).

afterwards (Fig. 2), and becoming an almost constant phenomenon 8 h after. Not all of the ganglionic layer cells were simultaneously affected. Only 8 h after injection did pycnosis appear in the external and inner granular layers. Disruption of the rod and cone cell layer was found from the 8 h group onwards. Ellipsoids and the glycogen containing paraboloids of the rods and accessory cones (Fig. 3), could be recognised up to the 12 and 22 h groups. The resistance of the cone ellipsoids of rabbits was observed by NOELL¹.

In the 4 days group, the different layers could hardly be recognised (Fig. 4). In the 6 and 7 days groups, only an apparently proliferated pigment layer was found, the remaining part of the retina being substituted by "fibroblast-like" cells (Fig. 5) (glial reaction?).

Discussion. Species differences probably explain the different time-course and characteristics of the iodoacetate effect on the retina in our material, when our results are compared with those reported earlier² on other animals. It is interesting to note that in this laboratory the intravenous administration of repeated 20 mg/kg doses of sodium monoiodoacetate to rats and mice did not induce lesions on the retina. The chick retina therefore seems the particularly sensitive to iodoacetate and should be good material for further cytological and biochemical study of its early effects. We refer to NOELL¹ for a discussion on the possible mechanism of action of the drug. Study of other more specific SH-combining drugs³ and protection experiments might elucidate the mechanism of the iodoacetate effect on the retina⁴.

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Résumé

Le monoiodoacetate de sodium injecté à des poulets par voie intraveineuse en une dose unique de 40 mg/kg, provoque des lésions très précoces dans toutes les couches cellulaires de la rétine. Une dégénérescence plus ou moins complète survient 6 à 7 jours après l'injection. On peut conclure que le poulet présente une sensibilité au monoiodoacetate plus grande que celle des mammifères précédemment étudiés (lapin, chat, macaque).

¹ W. K. NOELL, J. Cell Comp. Physiol. 40, 25 (1952).

² G. SCHUBERT und H. BORNSCHNEIN, Exper. 7, 461 (1951). – W. K. NOELL, J. Cell. Comp. Physiol. 40, 25 (1952). – P. KARLI, Arch. Anat. Histol. Embryol. 35, 1 (1952).

³ Z. M. BACQ, Exper. 2, 349 and 385 (1946). – E. S. G. BARRON, Adv. in Enzymol. 11, 201 (1951).

⁴ This work has been aided by grants from the Reitoria da Universidade de São Paulo and Conselho Nacional de Pesquisas. The advice of Prof. L. C. U. JUNQUEIRA is gratefully acknowledged.

Influence of Continuous Light or Darkness on Sexual Development in the Male Rabbit

Length of light has been shown to influence some of the female reproductive processes in certain species of animals, especially birds¹, ferrets², and sheep³, but not

rabbits¹. A perusal of the available literature shows that the influence of length of light on the various male reproductive processes of the rabbit has not been so far investigated (for review see YEATES, 1949). In view of the extensive use of the rabbit for laboratory work, a preliminary investigation was undertaken to study the effects of continuous light or darkness, for both short and long experimental periods on the weight of the male sex organs and on the production of fructose by the seminal vesicles in the male rabbit at different ages. Fructose has been shown to be a very important constituent of the seminal plasma, and its level in the seminal fluid indicates the degree of functional activity of the seminal vesicles. Moreover, it has also been found that the production of fructose in the seminal fluid is dependent upon the androgen status². Therefore, the level of fructose in the seminal fluid indirectly indicates the degree of functional activity of the interstitial cells which secrete the male sex hormone.

Groups of male rabbits (HAMMOND's inbred strains) of different ages were kept under continuous light, total darkness, and natural light conditions for both short and long periods at different times of the year. At the termination of each experiment, the rabbits were killed and their testes, prostate, seminal vesicles, adrenals, thyroid and pituitary were weighed separately. The fructose content of the seminal vesicles was estimated colorimetrically.

The results obtained from the present series of experiments indicate that continuous light or darkness for short or long periods did not affect the weight of the testes and accessory male organs in the inbred male rabbits when compared with those of the control groups at different ages. Microscopic examination of the testes of the experimental rabbits did not show any marked difference in their histological features. There was no significant difference between the fructose contents of the seminal vesicles of the control, total light and total darkness treated rabbits at different ages.

The present results show that the rabbit appears to be refractory to the effect of continuous light or darkness in so far as the development and functions of the male sex organs are concerned. However, length of light has been shown to influence some of the male reproductive processes in certain other species of animals viz. ferret³ and sheep⁴. It is, therefore, suggested that the question of the species of the animal should be taken into consideration while studying the subject of photoperiodicity in mammals.

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Animal Research Station, University of Cambridge, England, September 15, 1953.

Zusammenfassung

Gruppen von männlichen Kaninchen verschiedenen Alters wurden in verschiedenen Jahreszeiten unter Dauerbelichtung, Dauerverdunkelung und unter natürlichen Lichtverhältnissen gehalten, und zwar jeweilen

¹ G. K. SMELSER, A. WALTON, and E. O. WHETHAM, J. Expt. Biol. 11, 352 (1934).

² T. MANN and U. PARSONS, Nature, London 160, 294 (1947).

³ T. H. BISSONNETTE, Proc. Royal Soc. B. 110, 322 (1932).

⁴ N. M. T. YEATES, J. Agri. Sci. 39, 1 (1949).

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¹ W. ROWAN, Nature, London 115, 497 (1925).

² D. S. HART, J. Expt. Biol. 28, 1 (1951). – F. H. A. MARSHALL, J. Expt. Biol. 17, 139 (1940).

³ E. S. E. HAFIZ, Nature 166, 822 (1950). – D. S. HART, J. Agri. Sci. 40, 143 (1951). – N. M. T. YEATES, J. Agri. Sci. 39, 1 (1949).