der auf Grund gänzlich andersartiger Versuche zu der gleichen Annahme kam. Hiermit in Einklang stehen auch Messungen an Seeigeleiern von LINDAHL und HOLTER[1], die eine starke Verminderung der O_2-Aufnahme fanden, wenn die Oozyten in die Reifeteilung eingehen, also in der Phase, die bei *Sabellaria* gegen KCN und Monojodessigsäure unempfindlich ist. Ebenfalls nicht in Widerspruch zu obiger Ansicht sind die von ZEUTHEN[2] genau gemessenen Rhythmen in der O_2-Aufnahme während einzelner Furchungsschritte. Wie aus seinen neuen Untersuchungen hervorgeht, fällt die starke Atmung in die Interphase, während bei den eigentlichen Mitosephasen eine starke Reduktion der O_2-Aufnahme erfolgt.

Die hier gemachte Annahme scheint auf den ersten Blick den Befunden zu widersprechen, dass Teilungen unter anaeroben Bedingungen nicht ablaufen können und dass durch Steigerung der Atmung, zum Beispiel bei *Actinophrys sol*[3], die Meiose verfrüht ausgelöst werden kann. Auch die Blockierung der Furchungsmitosen von *Sabellaria* und anderen Objekten[4] durch KCN demonstriert die Bedeutung der Atmung für den Eintritt der Zelle in die Mitose. Nimmt man jedoch an, dass die *Bedeutung dieser aeroben Glykose für die Zellteilung in der Bildung energiereicher Phosphorverbindungen liegt,* von deren Anwesenheit der Mitoseeintritt abhängig ist, so hebt sich der Widerspruch auf. Die Annahme liegt nahe, dass sehr viele der gegensätzlichen Befunde auf verschiedenen Ausgangssituationen im Energiehaushalt der Zellen beruhen: Zellen mit einer grossen Menge gespeicherten ~ph werden eine oder mehrere Mitosen unter anaeroben Bedingungen ausführen können, während anderseits Zellen ohne wesentliche Mengen an ~ ph diese erst synthetisieren müssen, um überhaupt in die Teilung eintreten zu können. Diese Synthesen sind offenbar von der Atmungskettenphosphorylierung abhängig. Ihre Hemmung wird einen Mitosestopp herbeiführen, ihre Förderung eine schnelle Bildung von ~ ph und hierdurch den frühzeitigen Mitoseeintritt.

In diesem Zusammenhang sind von besonderem Interesse Versuche von HÄMMERLING[5] an *Acetabularia med.*: die Transplantation eines Hutes auf junge Zellen und Kerne löst verfrüht eine Vielzahl von Kernteilungen aus. Ebenso können sogenannte Sekundärkerne, die sich normalerweise nicht mehr teilen würden, hierdurch zu zahlreichen Teilungen gebracht werden. Es wäre denkbar, dass diese Teilungen dadurch zustande kommen, dass den Kernen durch das Transplantat energiereiche Phosphate (vielleicht Metaphosphate) zugeführt werden, die in jüngeren Stadien in nicht ausreichenden Mengen zur Verfügung stehen oder für Wachstumsprozesse in der Zelle verwendet werden (STICH[6]). Es soll versucht werden, unter diesem Gesichtspunkt diese Frage experimentell zu prüfen, um hierdurch einen weiteren Einblick in die eine Mitose auslösenden Faktoren zu erlangen.

H. STICH

Max-Planck-Institut für Meeresbiologie, Wilhelmshaven, den 23. November 1953.

Summary

The metabolic processes leading to meiosis in *Sabellaria spinulosa* were investigated with cytochemical methods (PAS-reaction for polysaccharides, and basic dyes in combination with ribonuclease applied for the detection of ribonucleic acid) and the use of specific inhibitors. Before the beginning of meiotic prophase, polysaccharides and later ribonucleic acid appear in the nuclear sap and become incorporated into the spindle. 2,4-dinitrophenol and NaN_3, which inhibit the formation of energy-rich phosphates, prevent the accumulation of polysaccharides and RNA in the nucleus and the formation of the meiotic spindle. KCN and mono-iodoacetic acid are without influence on polysaccharide and RNA metabolism of the nucleus. The first and second meiotic division are normal, but cleavage is completely suppressed. From these results the following conclusions may be drawn: (1) the division of the nucleus is dependant on the presence of energy-rich phosphate, (2) the division occurs without respiration, and (3) respiration is important for cell division only in so far as it provides the synthesis of energy-rich phosphates.

[1] E. LINDAHL und H. HOLTER, C.r. Labor. Carlsberg *24*, 49 (1941).
[2] E. ZEUTHEN, Pubbl. Staz. Zool. Napoli *23*, 47 (1951).
[3] J. STRAUB, Biol. Zbl. *70*, 24 (1951).
[4] O. WARBURG, Z. physiol. Chem. *66*, 305 (1910).
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[6] H. STICH, Z. Naturforschg. *8 b*, 36 (1953).

A Biliverdin-like Pigment in the Skull and Vertebrae of the Ocean Skipjack, *Katsuwonus pelamis* (Linnaeus)[1]

The occurrence of blue-green pigments in the skeleton of teleosts such as *Cottus, Belone, Zoarces, Strongylura* and others, has been reported by several workers[2]. The pigments have not been identified, although their similarity to bile pigments has been demonstrated. CAGLAR[3] believes that the skeletal pigment of *Belone* is biliverdin.

On rare occasions, the catches of skipjack in waters of Baja California include individuals whose endoskeletons, especially the skull and vertebrae, are strikingly green or blue-green. Such an individual was sent for our examination through the courtesy of the Van Camp Sea Food Company and M. B. SCHAEFER of the International Tropical Tuna Commission. Four years earlier, one of us (D.L.F.) had studied the pigment from the bony parts of a similarly coloured individual of the same species, made available through the kindness of HARRY C. GODSIL, of the California State Fisheries Laboratory.

Since the properties of such a pigment occurring in the *Katsuwonidae* have not been described, we have taken the opportunity of examining them. Unfortunately, both of the specimens which became available had been cooked, so that we cannot describe the distribution of the pigment except in the axial skeleton which was of a pea-green color.

In the vertebral column, it appeared especially abundant in the centra, neural arches and in the base of the haemal arches. Less appeared in the neural spines, and none in the notochord. In the skull, it appeared in both dermal and cartilage bones, being especially conspicuous in the prefrontals, pre-maxillae, maxillae, dentaries, median ethmoid, supra-orbitals, vomers, exoccipitals, articulars, quadrates and in the otic series. It was conspicuous in parts of the parietals, hyomandibulars, the opercular series, the branchial arches and in the sclerotic of the eye. Less was present in the

[1] Contribution from the Scripps Institution of Oceanography, New Series No. 677.
[2] M. CAGLAR, Nature *155*, 670 (1945). – D. L. FOX, *Animal Biochromes and Structural Colours* (Cambridge University Press, 1953), p. 279. – H. WILLSTAEDT, Enzymologia *9*, 260 (1941) (Chem. Abstr. *36*, 842).
[3] M. CAGLAR, Nature *155*, 670 (1945).

frontals, lachrymals and parasphenoid; little was present in the supra-occipital.

From the vertebrae, stripped of adhering flesh, washed in water and comminuted in a blender, the pigment was leached with methanol containing concentrated hydrochloric acid (9:1 by volume). The blue-green pigment was readily extracted from this solution by moderate dilution while agitating with chloroform.

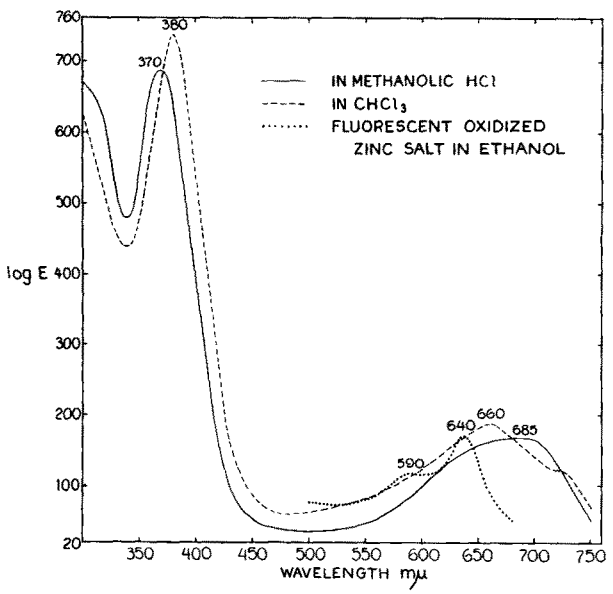

Fig. 1.–Comparative light absorption of the endoskeletal pigment dissolved in acid methanol and in chloroform. The absorption of the oxidized zinc complex dissolved in ammoniacal ethanol is also shown

The acid methanolic solution showed no fluorescence in ultra-violet light, but showed absorption peaks at 680–690 mμ and at 370 mμ and a minimum at 510 mμ (Fig. 1). The chloroform solution showed peaks at 660 and 380 mμ and a minimum at 480 mμ (Fig. 1). The absorption spectra thus resemble very closely those of biliverdin, a bilatriene tetrapyrrole (see Table, showing data from LEMBERG and LEGGE[1], TIXIER[2] and this paper).

Supporting these indications is the fact that the skeletal pigment, dissolved in chloroform, or in the form of a dry film, showed a clear positive reaction to the GMELIN test using fuming nitric acid. In chloroform the highly characteristic red, violet and blue colours appeared in the solvent, but treatment of the dry film on a porcelain dish yielded only the red colour.

The skeletal pigment dissolved in ammoniacal ethanol, to which zinc acetate and a few drops of iodine were then added, formed a characteristic blue-green solution showing an intense red fluorescence in ultra-violet light and marked absorption in the orange to red region of the spectrum (see Fig. 2). Such a reaction is characteristic of bilatrienes subjected to similar treatment (LEMBERG and LEGGE[1]).

The absorption spectrum of the zinc complex of the oxidized pigment in ammoniacal ethanol showed maxima at 590 and 640 mμ, agreeing with those given by TIXIER[2] for the zinc complex of an oxidized biliverdin methyl ester in neutral alcoholic solution. Moreover, the absorption

Pigment	Absorption maxima (mμ)		Absorption minimum (mμ)
	I	II	
Skeletal pigment of *Katsuwonus* in HCl-methanol	680–690	370	510
Biliverdin hydrochloride in HCl-methanol (LEMBERG and LEGGE)	680	377	approx. 500
Chloroform solution of biliverdin acidified by HCl-methanol (TIXIER)	675	–	492
Skeletal pigment of *Katsuwonus* in chloroform	660	380	480
Methanolic ester of biliverdin in chloroform (TIXIER)	665	384	490

properties of such solutions, in the range 580–660 mμ, correspond very closely with those exhibited by bilirubin when oxidized and subjected to the same treatment (Fig. 2). Indications are thus confirmed that the skeletal pigment of *Katsuwonus* is closely similar to biliverdin.

Two reactions of the pigment deserve mention. First, despite the foregoing, it consistently failed to show a pyrrole reaction when roasted, since the fumes failed to redden a pine splinter moistened with concentrated HCl. In view of this, the test was repeated on skatole, haematin and bilirubin. Though skatole gave a marked positive reaction, haematin and bilirubin failed completely. The negative result given by the skeletal pigment was perhaps, therefore, not surprising, and the test appears to have limited application.

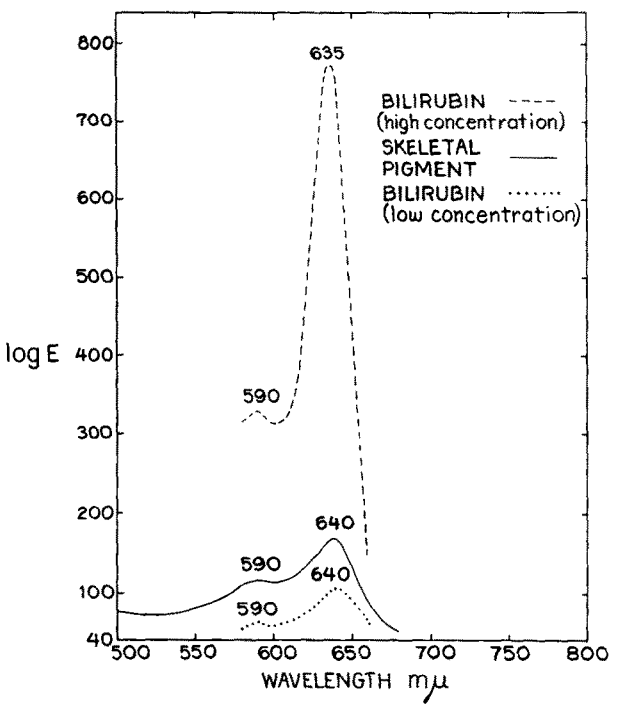

Fig. 2.–The light absorption of the oxidized zinc complex derived from the endoskeletal pigment, compared with that of solutions of the similar complex derived from bilirubin. All solutions were in ammoniacal ethanol.

[1] R. LEMBERG and J. W. LEGGE, *Haematin Compounds and Bile Pigments* (Interscience Publishers, New York, 1949), pp. 116–117.
[2] R. TIXIER, Ann. Inst. Oceanogr. *22* (Fasc. 5), 344 (1945).

Second, the skeletal pigment was found to react with benzidine and hydrogen peroxide, producing a pea-green colour (cf. WILLSTAEDT[1]). 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.

D. L. FOX and N. MILLOTT[2]

Division of Marine Biochemistry, Scripps Institution of Oceanography, University of California, La Jolla, Calif., November 9, 1953.

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 mμ, die Chloroformlösung bei 660 und 380 mμ. 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 Fluoreszens und Absorptionsmaxima bei 590 und 640 mμ. Das Pigment besitzt somit die Eigenschaften eines Bilitrientetrapyrrol 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 rabbit[1], cat and monkey[2], 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.

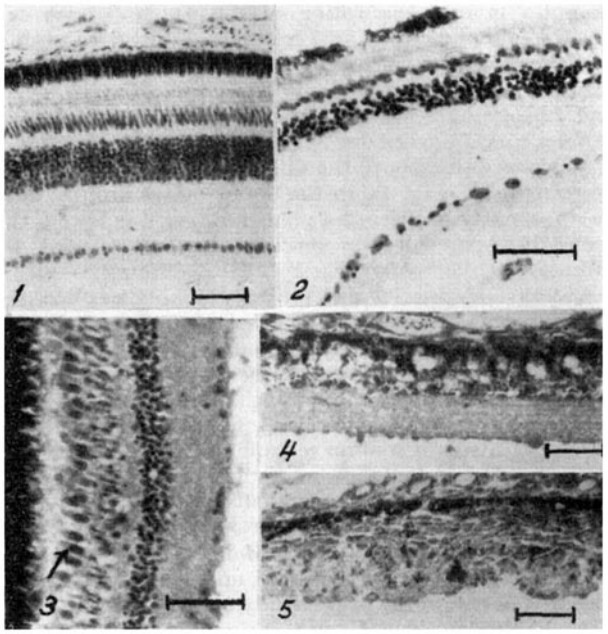

Fig. 1.–Retina of control chick; gallocyanin-chromalum stain.

Fig. 2.–Chick retina 2 h after iodoacetate injection. Pycnosis in the ganglionar and inner and outer granular layers; gallocyanin-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; gallocyanin-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 $^3/_4^{ths}$ 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 $^3/_4^{ths}$ of the treated animals. Pycnosis of the cells of the ganglionar layer was seen 30 min after the injection of the drug, increasing

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