structure so that the less dense band now represents material which more readily associates with nonribosomal protein. This association would take place during the early stages of centrifugation before the soluble protein could move to the less dense region of the gradient. The pattern of Fig. 3 for a mixture of soluble protein labeled with lysine-H^3 and nonlabeled ribosomes, indicates that this less dense band does not adsorb nonribosomal protein.

Repeated tests of the effects on partially purified ribosomes of Cs_2SO_4 centrifugation with both the Spinco SW-39 rotor and the Spinco model E analytical centrifuge, with both ultraviolet and Schlieren optics for analyses, indicate that the results are variable with respect to the formation of one or two bands. Most of the time there are two peaks but the relative amount of ultraviolet-absorbing material in one band compared with that in the other varies. Bentonite treatment of these partially purified ribosomes results in Cs_2SO_4 centrifugation patterns in which almost all the ultraviolet-absorbing material appears in one broad band.

The above technique allows further purification of partially purified ribosomes, but it is not designed to give high resolution and purification of ribosomes from very crude cell extracts. It will permit analytical studies to be made on the alteration of the structure of the ribosome (and its various complexes) brought about by treatment prior to Cs_2SO_4 centrifugation.

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Reversible, Light-Screening Pigment of Elasmobranch Eyes: Chemical Identity with Melanin

Abstract. *There lies directly beneath the tapetum lucidum, in the eyes of many elasmobranch fishes, a layer of darkly pigmented choroid cells which, in bright light, extend individual strands that aggregate to form a dark, compound curtain which shields the reflecting tapetal cells. This process is reversed in dim light or in darkness; the tapetum is exposed and visual sensitivity presumably increased. The black choroid pigment has been isolated, analyzed, and shown to possess the properties of melanin.*

Elegant studies by Nicol (*1, 2*), Denton and Nicol (*3*), earlier work by others cited by them, and a lucid review by Gilbert (*4*) focus attention upon the choroidal tapetum lucidum underlying the retina in elasmobranchs. In many sharks and in some rays, the tapetum is occlusible by a dark, compound curtain. In bright light, a layer of black, migratory cells beneath the tapetal layer extend individual, dark, finger-like processes (or dark microgranules through permanent, clear-walled channels) over the plate-like, guanine-laden reflecting cells of the tapetum lucidum. On return to darkness or to very dim light, the animal's eyes regain their eye-shine as the choroid pigmentary migration is reversed, leaving unshielded the bright, specular tapetum.

The dark, stationary choroid pigment in certain other animals, cattle, for example, is reportedly melanin (*5*, p. 226). The pigment involved in the tapetum-screening eyes of elasmobranchs has also been assumed to be melanin. However, there exist other dark pigments, such as chromolipids (*5*, pp. 69, 191 ff.), ommochromes, and sclerotins (*6*), that superficially can be confused with melanin. Since we were unaware of any analytical studies bearing on this material, we undertook diagnostic chemical examinations of elasmobranch choroid pigment.

Melanin, being an end-product of enzymic oxidation of tyrosine, is not a stoichiometric molecule, but a complex, oxidized, relatively inert polymer. It cannot, therefore, be identified with finality by any single test, but is characterized by a positive response to each of a number of chemical tests. Accordingly, the procedures and observations outlined below were applied to one or more of the species-products as designated.

We used 32 fresh eyes from the thornback ray (*Platyrhinoidis triseriata*), four fresh eyes from the horned shark (*Heterodontus francisci*), and two large, formalin-preserved eyes from the great blue shark (*Prionace glauca*). The cornea, lens, iris, and intraocular fluid were removed from each eye; the orb was then cut fully open, the exposed retina lifted away with fine forceps, and the dark choroid readily separated from the underlying sclera. The choroids from *P. glauca* were leached for a few days in distilled water to remove formalin.

A *Heterodontus* specimen showed dark tapetal occlusion in an eye which had been exposed to bright illumination, while the animal's other eye, which had been masked, kept its bright tapetum unshielded. *Platyrhinoidis*, whose choroid cells are less extensively mobile, notably in the ventral half, manifested, after exposure to bright light, a darkening of the dorsal half, with partial shading of its tapetum, both dorsally and ventrally, by melanophoric extensions. Another specimen, maintained for several hours in darkness, displayed bright tapeta from which the dorsal melanistic shading had been withdrawn.

Although a living specimen of the great blue shark (*Prionace*) was not available for similar experimentation, we share Dr. Gilbert's view (personal communication) that this predacious, pelagic, oceanic species almost certainly is capable of shading the tapetum in bright light.

For complete recovery of melanin, we applied the assay method evolved by Sumner and Doudoroff (*7*), based largely on the original methods of Gortner (*5*, p. 225). The ethanol-dehydrated material, dried and extracted of lipids with ethanol-ether in a Soxhlet apparatus, was digested in 0.2 percent NaOH under reflux. The filtrate from this digestion produced, on mild acidification with dilute HCl, a dark, powdery precipitate. After decantation or centrifugation, the dark residue was redissolved several times in cold, dilute

alkali and reprecipitated with dilute acid which was then decanted; the residue was washed and dried, and yielded a black, readily friable powder. The diagnostic tests that were made for melanin are outlined in Table 1.

The reactions given in this table are characteristic of melanins throughout. Special attention is directed to (i) the evolution of pyrrole on roasting the dry pigment; (ii) the reduction of silver ion; and (iii) the positive reaction for indolic or pyrrolic derivatives, or both, after treatment of the dried material with fused KOH.

We modified the customary methods for tests (ii) and (iii) in the interest of increased certainty. In exploring for the reduction of the ammoniacal silver ion to the free metal, we chose a more searching procedure than appears to be afforded by the usual microscopic method (*8*). We performed the work in glass vessels instead of on microscope slides and followed the procedure given below.

The black, powdery material was dissolved in 5 percent NH_4OH and gave a coffee-colored filtrate. To a sample was added about one-fourth its volume of 5-percent $Ag(NH_3)_2NO_3$, and to control an equal proportion of distilled water. Both the test sample and the control were stoppered under an atmosphere of nitrogen. After storage overnight in the dark at room temperature, the silver-containing sample was noticeably darker than the control and showed traces of fine, dark, solid material on the bottom. The silver-treated sample was passed through fine filter paper (Whatman No. 50) to retain any elementary silver, and the paper was washed first with dilute NH_4OH and finally with distilled water until fresh filtrate showed no trace of AgCl on addition of chloride. The paper and the residue it contained were ashed in a crucible, and the ignited residue was treated with hot, concentrated HNO_3, diluted, and then filtered. A faint cloud in the filtrate (presumably due to traces of residual chloride in the ignited material) was intensified by the addition of NaCl, and all turbidity was cleared by the addition of NH_4OH, which indicates the presence of AgCl.

For the so-called indole reaction, the following procedure was used: Some of the dark powder was dropped into pure, fused KOH; the mixture was brought to a boil (when a mildly fecal odor suggestive of indole or skatole was noticed), and then cooled. The dark solid mixture was dissolved in distilled water, a comparable volume of saturated NaCl solution was added (to favor partition of indolic compounds into the extracting fluid), and this mixture was shaken with diethyl ether. To a few milliliters of the colorless ether extract were added a few drops of Ehrlich's reagent (4 g of *p*-dimethylaminobenzaldehyde in 380 ml of ethanol and 80 ml of concentrated HCl) with the result that the coral-pink, violet-red, or magenta colors characteristic of indole or pyrrole derivatives were produced.

Table 1. Diagnostic tests for melanin.

Test	Reaction	Source of choroid pigment
Solubility		
Inorganic solvents		
Dilute alkali	Very soluble (dark brown sol.)	All three spp.
Dilute acid	Precipitated thereby, as black powder	All three spp.
Conc. H_2SO_4	Soluble (brown sol.)	Blue shark
Lipid solvents		
Ethanol	Insoluble	Blue shark
Diethyl ether	Insoluble	Blue shark
Acetone, warm	Insoluble	Blue shark
Chloroform, warm	Insoluble	Blue shark
Specific solvents		
Pyridine	Slightly soluble (pale brown sol.)	Blue shark
Chloroethanol	Soluble (dark brown sol.)	Blue shark
Oxidizing agents		
Chlorine	Bleaching, usually complete in 20 hours	All three spp.
Peroxide (peracetic acid)	Bleaching, usually complete in 20 hours	All three spp.
Ammoniacal silver ion	System darkened, liberating Ag	Blue shark; horned shark
Degradation		
Roasting	Vapors reddened a pine splinter moistened with conc. HCl (pyrrole)	Blue shark; horned shark
Fusion in KOH (*9*)	Ether extract + Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in acidic ethanol) gave violet-red or magenta color produced by indolic or pyrrolic compounds	Blue shark

Our introduction of the step calling for dissolving the cooled solid in water, rather than attempting to extract the ground material directly with ether or alcohol-ether, was found to render the test far more reliable and delicate, and to be critically important when only small amounts of test material were available.

Pure tryptophan or pure indoleacetic acid gave no color with Ehrlich's reagent, but material extracted with diethyl ether from solutions of the KOH-fusion of each of these compounds gave immediate color reactions with the reagent. The absorption spectrum of each of the resulting solutions agreed precisely with the spectrum manifested by pure indole, which, when tested without previous fusion, gave a single maximum at 566 mμ and a shoulder at 536 mμ.

A solution of pyrrole in ethanol, decolorized with active charcoal and treated with Ehrlich's reagent, gave an immediate violet-red color, fading in the cuvette, but showing a decreasing maximum at 545 mμ and a slightly increasing secondary absorption at 470 mμ.

Our product from the black choroid gave, with the same reagent, a single high maximum at 542 mμ. The solution did not fade in color, but the next day showed additional general absorption toward the blue-violet end. Its initial absorption profile agreed very well with that obtained earlier with the use of black-feather melanin and with the melanoid magenta chromogen from the jellyfish *Palagia* (now *P. colorata*) (*9*). In the earlier work, indole, after fusion in air with KOH, gave spectral absorption maxima at about 537 and 465 mμ with Ehrlich's reagent, and the colored derivative from alkali-fused tryptophan showed a single maximum at about 510 mμ, which suggested that additional heating might produce further breakdown (*9*).

A considerable number of hydroxylated indoles and carboxy-substituted pyrroles have been recovered as products of alkaline fusion or drastic oxidation of purified melanin from *Sepia* ink (*10–14*). The degradation products obtained from choroid melanin or from black-feather melanin, which exhibited closely similar absorption spectra when treated with Ehrlich's reagent, could not be identified with a known indolic or pyrrolic compound.

It has long been known, notably from the experiments of Sumner (*15*),

that melanin microgranules gradually disperse within dendritic epidermal melanophores of teleost fishes as a response to dark backgrounds and reaggregate in the center of the cell, giving the animal a pale appearance in white-reflecting aquariums. Such light-induced reactions are regarded by many naturalists (*16*) as being valuable for survival by contributing to the animal's concealment from predators.

Direct or reflected illumination as opposed to dim light, however, evokes responses opposite from the above in the choroid melanophores of elasmobranchs, inducing a dispersal of light-shielding melanin over the specular tapetum in bright light and, in darkness, retreat of the pigment into the mother-cells. The tapetal surface is thus exposed in dimly illuminated environments. Gilbert (*4*) quotes and agrees with Nicol, who pointed out that visual acuity is likely to be improved on a black, absorbing surface, and sensitivity increased by a specular tapetum. It is conceivable that a shark, adapting itself for improved vision in moderately lighted waters by partially shading its tapetum, may also profit to some degree through diminution of its conspicuous eye-shine, but that at deeper and darker levels the increased tapetal exposure, augmenting the predator's visual perceptiveness, should more than compensate for the enhancement of the eye-shine and its incidental warning signals.

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Regulation of Cockroach Fat-Body Metabolism by the Corpus Cardiacum in vitro

Abstract. *Incubation in vitro of young, adult, male* Leucophaea maderae *fat body with extracts of corpora cardiaca or intact corpora cardiaca results in stimulation of oxygen consumption but reduction in carbon dioxide evolved from carbohydrate. The carbohydrate is preferentially used for trehalose synthesis, and the endogenous metabolism of the fat body appears to be supported by increased lipid utilization. A hormone from the corpus cardiacum is most likely responsible for these effects and may act at two points, at least, in the glycolytic pathway.*

The corpus cardiacum is an important component of the neuroendocrine axis that regulates the growth and development of insects. It is located between, and connected to, the brain (source of the brain hormone) and the corpus allatum (source of the juvenile hormone). In addition to the proposed role of the corpora cardiaca as storage centers or modifiers of the brain hormone during the molting cycle, these structures have also been implicated as regulators of physiological processes vital to the existence of the organism. For example, the corpus cardiacum stimulates the muscular contraction of the insect heart, hindgut, and malphigian tubules (*1*); it depresses the spontaneous activity of the central nervous system (*2*); and it blocks inhibitory centers of the nervous system (*3*). The release of the humoral substance or substances from the corpus cardiacum may be a consequence of normal feeding (*4*) or may be induced by enforced hyperactivity or electrical stimulation (*5*). The chemical nature of the factor or factors responsible for the various actions of this gland is not known with certainty, although there have been some attempts to separate the components of the gland by differential centrifugation and solvent partitioning (*6*).

The injection of corpus cardiacum extracts into cockroaches (*Periplaneta americana* and *Blaberus discoidalis*) results in the mobilization of fat-body glycogen and a consequent increase in blood trehalose (*7*). These alterations in substrate concentration may be due to an increase in the concentration of active phosphorylase (*8*). We have shown with techniques in vitro that the corpus cardiacum stimulates the release of trehalose from the fat body of *Leucophaea maderae*, with a concomitant decrease in fat-body glycogen (*9*). In the course of experiments designed to perfect a quantitative bioassay for this "hyperglycemic hormone," we observed the release of trehalose from the C^{14}-labeled glycogen from the fat body and noted new effects of the corpus cardiacum on fat-body metabolism that have bearing on the mechanism of action of the hormone.

The experimental insects were male adults 4 to 7 weeks old; they were raised at 26°C, 85-percent relative humidity, on dog biscuits and water. Experimental conditions in vitro were designed to minimize effects on other organs that could respond to the hormone and secondarily affect the fat body. Hormone extracts were prepared by homogenizing corpora cardiaca in buffered Ringer solution (0.005*M* tris, *p*H 7.5), heating in a water bath at 100°C for 7 minutes, and using the supernatant after centrifugation of the boiled homogenate at 3000*g* for 5 minutes (*7*). The fat bodies from 8 to 12 decapitated cockroaches were dissected out under cold buffered Ringer solution, gently separated into small pieces, and randomly distributed in four to six reaction flasks. Incubation at 30°C in an oscillating water bath was followed by removing and rinsing the tissues, and homogenizing them in a mixture of chloroform and methanol (2:1) to extract the lipid. The "lipid-free dry weight" or lean dry weight was determined gravimetrically and was a convenient and reproducible representation of the tissue mass. Glycogen was extracted from the lipid-free material with trichloroacetic acid and subsequently precipitated with ethanol. Preparation of sodium palmitate and assay of the lipid, glycogen, and CO_2 fractions by liquid-scintillation spectrometry was as previously described (*10*), except that in the present work the glycogen was