Michigan. The burrows were at the margin of a pit dug in the base of the quarry. All lay within about 20 meters of each other; two were somewhat displaced by digging equipment, but all had come from approximately the same level. The character of the matrix appears to be uniform from the base of the pit to a few meters above the burrows. It is a soft, light gray shale used in making vitrified tiles. Since the burrows were discovered, this pit has become filled with water which now just covers the level of the burrows.

According to Arnold (*5*), the lower beds exposed in the quarries at Grand Ledge are at approximately the level of cycle "A" of Kelly (*6*), in the Saginaw group, Pottsville series. Although these burrows are of somewhat greater diameter and are considerably older than the lungfish burrows from Texas, they may have been made by the same genus, *Gnathorhiza*, which is reported from the Danville formation, lower Conemaugh (*7*), stratigraphically only slightly above the Michigan beds.

This discovery extends by almost a full period our knowledge of the aestivation of lungfish and reinforces Romer and Olson's suggestion of a very early separation of the aestivating line of lungfish from the more primitive, nonaestivating forms such as *Ceratodus* and *Epiceratodus*.

The presence of lungfish burrows raises again the question of why the Michigan coal basin, so rich in plant material, has so far revealed no terrestrial vertebrates and only a few scraps of aquatic vertebrates. Except for a pleuracanth spine (*8*) and several other undescribed fish spines, no other vertebrate remains have been reported from the Pennsylvanian of Michigan.

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23 February 1965

Drosophila Phenol Oxidases

Abstract. *The phenol oxidase systems of* Drosophila melanogaster *arise from at least four protein components. One A component plus the P preparation yields tyrosinase, while the other two (A_2 and A_3) yield primarily 3,4-dihydroxyphenylalanine oxidases. Probably the four components represent specialized subunits of the oxidases produced.*

Beginning with a primary interest in the mechanisms for the regulation of the onset of melanin pigmentation about the end of the third day of the pupal life of *Drosophila melanogaster*, we have made an extensive study of the phenol oxidase system in this organism. The specific aspect of the problem that is considered here is derived from the original observation of Horowitz and Fling (*1*), who demonstrated that in adult flies tyrosinase activity in dilute extracts appears only after a period of incubation at 0°C. Evidence was presented for the existence of an inactive proenzyme and a separable activator protein. A similar situation was indicated for *Drosophila* larvae by Ohnishi (*2*) and for *Calliphora erythrocephala* diphenol oxidase by the extensive investigations of Karlson and collaborators (*3*). Thus, one phase of our program has been concerned with obtaining more specific information on the nature and number of components that enter into the formation of the phenol oxidases. The data presented here show that in *Drosophila melanogaster* there are at least four components. Three are designated A_1, A_2, and A_3; each gives rise to a phenol oxidase by reaction with the fourth component, designated P.

For preparation of A components a 1-g sample of wild-type (Oregon-R) pupae, aged 46 to 48 hours from puparium formation (*4*), was frozen at −80°C and then ground for 2 minutes at 0°C in a glass cone grinder with 20 ml of cold buffer [0.1*M* potassium phosphate, *p*H 6.3, containing 10 mg of disodium ethylenediaminetetraacetate (EDTA) per milliliter]. The homogenate was centrifuged at 0°C for 5 minutes at 18,000*g*, and the supernatant solution was subjected to fractionation with ammonium sulfate. The salt was added as a cold saturated solution in buffer, but the resulting *p*H 5.2 was not readjusted after saturation of the buffer with ammonium sulfate. Fractions retained after the addition of $(NH_4)_2SO_4$ were those resulting from saturations of 36 to 41 percent, 41 to 43 percent, 43 to 47 percent, and 47 to 56 percent, respectively. The first three fractions were washed once with ammonium sulfate solution, and each of the samples was dissolved in phosphate buffer (5 ml). Portions (0.05 ml) were subjected to electrophoresis on acrylamide gels for 3 hours at 4°C and 1.5 milliamperes per tube. The standard equipment and gel solutions obtained from Canalco (*5*) were used, with the exception that the lower gel was diluted to 6.5 percent.

For detection of A components on gels, a preparation of the fourth component, P, was obtained by homogenizing pupae in buffer as already described, but without addition of EDTA, which slows activation and alters the properties of P. The homogenate was centrifuged immediately, and the supernatant solution was adjusted quickly to 41 percent saturation with $(NH_4)_2SO_4$. The precipitate was washed with the salt at 41 percent saturation and then dissolved in phosphate buffer (10 ml per gram of pupae). Particulate matter was then removed by centrifugation at 105,000*g* for 1 hour at 0°C. The supernatant solution was used directly or stored

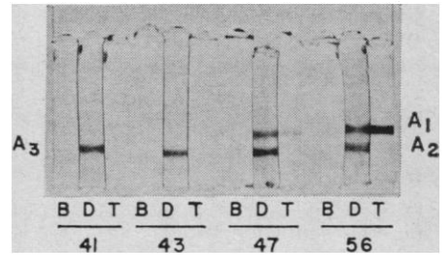

Fig. 1. Gel electrophoresis of A components from 48-hour pupae of *Drosophila melanogaster*. The origin of migration of proteins is at the bottom. Each gel of the four groups of three received 50 μl of solution from the $(NH_4)_2SO_4$ fraction indicated. Gels marked *B* were incubated with buffer and then with 3,4-dihydroxyphenylalanine. Those marked *D* and *T* were incubated first with the P fraction, then with the phenylalanine for *D* and with tyrosine for those marked *T*. Distinct A components are indicated by A_1, A_2, and A_3. Gels not containing A components but incubated with P and then the phenylalanine show no oxidase bands.

at −80°C. In use, extruded gels containing A components were immersed in the solution of P at 4°C for a period of 3 to 5 hours. Gels were then washed twice with buffer and incubated further with 3,4-dihydroxyphenylalanine or tyrosine (0.4 mg/ml in phosphate buffer) to show the locations of the phenol oxidases produced. The gels were incubated (Fig. 1) for about 20 hours at 4°C before the photographs were made, but the bands could be detected in less than 1 hour; they appeared initially as red rings and turned black in a short time.

Obviously from the results (Fig. 1) the components A_1 and A_2 differ both in electrophoretic behavior and in specificity with respect to oxidase activity. A_1, which is present in the 47- and 56-percent ammonium sulfate fractions, yields primarily a tyrosinase, whereas A_2 which appears also in the 43-percent fraction yields primarily a 3,4-dihydroxyphenylalanine oxidase. The latter oxidase gives a weak reaction with tyrosine after several days, but this may be due to oxidation in air of tyrosine to dihydroxyphenylalanine. In any case there is an extreme difference in reaction rates. On electrophoresis the component A_3 in the 41-percent fraction moves only slightly differently from A_2, but it is very nearly completely separated by the salt fractionation. As shown, A_3 also yields primarily a diphenol oxidase.

As to the activation processes which yield the phenol oxidases, it may be noted that if activation is permitted prior to electrophoresis then A_1, A_2, and A_3 do not appear on the gels, and oxidase function is found at or near the starting gel interface. After activation, furthermore, oxidase function is easily sedimented in the centrifuge just as observed (*1*) for the crude extracts of adult flies. For these reasons activation may be represented as a process of assembling the enzyme from the subunits represented as A_1, A_2, or A_3 plus a component of the P fraction. Our data do not rule out a participation of proteolytic action in activation, as indicated for diphenol oxidase in *Calliphora* (*6*); in fact the P fraction does contain two components of which one functions catalytically in the activation process.

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Annelid Ciliary Photoreceptors

Abstract. *The photoreceptor cells of the tube-dwelling polychaete* Branchiomma vesiculosum *contain stacked disc-shaped membranous sacs which are the expanded and flattened outer membranes of cilia.*

The light-sensitive organelle of vertebrate photoreceptors is a stack of membrane-covered discs originating from a cilium-like outgrowth of rod or cone cell (*1*). The photosensitive organelle of the arthropod retinula cell is a field of microvilli apparently unassociated with ciliary apparatus at any stage of development (*2*). Having surveyed the evidence on the fine structure of presumed light-sensitive organelles in several phyla, Eakin (*3*) suggests with some reservation that photoreceptors of ciliary origin may be restricted to echinoderm-vertebrate stock, while those of nonciliary (rhabdomeric) organization may be the rule for the annelid-arthropod line.

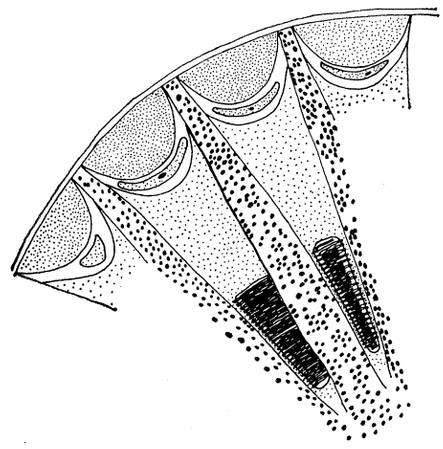

Fig. 1. A schematic drawing of part of a radial section through an eye of *B. vesiculosum* to illustrate its gross organization. Nuclei of lens cells are shown as crescent-shaped structures; those of the receptor cells are off the plane of section. The two completely drawn units are depicted as though cut at right angles to each other and they correspond to the left and right units, respectively, of Hesse (*5*, Fig. 64).

We wish to draw attention to an annelid photoreceptor whose presumed photosensitive structures are of distinct ciliary origin. The tip of each branchial filament of the tube-dwelling polychaete *Branchiomma vesiculosum* possesses a dark, spherically shaped eye which is an aggregate of some 40 to 80 cone-shaped receptive elements separated from one another by dense pigmentation (Fig. 1). Each conical element is composed of a lens cell sitting atop a receptor cell. According to Brunotte (*4*) the apical (deep) half of each receptor cell is filled by a body of lamellar appearance. Hesse (*5*) de-

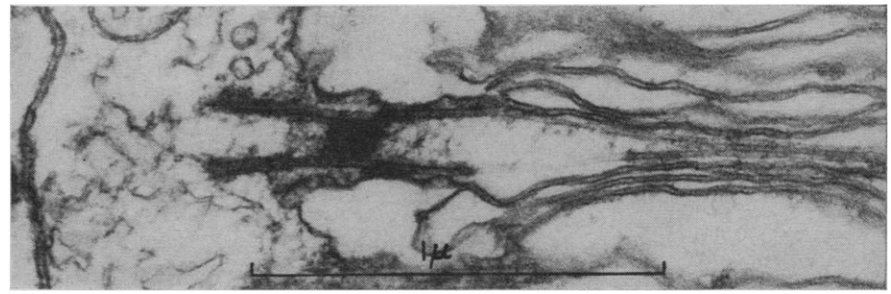

Fig. 2. Electron micrograph of a cilium emerging from the cytoplasmic wall (left) of the apical cavity and projecting into it (toward the right). The plane of section corresponds to that of Fig. 1. The outer membrane of the cilium and two of its axial filaments can be followed into the "lamellar" region, whose edge is shown at the right of the micrograph. Above and below this "cilium" are the edges of discs arising from other basal bodies.