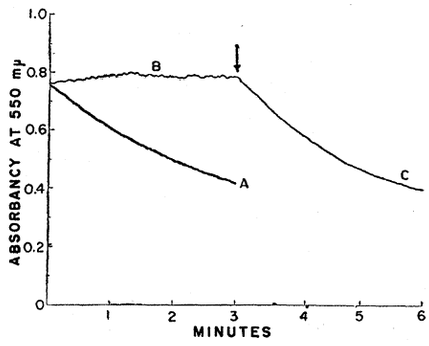

Fig. 2. Spectrophotometric demonstration of inhibition of cytochrome *c* oxidase by protamine sulfate and reversal of protamine inhibition by polyglucose sulfate. Curve *A* (control), oxidation of dithionite-reduced cytochrome *c* by untreated deoxycholate-solubilized oxidase preparation. Curve *B*, complete inhibition of oxidase by incorporation of protamine sulfate (final concentration 33 μg/ml) into the system. Wavering of absorbancy trace was caused by slight turbidity arising from protamine sulfate combination with deoxycholate preparation. Arrow, readings interrupted for 20 to 30 seconds while protamine sulfate (final concentration 33 μg/ml) was added to cuvette with rapid mixing. Curve *C*, reversal of protamine inhibition by polyglucose sulfate addition.

it is shown that not only was the inhibition of the protamine completely reversed by the polyglucose sulfate addition, but, as evidenced by the greater slope of curve *C*, the reaction rate was increased in comparison with that of the untreated control shown in curve *A*. This increase in activity beyond control values resulting from polyglucose sulfate addition is a real phenomenon. It is undoubtedly a result of the fact that in addition to reversal of the protamine inhibition, per se, the amount of polyglucose sulfate used was also sufficient to reactivate some reversibly denatured oxidase in the original enzyme preparation. This finding has also been established by separate heat-denaturation experiments.

Similar reversible inhibitions of cytochrome *c* oxidase have also been demonstrated, using fresh whole rat heart homogenates and the Keilin and Hartree type of insoluble beef heart muscle oxidase preparations. In addition, histone, ribonuclease, and lysozyme were also capable of inhibiting the cytochrome *c* oxidase activity of the above preparations. As with the protamine inhibitions, those produced by the other basic proteins mentioned above could be reversed by polyglucose sulfate.

It is important to stress that the type of reversible inhibition described in this report is in no way specific for cytochrome *c* oxidase. Similar behavior has been established for a number of other enzymes (*9*). However, the fact that cytochrome *c* oxidase may be reversibly inhibited by polyionic macromolecules is of great significance. Until now, the most important reversible inhibitions of cytochrome *c* oxidase have been those accomplished with cyanide, azide, and carbon monoxide. Spectroscopic and spectrophotometric studies of these classic inhibitions have contributed much to our understanding of the enzyme, and in particular, its heme component. It is therefore of great interest that, thus far, spectral changes or shifts during the inhibitions by the cationic macromolecules reported in this paper have not been demonstrable in a large number of experiments. This leads us to suspect that charge-density influences upon the configuration of the protein (and other nonheme components) of the oxidase aggregate may be operating in the present type of inhibitory action. Effects that involve electron transfer through the nonheme moieties of the enzyme are now under consideration.

The disclosure that substances such as protamine, histone, and ribonuclease, which are important intracellular proteins, can exert a reversible inhibition of a major enzymatic component of cell mitochondria also has important implications for the study of nuclear-cytoplasmic metabolic interrelations. Finally, since spectrophotometric evidence, to date, indicates that the heme component of the oxidase is not involved in these inhibitions, we believe that a means is now available for approaching the study of the contributions of the nonheme components of cytochrome *c* oxidase to the electron transfers accomplished by the enzyme.

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References and Notes

1. P. Person and A. Fine, *Nature* **183**, 610 (1959).
2. ———, *Arch. Biochem. Biophys.* **84**, 122 (1959).
3. L. Smith and H. Conrad, *Federation Proc.* **17**, 313 (1958).
4. J. W. Lash, *Science* **130**, 334 (1959).
5. ——— and M. W. Whitehouse, *Biochem. J.* **74**, 351 (1960).
6. D. Keilin and E. F. Hartree, *Proc. Roy. Soc. London* **B127**, 167 (1939).
7. B. Eichel, W. W. Wainio, P. Person, S. J. Cooperstein, *J. Biol. Chem.* **183**, 89 (1950).
8. J. W. Wood and P. T. Mora, *J. Am. Chem. Soc.* **80**, 3700 (1958). Polyglucose sulfate was obtained through the generosity of Peter T. Mora, National Cancer Institute, Bethesda, Md.
9. J. S. Roth, *Arch. Biochem. Biophys.* **44**, 265 (1953); P. T. Mora and B. G. Young, *ibid.* **82**, 6 (1959).

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Trophic Substances in a Blind Cave Crayfish

Abstract. The eyestalks, supraesophageal ganglia, and circumesophageal connectives of the blind cave crayfish *Orconectes pellucidus australis* contain a red pigment-concentrating substance and a distal retinal pigment light-adapting one. Assays were performed on the dwarf crayfish, *Cambarellus shufeldti*. The significance of these findings is discussed in relation to endocrine regulation of pigmentary effectors in crayfishes.

The crayfishes *Cambarellus shufeldti* and *Orconectes clypeatus* produce some substances that concentrate chromatophoral pigments and others that cause the distal retinal pigment to migrate toward the fully light-adapted position (*1–4*). No one has determined whether such trophic substances occur in cave crayfishes which lack chromatophores and retinal pigment cells.

Recently we were fortunate to obtain enough specimens of *Orconectes pellucidus australis* (*5*) to learn whether this organism produces a red pigment-concentrating hormone and a distal retinal pigment light-adapting substance. These crayfish were collected in Shelta Cave, Huntsville, Alabama. The specimens of *Cambarellus shufeldti* used as assay

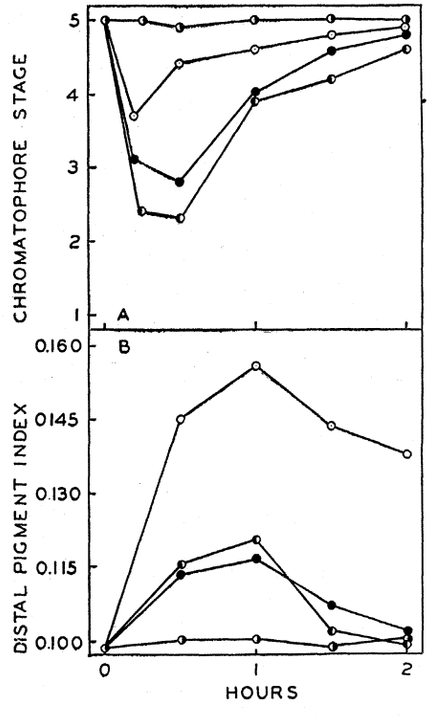

Fig. 1. Responses of (*A*) dark red chromatophores and (*B*) distal retinal pigment of dwarf crayfish to extracts of eyestalks (circles), supraesophageal ganglia (dots), and circumesophageal connectives (circles half-filled on left) of blind cave crayfish. Control, circles half-filled on right.

animals were collected in roadside ditches at Hickory, Louisiana.

The method of Sandeen and Brown (*6*), as modified by Fingerman (*3*) for use with crayfishes, was employed to determine the effect of an extract on the distal retinal pigment of *Cambarellus*. Crayfish were placed one at a time, ventral surface down, on the stage of a stereoscopic dissecting microscope. With the aid of an ocular micrometer and transmitted light two measurements were made: (i) the width of the translucent distal portion of the compound eye in a plane parallel to the long axis of the eyestalk and (ii) the length of the eye from the corneal surface to the apex of the notch at the proximal portion of the eye. The ratio of length of clear area (measurement i) to total length (measurement ii) is known as the distal pigment index.

The dark red chromatophores in the portion of the carapace of *Cambarellus* dorsal to the heart were staged according to the system of Hogben and Slome (*7*). Stage 1 represents maximal concentration of pigment; stage 5 maximal dispersion; and stages 2, 3, and 4 the intermediate conditions.

Student's *t*-test was used for determination of the level of significance between means. The 5 percent probability level was considered the maximum for a significant difference. The results of the statistical analyses comprise Table 1.

Table 1. Summary of the statistical analyses. *N* is number of chromatophore indices or distal pigment indices used in the analysis; S.D. is standard deviation; S.E. is standard error of the difference between the means; *t* is Student's *t*; *p* is probability value. The letters signify extracts of: (C) control, (CC) circumesophageal connectives, (ES) eyestalks, (SG) supraesophageal ganglia.

Analysis	Extract	*N*	Mean	Range	S.D.	S.E.	*t*	*p*
			Red pigment concentration					
1	ES	23	4.00	2.0 –5.0	0.724	0.157	5.75	0.001
	C	24	4.97	4.0 –5.0	.204			
2	SG	24	2.91	1.0 –4.0	.865	.181	10.71	.001
	C	24	4.97	4.0 –5.0	.204			
3	CC	24	2.37	1.0 –4.0	.672	.144	13.98	.001
	C	24	4.97	4.0 –5.0	.204			
4	ES	23	4.00	2.0 –5.0	.724	.232	4.54	.001
	SG	24	2.91	1.0 –4.0	.865			
5	ES	23	4.00	2.0 –5.0	.724	.204	6.84	.001
	CC	24	2.37	1.0 –4.0	.672			
6	SG	24	2.91	1.0 –4.0	.865	.224	2.18	.05
	CC	24	2.37	1.0 –4.0	.672			
			Light adaptation of distal pigment					
7	ES	22	0.150	0.10–0.19	0.0250	0.00604	12.83	0.001
	C	26	.100	.08– .13	.0118			
8	SG	26	.114	.09– .13	.0115	.00322	3.89	.001
	C	26	.100	.08– .13	.0118			
9	CC	26	.117	.08– .16	.0197	.00451	3.63	.001
	C	26	.100	.08– .13	.0118			
10	ES	22	.150	.10– .18	.0250	.00603	9.18	.001
	SG	26	.114	.09– .13	.0115			
11	ES	22	.150	.10– .18	.0250	.00680	6.22	.001
	CC	26	.117	.08– .16	.0197			
12	SG	26	.114	.09– .13	.0115	.00448	0.637	>0.5
	CC	26	.117	.08– .16	.0197			

Eyestalks, supraesophageal ganglia, and circumesophageal connectives of three crayfish were each extracted in 0.6 ml Van Harreveld's solution (*8*). The extracts were assayed for red pigment-concentrating hormone and distal retinal pigment light-adapting hormone on six dwarf crayfish in black containers maintained under an illumination of 29 ft-ca. Each assay animal received 0.02 ml of extract. Each control animal received 0.02 ml of Van Harreveld's solution. The second time the experiment was performed each extract was injected into seven animals. The results of the two experiments were essentially the same and were averaged (Fig. 1).

Chromatophore stages, determined 15 and 30 minutes after the extracts were administered, were subjected to statistical analysis. The amount of red pigment concentration produced by each extract was statistically significant (analyses 1–3). Furthermore, the response to the eyestalk extracts was significantly less than the response to extracts of either the supraesophageal ganglia or circumesophageal connectives; the response to the supraesophageal ganglia was in turn significantly less than the response to the circumesophageal connectives (analyses 4–6).

Distal pigment indices obtained 30 and 60 minutes after the extracts had been injected were also subjected to statistical analysis. The amount of light-adaptation produced by each extract was statistically significant (analyses 7–9). The degree of light-adaptation produced by the eyestalks was greater than that produced by either the supraesophageal ganglia or the circumesophageal connectives (analyses 10 and 11), but no significant difference was found between the responses to the extracts of the supraesophageal ganglia and the circumesophageal connectives (analysis 12).

Fingerman (*1*, *2*) found that the circumesophageal connectives of a specimen of *Cambarellus shufeldti* or *Orconectes clypeatus* contain more red pigment-concentrating hormone than do the supraesophageal ganglia, which in turn contain more of the hormone than is present in a pair of eyestalks. The same order was found for organs of *Orconectes pellucidus australis* (Fig. 1*A*).

Fingerman, Mobberly, and Sundararaj (*4*) found that the supraesophageal ganglia of *Cambarellus* produce more light-adaptation of the distal retinal pigment than do the eyestalks. In contrast, eyestalks of *Orconectes clypeatus* contain more light-adapting hormone than the supraesophageal ganglia (*4*) just as was observed with organs of *Orconectes pellucidus australis* (Fig. 1*B*).

The cave crayfish used in this investigation possesses neither retinal pigments nor chromatophores. Presumably, this cave dweller descended from eyed forms that possessed chromatophores and retinal pigments as well as mechanisms to regulate migration of their chromatophoral and retinal pigments. The persistence of activators of these pigments in blind specimens indicates that either the loss of the controlling mechanism takes longer than the loss of the end organ, or blind forms have given these trophic substances a new function (*9*).

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References and Notes

1. M. Fingerman, *Tulane Stud. Zool.* **5**, 137 (1957).
2. ——, *Am. Midland Naturalist* **60**, 71 (1958).
3. ———, *J. Cellular and Comp. Physiol.* **50**, 357 (1957).
4. M. Fingerman, W. C. Mobberly, Jr., B. I. Sundararaj, *Am. Midland Naturalist* **62**, 429 (1959).
5. The authors are indebted to Dr. G. H. Penn and Dr. R. D. Suttkus of Tulane University for supplying the cave crayfish.
6. M. I. Sandeen and F. A. Brown, Jr., *Physiol. Zool.* **25**, 223 (1952).
7. L. T. Hogben and D. Slome, *Proc. Roy. Soc. London*, **B108**, 10 (1931).
8. A. Van Harreveld, *Proc. Soc. Exptl. Biol. Med.* **34**, 428 (1936).
9. This investigation was supported by grant No. B-838 from the National Institutes of Health.

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