11434

Squid Melanin: A Naturally Occurring Reversibly Oxidizable Pigment.*

FRANK H. J. FIGGE.

From the Department of Anatomy, University of Maryland Medical School, Baltimore, Md., and the Marine Biological Laboratory, Woods Hole, Mass.

It was reported previously that synthetic melanin resulting from either the enzymatic oxidation of tyrosine or auto-oxidation of dihydroxyphenylalanine was reversibly oxidizable.[1] It was also found that natural melanin was reversibly oxidizable but that the reaction was far more sluggish and barely perceptible. This was thought to be due to the necessarily low concentrations obtainable and the contaminations present in the solutions of natural melanin.[1]

The change in percentage light absorption on reduction of synthetic melanin was very marked. It was thought that these could be duplicated if a source of relatively pure and concentrated natural melanin could be found. Such a source was found in the so-called ink of the ink-sac of the squid (*Loligo pealii*). The ink is a highly concentrated, relatively pure colloidal solution of melanin. The particles were so small that they were invisible under the ordinary microscope, but visible with dark field illumination.

The ink was collected by removing the squid from the water with a net as carefully as possible to prevent discharge of the ink. The mantle was slit immediately with a pair of long scissors. The ink-sac was then dissected out without much danger of being discharged. Many attempts to anesthetize or immobilize the animal by cooling almost always caused the discharge of most of the ink. The ink was withdrawn with a syringe or expressed into a vial after the sphincter had been removed. The ink collected in this manner was extremely concentrated. After diluting with 300 volumes of water, an ink solution, approximately 1 cc in thickness, would transmit an amount of light that was barely registered by a sensitive photoelectric colorimeter.

For purposes of comparison, a solution of dopa melanin was prepared by auto-oxidation in air. The concentrations of the squid melanin and the dopa melanin solution were adjusted so that they

* Aided by a grant from the American Association for the Advancement of Science.

1 Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 127.

both absorbed 90% of the light. Both solutions were then reduced with sodium hydrosulphite and the light absorption values again determined. The reduced dopa melanin absorbed 66% of the light, while the reduced squid melanin absorbed 76% of the light. The melanin solutions were then re-oxidized with potassium ferricyanide. The re-oxidized dopa melanin absorbed 89% of the light, while the re-oxidized squid melanin absorbed 88.5%. This experiment was repeated 22 times with ink from 35 squid. The results were always uniform.

It may be seen from the light absorption values that the reversibility of the oxidation of the natural squid melanin approached that of synthetic dopa melanin. The figures indicate that the squid melanin was contaminated with some substance that absorbed about 10% of the light and which was not reversibly oxidizable. This substance may have been melanin that had aged in the squid ink-sac because even synthetic melanin 6 months old was not reversibly oxidizable.

11435 P

Relationship between "Spreading Factor" and Hyaluronidase.*

KARL MEYER, GLADYS L. HOBBY, ELEANOR CHAFFEE, AND MARTIN H. DAWSON

From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, The Institute of Ophthalmology, and the Edward Daniels Faulkner Arthritis Clinic Presbyterian Hospital, New York.

Duran-Reynals[1] demonstrated the presence of an extractable factor in certain tissues and bacteria which enhances the invasiveness of some pathogenic agents. This factor has been extensively studied by Duran-Reynals and by others.[2, 3] Chain and Duthie[4] reported that testis extracts containing "spreading factor" decrease the viscosities of synovial fluid and vitreous humor with the liberation of reducing substances. They suggested that "spreading factor"

* This work has been supported in part by a grant from the John and Mary Markle Foundation.

1 Duran-Reynals, F., *Compt. rend. Soc. biol.*, 1928, **99**, 6; *J. Exp. Med.*, 1929, **50**, 327; *ibid.*, 1933, **58**, 161; *Yale J. Biol. and Med.*, 1939, **11**, 601.

2 McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045; *ibid.*, 1936, **42**, 477.

3 Claude, A., *J. Exp. Med.*, 1937, **66**, 353; Claude, A., and Duran-Reynals, F., *J. Exp. Med.*, 1937, **65**, 661.

4 Chain, E., and Duthie, E. S., *Nature*, 1939, **144**, 977.