temperature of the cathode was raised further until active electron emission occurred. The electron source was imaged upon the fluorescent screen with appropriate magnetic lenses. Photographs were then made of the luminescent image.

After many tests with pure salts of magnesium and calcium it was determined that the electron source, responsible for the image, was these elements. Examination of such preparations enabled us to localize with some accuracy magnesium and calcium in striated muscle from several mammalian species. Due to the preliminary freezing process muscle is thrown into many strong contractions. In every case we could localize magnesium and calcium in these contraction bands. Little or none of these elements was present in the remainder of the sarcoplasm. It is also of some interest that there was no magnesium or calcium detected in the "tissue spaces" of Fenn.[5] Apparently the bulk of these elements existing in muscle is to be found in the muscle cells and not in the connective tissue. Naturally some must be present there since both magnesium and calcium must be transported to and from capillary and muscle cell.

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Preliminary *in vitro* Studies of Melanophore-Principle Activity of the Pituitary Gland.*

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An attempt was made to correlate the melanophore-dispersing hormone of the hypophysis with melanin production through the use of a known oxidase system (tyrosine-tyrosinase). The oxygen consumed in this system was measured with the Warburg apparatus. The melanophore-containing fractions were prepared by a modification of Stehle's method.[1] Acetone-desiccated powders prepared from (a) anterior and (b) posterior lobe beef pituitary glands, and (c) from the anterior lobe of sperm whale pituitary glands served as source material for the preparation of the various melanophore

[5] Fenn, W. O., *Symposia on Quantitative Biology*, Biological Laboratory, Cold Spring Harbor, N. Y., 1936, **4**, 252.

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[1] Stehle, R. S., *J. Pharm. Exp. Therap.*, 1936, **57**, 1.

fractions used in this study. Melanophore-dispersing activity of each fraction was assayed by injecting 1 cc doses of varying dilution into hypophysectomized frogs, *Rana pipiens*.[2] Hormone fractions obtained from whale anterior pituitary had activity in dilutions as high as 1×10^{-6}. Pressor and oxytocic hormone assays were run on the melanophore fractions obtained from posterior beef pituitary powder because of the known inhibitory effects of these hormones on melanophore activity.

For the enzyme, a tyrosinase solution was prepared by triturating 2 g of meal worms in 10 cc of buffer solution and centrifuging out extraneous material. Such solutions were of a crude nature and made up a part of the substrate, hence more gas was consumed than would theoretically be taken up in these oxidations. This was checked by adequate controls. The substrate used was recrystallized tyrosine. It was dissolved in a phosphate buffer at a pH of 6.85, or 7.33. Most of the experiments were conducted at a pH of 6.85 since the enzyme activity is slower in this range. All solutions of hormone, enzyme, and substrate were freshly prepared before each determination. The temperature of the bath was 38°C. The typical experiment consisted of 1.5 cc of .0033 molar tyrosine, 0.3 cc of tyrosinase solution, and 0.3 cc of a 1×10^{-3} solution of melanophore hormone, the controls containing 0.3 cc of distilled water in place of the hormone solution.

The presence of the melanophore hormone from posterior beef pituitary in the reaction mixture was associated with a 12% increase in gas uptake over the control determinations by the end of a 5½ hour period. Destruction of the pressor and oxytocic hormones by alkali previous to use in this system, increased the gas uptake to 54% more than that of the controls by the end of the same 5½ hour period. This indicated that the pressor and oxytocic hormones were antagonistic to the accelerating effect which the melanophore hormone had on the oxidase system. Melanophore hormone obtained from freshly removed anterior lobes of beef pituitary showed a 30% increase in gas uptake by the end of 6 hours. Injection of the melanophore hormone fractions from anterior and posterior lobes of beef pituitary into hypophysectomized frogs showed a marked difference in reactivity. Apparently the presence of the melanophore hormone, over a wide dilution range, will accentuate the oxidase system. This latter point is being further investigated. A

[2] Teague, R., Noojin, R., and Geiling, E. M. K., *J. Pharm. Exp. Therap.*, 1939, **65**, 115.

melanophore fraction of the anterior lobe of sperm whale[3] has been found to accelerate this oxidase system more than 100% in a period of 4 hours. Addition of standard pituitary solution decreased this acceleration 36%, due to the presence of the pressor and oxytocic principles. Further information as to the inhibitory nature of the pressor and oxytocic hormones was obtained when 0.3 cc of an acetic acid extract of the neural lobe of the sperm whale, which contains pressor and oxytocic hormones, but no melanophore hormone, decreased the oxidation rate of this oxidase system 12% as compared to the controls at the end of a 3-hour period. Destruction of the melanophore-containing fraction by tryptic digestion previous to use in the oxidase system resulted in a failure to accelerate this system and also indicated that the melanophore hormone did not act as additional substrate. The melanophore hormone alone has not been found to act as a substrate for tyrosinase. However, in a tyrosine-tyrosinase-melanophore hormone system the melanophore hormone is invariably partially destroyed during the oxidation of the substrate, as determined by injecting the resulting melanin product into hypophysectomized frogs. The addition of 8-hydroxy quinoline to the system prevents the oxidation even when the melanophore hormone is present.

The results of these experiments are being correlated with investigations on the thermostable metabolic stimulant that has recently been cited by O'Donovan and Collip[4] as being present in pituitary preparations rich in melanophore principle. The experiments so far completed point to at least one possible physiological rôle which the melanophore hormone may play in higher animals, namely, a stimulation of an oxidase system or systems in the body. It also indicates a probable rôle of this hormone in lower forms. The influence of this hormone on other oxidase systems is now being studied.

Summary. (1) *In vitro* experiments indicate that one of the biological mechanisms of the melanophore hormone is that of an accelerator of an oxidase system such as tyrosine-tyrosinase. (2) The pressor hormone, and perhaps the oxytocic hormone also, act to inhibit this oxidase system. (3) Melanophore hormone has been obtained from anterior sperm whale pituitary which results in a black coloration of hypophysectomized frogs when 1 cc of a 1×10^{-6} dilution of this hormone is injected. The black coloration persists for at least 24 hours.

[3] Geiling, E. M. K., *Bull. Johns Hopkins Hosp.*, 1935, **62**, 123.

[4] O'Donovan, D. K., and Collip, J. B., *Am. J. Physiol.*, 1938, **123**, 157 (Proc.).