ON THE PRESENCE OF MYOGLOBIN AND CYTOCHROME OXIDASE IN THE CARTILAGINOUS ODONTOPHORE OF THE MARINE SNAIL, BUSYCON [1]

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Early studies of cartilage metabolism revealed the tissue to be almost completely devoid of aerobic oxidative metabolism (Kuwabara, 1932; Bywaters, 1937). That aerobic processes do occur, however, was subsequently shown by Boyd and Neuman (1954), who found chondroitin sulfate synthesis to be accompanied by significant oxygen utilization in chick embryo cartilage, and by Person and Fine (1959a, 1959b), who more recently demonstrated cytochrome oxidase and succinoxidase activity in invertebrate and vertebrate cartilages from very young animals. Additional evidence for the existence of aerobic processes in an invertebrate cartilaginous tissue will be presented in this report.

It was observed initially (Lash, 1959) that the odontophore, a cartilaginous structure which supports the radula of the whelk, *Busycon canaliculatum,* was colored red. On analysis, the red color was found to result from the presence of myoglobin in the tissue. The myoglobin was very similar to that found by Ball and Meyerhof (1940) in the radular musculature of the same animal. Additional studies revealed that the tissue possessed readily demonstrable cytochrome oxidase activity. In this paper we present a spectrophotometric characterization of the cartilage myoglobin and its pyridine hemochrome, and evidence for the existence of cytochrome oxidase activity in homogenates of the tissue.

Materials and Methods

Odontophores were obtained by knocking off the hard calcareous shell of the animal and exposing the proboscis. The proboscises were cut off, the odontophores dissected out, carefully trimmed of adherent muscle, and placed on aluminum foil in a beaker of cracked ice. Complete removal of muscle tissue from the odontophore was checked by means of a magnifying lens and also by examination of histological sections. For spectrophotometric and manometric studies the tissue was homogenized in a ground-glass homogenizer in 0.1 *M* $Na_2HPO_4-KH_2PO_4$ buffer, pH 7.4, or water, and used immediately. Some preparations were lyophilized immediately following homogenization in glass-distilled water.

Spectrophotometric studies were made with a Beckman Model DU Spectro-

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photometer and a Process and Instruments Recording Spectrophotometer, Model RS 3. For determinations of cytochrome oxidase activity, the oxidation of reduced cytochrome *c* by the tissue homogenate was followed at 550 mμ (Wainio *et al.*, 1951). Manometric determinations of oxygen uptake in a system limiting for cytochrome oxidase were made using hydroquinone as substrate in the presence of added cytochrome *c* (Sigma) (Eichel *et al.*, 1950).

Experimental and Results

1. *Absorption spectra of myoglobin*

The odontophore myoglobin was very soluble and easily dissolved out of the tissue by either glass-re-distilled water, or 0.1 *M* PO_4 buffer at pH 7.4. The curves presented in Figure 1 were obtained by homogenizing 236 mg. wet weight of freshly dissected tissue in 5.0 ml. of buffer at 4° C. The homogenate was spun in the centrifuge at 4° C. at 1700 × g for twenty minutes. The supernatant solution was decanted and used for spectrophotometric study. Reduction of the pigment was accomplished by the addition of sodium dithionite.

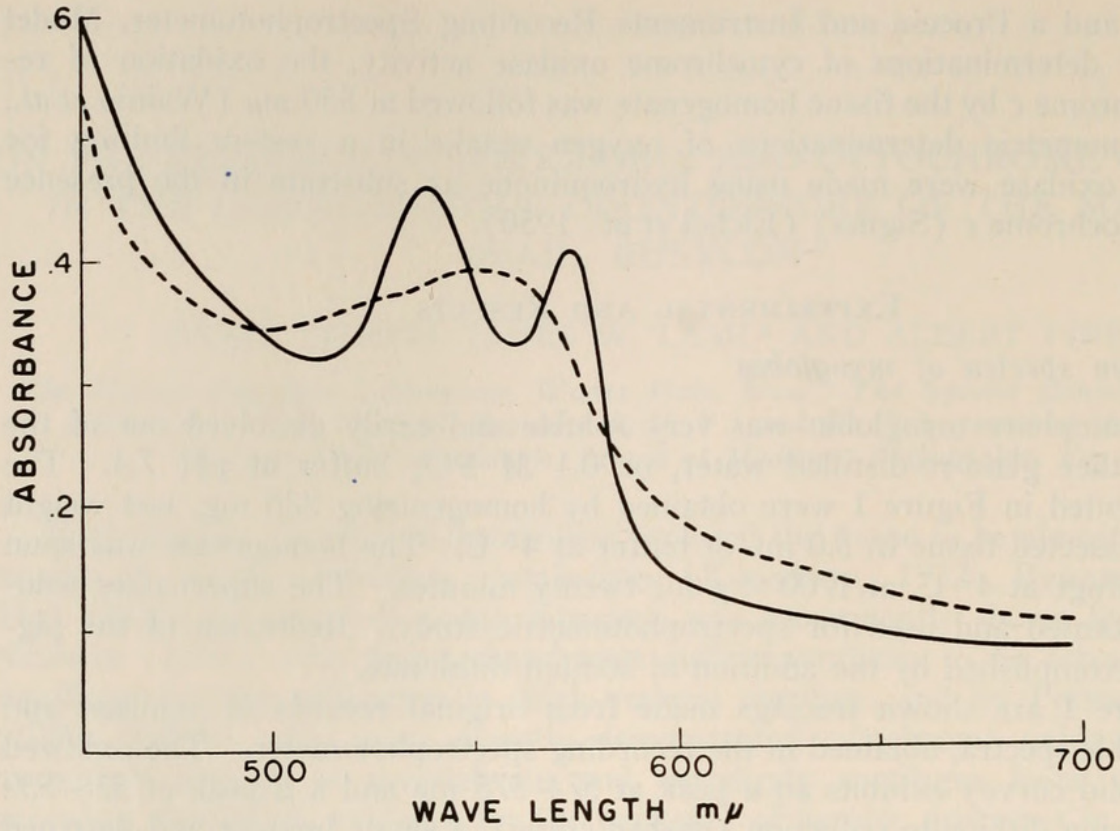
In Figure 1 are shown tracings made from original records of oxidized and reduced visible spectra, obtained in the recording spectrophotometer. The oxidized pigment (solid curve) exhibits an α peak at 574–575 mμ and a β peak at 538–539 mμ. Following dithionite reduction (dashed curve), a single broader and flattened absorption occurs at 540–565 mμ. These absorption maxima are similar to those determined (with a hand-spectroscope) by Ball and Meyerhof (1940) for the muscle myoglobin of Busycon, *i.e.*, oxidized compound, α peak, 570–580 mμ; β peak, 540–545 mμ. The discrepancies in location of the peaks may be the result of difference in instrumentation.

For study of the ultraviolet absorption of the myoglobin, the solution described above was diluted 1 : 5 with the same phosphate buffer. In Figure 2 the ultraviolet spectrum is shown. In the oxidized form (solid curve) a γ or Soret peak is present at 415–416 mμ. A broad, flat elevation encountered at 320–360 mμ is associated with the porphyrin moiety of the pigment (Barron and Flood, 1952). The sharper peak at 290 mμ is due to the presence of protein. A well-defined peak is seen at 238–239 mμ, possibly due to the presence of fatty acids in the preparation.

The spectrum of dithionite-reduced material exhibits a γ peak at 430 mμ; the remainder of the ultraviolet spectrum could not be obtained in the dithionite-reduced material because of the absorption of ultraviolet light by dithionite itself.

2. *Absorption spectra of pyridine hemochrome*

For preparation of the pyridine hemochrome of the myoglobin prosthetic group, 230 mg. wet weight of freshly trimmed odontophore were homogenized at 4° C. in 5 ml. of reagent pyridine (Merck and Co.). The homogenate was spun at 4° C. at 1700 × g for twenty minutes, and the supernatant solution decanted into a cuvette. Reagent pyridine was used in the reference cuvette. The visible absorption spectrum of the reduced hemochrome is shown in Figure 3. The α peak is located at 554–555 mμ, the β peak at 524–525 mμ. A broader and lower peak is present at 480 mμ. For determination of the ultraviolet absorption, shown in Figure 4, the pyridine supernatant described above was diluted 1 : 12 with addi-



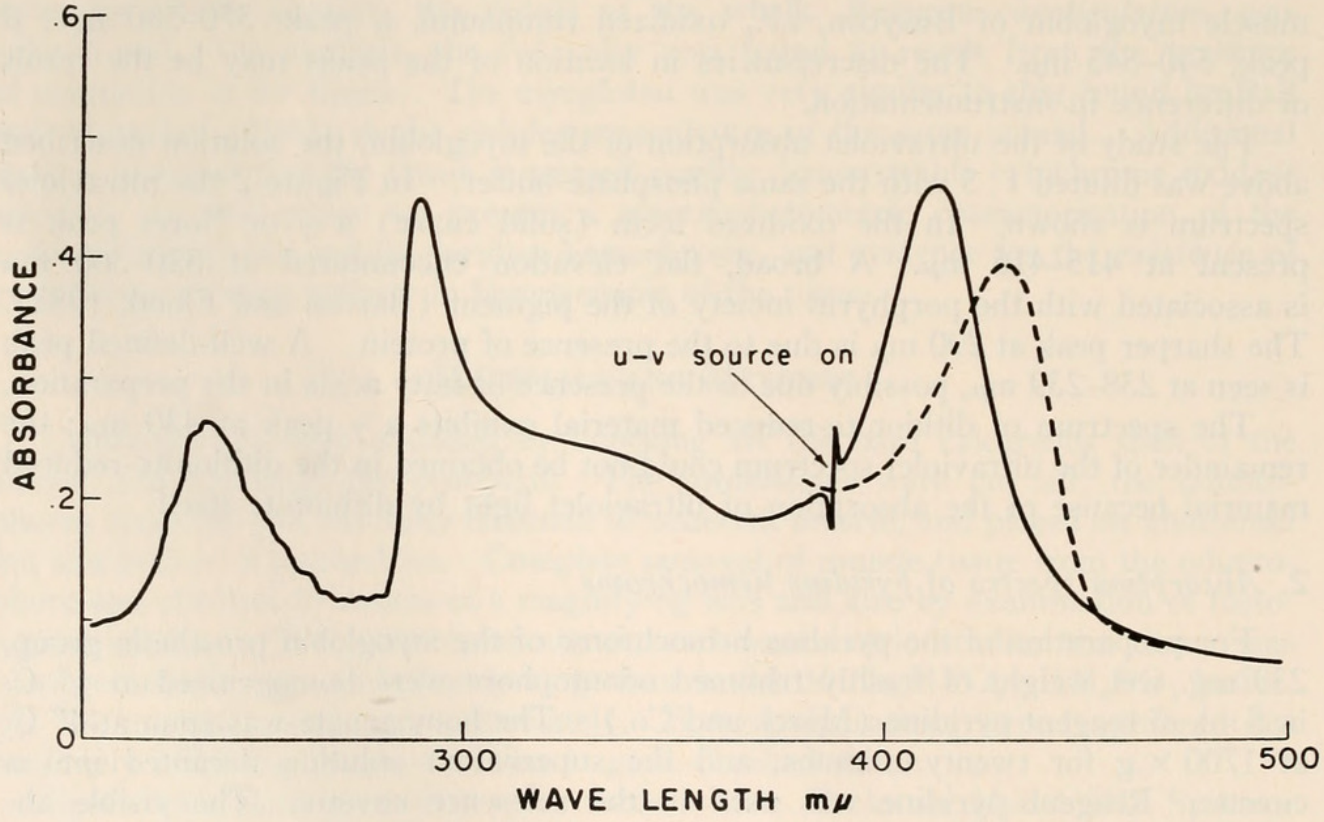
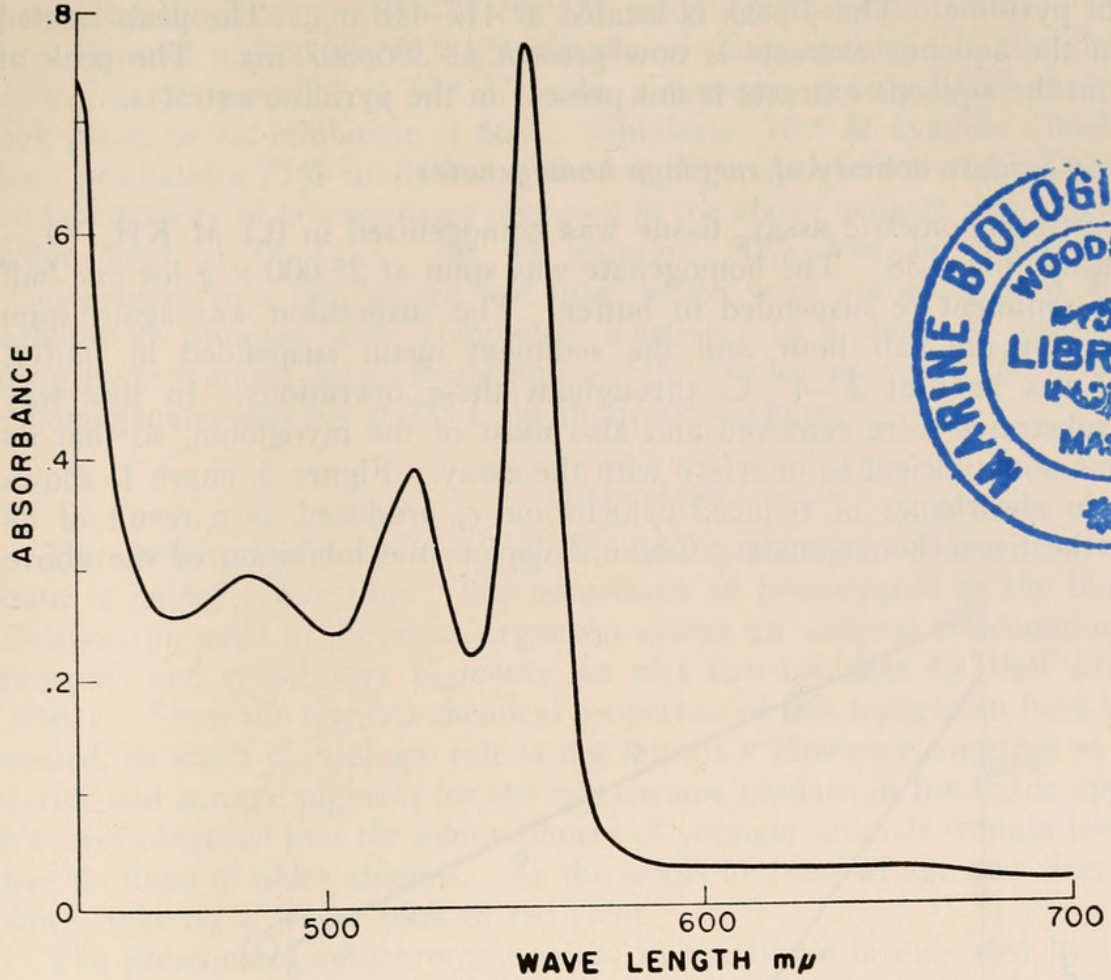


FIGURE 1 (above). Visible absorption of odontophore myoglobin. Solid curve: oxidized pigment; dash curve: dithionite-reduced pigment.

FIGURE 2 (below). Soret and ultraviolet absorptions of odontophore myoglobin. Solid curve: oxidized pigment; dash curve: dithionite-reduced pigment.



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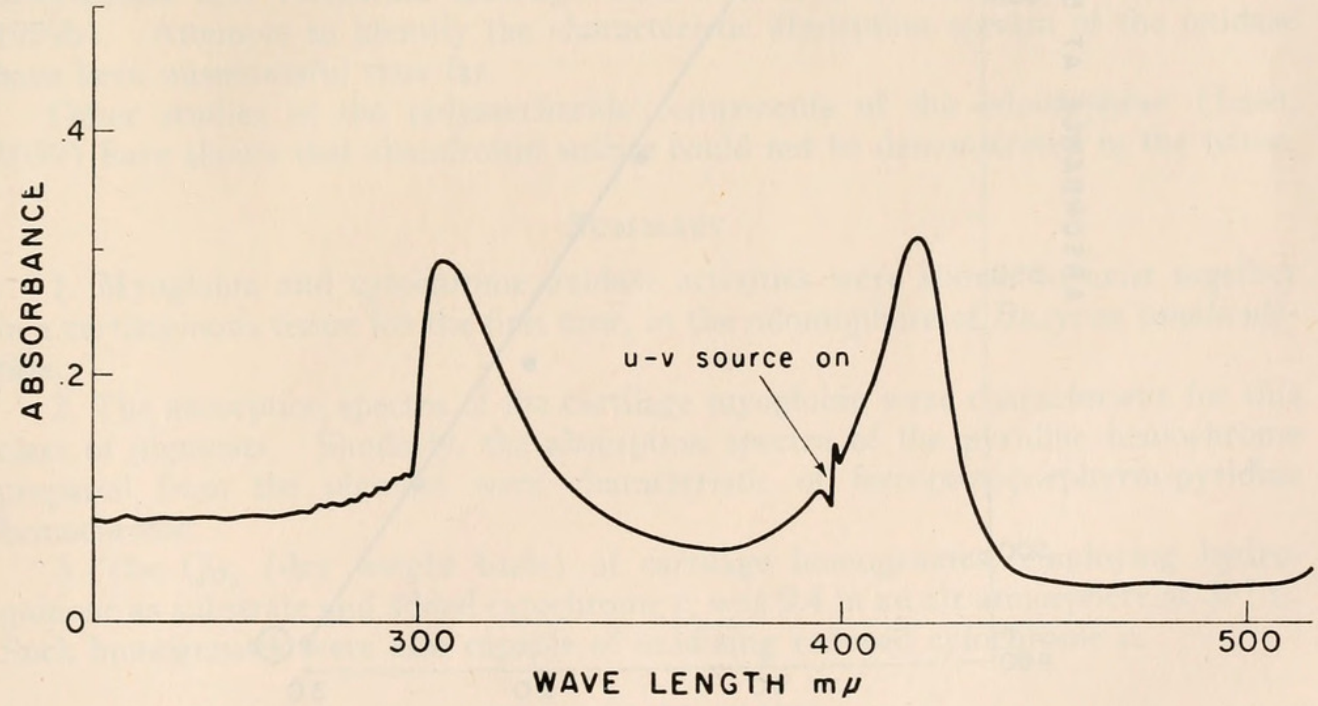

FIGURE 3 (above). Visible absorption spectrum of pyridine hemochrome prepared from odontophore myoglobin.

FIGURE 4 (below). Soret and ultraviolet absorptions of pyridine hemochrome prepared from odontophore myoglobin.

tional reagent pyridine. The γ peak is located at 417–418 mμ. The peak located at 290 mμ in the aqueous extracts is now present at 306–307 mμ. The peak at 238–239 mμ in the aqueous extracts is not present in the pyridine extracts.

3. *Cytochrome oxidase activity of cartilage homogenates*

In the spectrophotometric assay, tissue was homogenized in 0.1 *M* KH_2PO_4–Na_2HPO_4 buffer, pH 7.38. The homogenate was spun at 25,000 × g for one-half hour and the sediment re-suspended in buffer. The suspension was again spun at 25,000 × g for one-half hour and the sediment again suspended in buffer. Temperature was kept at 2°–4° C. throughout these operations. In this way endogenous substrates were removed and also most of the myoglobin, so that its absorption was not sufficient to interfere with the assay. Figure 5, curve 1, shows the decrease in absorbance of reduced cytochrome *c*, produced as a result of its oxidation by the tissue homogenate. Curve 2 depicts the inhibition of the above

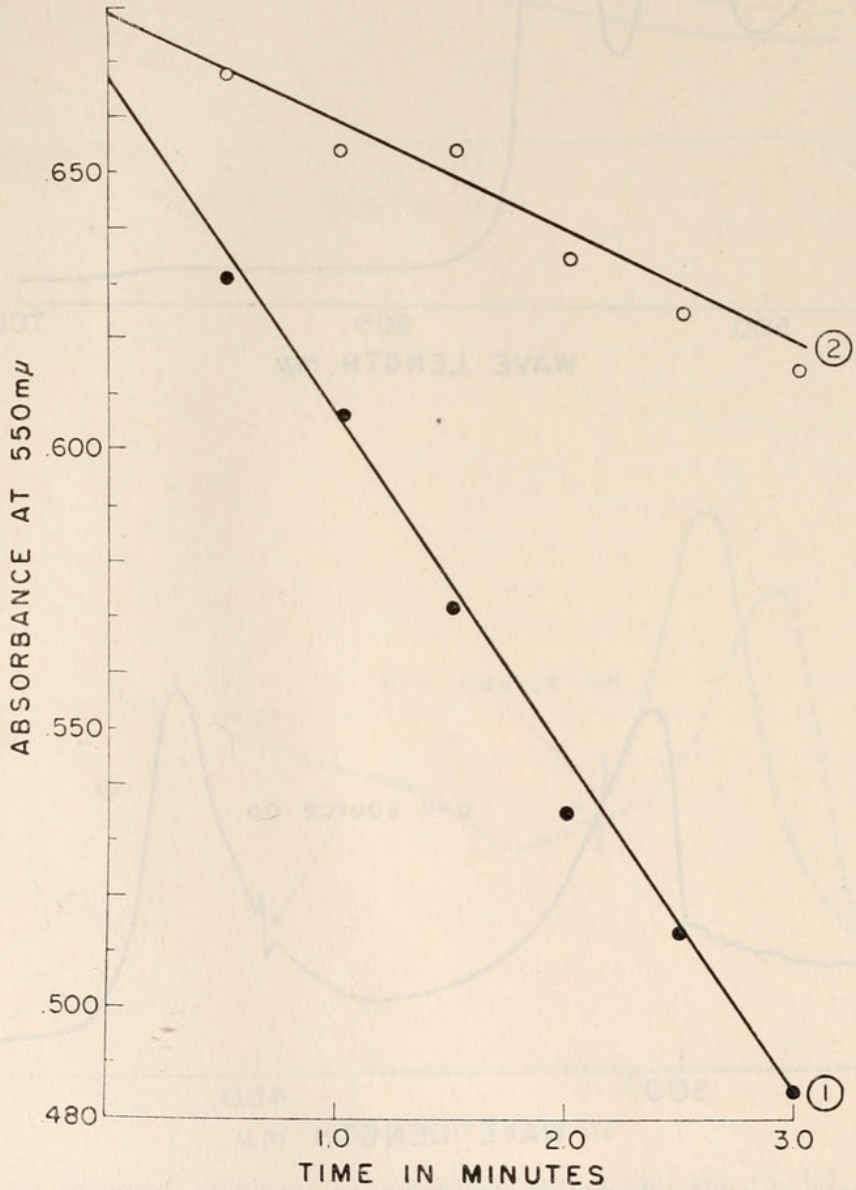

FIGURE 5. Cytochrome oxidase activity of odontophore homogenates as determined (spectrophotometrically) by oxidation of reduced cytochrome *c*. Curve (1) : untreated homogenate; Curve (2) : homogenate in presence of 10^{-4} *M* azide.

system by the incorporation of 10^{-4} *M* azide (final concentration). Whereas in three minutes a decrease of 0.171 absorbance units occurred in the absence of inhibitor, in the presence of 10^{-4} *M* azide, a decrease of only 0.058 absorbance units took place, or an inhibition of 66%. Similarly, 10^{-4} *M* cyanide (final concentration) produced a 75% inhibition of the cartilage oxidase activity. The cytochrome oxidase activity of homogenates prepared in the above manner was extremely labile. When kept at 0°–1° C. for 45 minutes, 50% loss in activity was noted. Homogenates in a thin-walled test tube, which were immersed in a boiling water bath for 25 minutes, were completely inactive. In a standard manometric assay using hydroquinone as substrate and added cytochrome *c*, the Q_{O_2}/(mg. dry wt.) of homogenates was 9.4 at 37° C. in an air atmosphere.

Discussion

The combined presence of myoglobin and cytochrome oxidase in a cartilaginous tissue is indeed interesting. The occurrence of hemocyanin as the blood oxygen-transport pigment in the same organism makes an unusual combination of oxygen transport and respiratory pigments, as was noted earlier by Ball and Meyerhof (1940). Since the physico-chemical properties of this myoglobin have not yet been studied, its exact physiologic role is not known. However, function as an oxygen-carrier and storage pigment for the cytochrome oxidase in the tissue appears likely. We have observed that the odontophores of younger animals contain less myoglobin than do those of older animals. As the snails increase in age and size, the odontophores take on a deeper pink to red color.

The presence of cytochrome oxidase in the tissue is indicated by its ability to oxidize reduced cytochrome *c*. We have been able to detect such activity in other invertebrate and vertebrate cartilage tissues as well (Person and Fine, 1959a, 1959b). Attempts to identify the characteristic absorption spectra of the oxidase have been unsuccessful thus far.

Other studies of the polysaccharide components of the odontophore (Lash, 1959) have shown that chondroitin sulfate could not be demonstrated in the tissue.

Summary

1. Myoglobin and cytochrome oxidase activities were shown to exist together in a cartilaginous tissue for the first time, in the odontophore of *Busycon canaliculatum*.

2. The absorption spectra of the cartilage myoglobin were characteristic for this class of pigments. Similarly, the absorption spectra of the pyridine hemochrome prepared from the pigment were characteristic of ferroprotoporphyrin-pyridine hemochrome.

3. The Q_{O_2} (dry weight basis) of cartilage homogenates, employing hydroquinone as substrate and added cytochrome *c*, was 9.4 in an air atmosphere at 37° C. Such homogenates were also capable of oxidizing reduced cytochrome *c*.

LITERATURE CITED

Ball, E. G., and B. Meyerhof, 1940. On the occurrence of iron-porphyrin compounds and succinic dehydrogenase in marine organisms possessing the copper blood pigment hemocyanin. *J. Biol. Chem.*, **134**: 483–493.

BARRON, E. S. G., AND V. FLOOD, 1952. Studies on the mechanism of action of ionizing radiations IX. *Arch. Bioch. Biophys.,* **41**: 203–218.

BOYD, E. S., AND W. F. NEUMAN, 1954. Chondroitin sulfate synthesis and respiration in chick embryonic cartilage. *Arch. Bioch. Biophys.,* **51**: 475–485.

BYWATERS, E. L. G., 1937. The metabolism of joint tissues. *J. Path. Bact.,* **44**: 247–268.

EICHEL, B., W. W. WAINIO, P. PERSON AND S. J. COOPERSTEIN, 1950. A partial separation and characterization of cytochrome oxidase and cytochrome *b*. *J. Biol. Chem.,* **183**: 89–103.

KUWABARA, G., 1932. Über den Stoffwechsel des Knorpel- und Kallusgewebes. *J. Biochem. (Japan),* **16**: 389–402.

LASH, J. W., 1959. The presence of myoglobin in the cartilage of the marine snail, Busycon. *Science,* **130**: 334.

PERSON, P., AND A. FINE, 1959a. Cytochrome oxidase and succinoxidase activity in Limulus gill cartilage. *Arch. Bioch. Biophys.,* **84**: 123–133.

PERSON, P., AND A. FINE, 1959b. Oxidative metabolism of cartilage. *Nature,* **183**: 610–611.

WAINIO, W. W., P. PERSON, B. EICHEL AND S. J. COOPERSTEIN, 1951. The reaction between cytochrome oxidase and ferrocytochrome *c*. *J. Biol. Chem.,* **192**: 349–360.

Person, Philip, Lash, James W, and Fine, Albert. 1959. "ON THE PRESENCE OF MYOGLOBIN AND CYTOCHROME OXIDASE IN THE CARTILAGINOUS ODONTOPHORE OF THE MARINE SNAIL, BUSYCON." *The Biological bulletin* 117, 504–510. https://doi.org/10.2307/1538863.

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