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HEMOGLOBIN AND MYOGLOBIN IN MAMMALS, SEVERAL SPECIES OF FISH AND THE NORTHERN WHELK *(Buccinum undatum)*

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Current concepts in molecular evolution emphasize the hypothesis that myoglobin is likely older than hemoglobin and that hemoglobin (a tetramer) evolved from the monomer by phenomena of successive duplication and translocation. The purpose of this study was to extend the spectrophotometric observations to species below the evolutionary level of mammals comparing in this manner their hemoglobins and myoglobins to those of the higher animals.

A standard technique was used to isolate the principal hemeproteins from both erythrocytes and skeletal muscle. Freshly obtained samples were extracted by first mixing them with small quantities of 0.1 M phosphate buffer, ph 7.4 and then twice freeze-thawing to disrupt the cells. The myoglobin samples were initially ground with small quantities of prepared beach sand. DEAE-Sephadex-50 columns equilibrated with .01 M Tris-HCl buffer pH 8.2 separated hemoglobin and myoglobin in the muscle preparations. Hemoglobin from each species was similarly treated. The dilute eluates were concentrated against polyethylene glycol (M.W. 20,000) in 0.1 M phosphate buffer, ph 7.40, through Visking cellophane bags over 18 hours and the final samples thus obtained were diluted with the same buffer so that the Soret maxima read approximately 1.500 optical density units on the Hitachi-Perkins Elmer spectrophotometer with a digital read-out attachment. About 15 minutes prior to reading, several crystals of dithionite were added and oxygen was then gently bubbled through the hemeprotein solution. Fish muscle preparations in general were cloudy (opalescent) and virtually impossible to "read." A successful method of clearing was found by using a Calcium-Phosphate Gel method which overcame this problem. All procedures were carried out at 4° C except the actual spectrophotometry. The visible and near ultraviolet light spectra, with their maxima and minima, are depicted in nanometers for the hemoglobins in Table 1. We were unable to isolate red cells or any circulatory red pigment from the Northern Whelk (*Buccinum undatum).*

There was essentially no difference in the complete spectrum between the hemoglobin of any of the specimens studied and in addition we have also established spectral characteristics not heretofore reported. The most primitive species from which hemoglobin was isolated (Hagfish) was similar to man in this study.

Table II displays the myoglobin readings in the same manner that table I applies to the hemoglobins. Although muscle samples from *homo sapiens* could not be done simultaneously during these experiments; the spectrum derived during previous studies was included.

The myoglobin observations point out that the alpha peak of myoglobin (that peak which first

TABLE I

OXYHEMOGLOBIN SPECTRA

SPECIE*	MAXIMA					MINIMA			
1. Homo sapiens	270	350	414	540	575	310	370	510	560
2. Phoca vitulina	270	350	413	540	575	310	370	510	560
3. Scomber scombrus	270	350	413	540	578	310	370	510	560
4. Squalus acanthias	270	350	415	540	575	310	370	510	560
5. Myxine glutinosa	270	350	414	540	575	310	370	510	560

*Man, Seal, Mackerel, Dogfish and Hagfish (in this order).

TABLE II

OXYMYOGLOBIN SPECTRA

SPECIE*	MAXIMA					MIMINA			
1. Homo sapiens	280	350	413	542	583	310	365	515	565
2. Phoca vitulina	280	350	411	540	582	310	365	520	570
3. Scomber scombrus	270	350	412	540	576	320	365	515	560
4. Squalus acanthias	270	345	412	540	578	315	370	515	560
5. Myxine glutinosa	270	---	410	540	578	---	365	515	560
6. Buccinum undatum	270	345	415	540	576	315	370	510	560

*Man, Seal, Mackerel, Dogfish, Hagfish and Northern Whelk (in this order)

appears for oxyhemeproteins when one reads the spectrum "down" from 700 nm) is established for adult oxymyoglobin in man (583 nm) and the seal (582 nm) only; whereas in the four other species in table II the alpha peak was very close to that reported for the hemoglobins in table I and fetal skeletal muscle myoglobin in a number of mammals (Nature 213:75, 1967). The second apparently distinguishing feature of mammalian adult skeletal myoglobin was the 280 nm peak. This differed also from the hemoglobins where the 270 nm maximum predominated. Why the hagfish *(myxine glutinosa)* myoglobin did not show a maximum around 350 nm or a minimun in the 310-320 nm region we do not know.