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EFFECTS OF DIVING ON PERIPHERAL VENOUS RENIN ACTIVITY IN THE HARBOR SEAL *Phoca vitulina*

A.P. Shapiro, Henry Berliner, H.V. Murdaugh, Jr., Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania

Previous studies performed by investigators at Mount Desert Island Biological Laboratory and other marine stations have indicated that blood flow to the kidney ceases at the onset of diving in the harbor seal. Early studies using renal clearance demonstrated absence of urine production during trained diving followed by a transient impairment in concentration of urine suggesting but not demonstrating this change in renal blood flow. Arteriograms before and during diving documented the cessation of renal blood flow during the early dive period. Cardiac output in the seal decreases from seven to 10 percent of control values at onset of dive but increases slightly as the dive is prolonged to 15 minutes. The latter finding suggested the possibility that renal artery constriction did not persist during the entire duration of the dive. The availability of renin activity assay provided the opportunity to look into this last question. Accordingly extradural vein renin activities were monitored before, during, and after eight-minute dives in the harbor seal.

The seals were trained to dive under laboratory conditions as previously reported by the investigators. Diving was simulated using a teeter board with the seal comfortably restrained. A polyethylene catheter was inserted into the extradural vein via a thin walled needle using procaine anesthesia. Samples of blood with EDTA added as anticoagulant were obtained in duplicate before diving and sequentially during and after the dive. Samples were chilled, rapidly centrifuged, and the separated plasma was frozen. Renin activity assays were carried out using a modification of the Haber radio-immunoassay technique.

We completed four studies in three seals. During diving the renin activity did not increase but appeared to decrease slightly in three of the four studies. Post-dive, there was a brisk but transient rise in renin activity to as much as three times control value.

These data are consistent with persistence of renal arterial constriction throughout the period of the dive. The transient post-dive increase in plasma renin activity is presumably a result of renin production that occurred in the kidney during diving.

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THE MOLECULAR CHARACTERISTICS OF CERTAIN MAMMALIAN AND PISCINE MYOGLOBINS AND HEMOGLOBINS

George B. Theil, Mary F. Theil, The Medical College of Wisconsin, Milwaukee and the Veterans Administration Hospital, Wood, Wisconsin

The structural development of the globin protein of hemoglobin (Hb) and myoglobin (Mb) evolved from a common monomeric globular molecule with a molecular weight of about 17,000.

The phylogenetic tree then branched from this ancestral protein; one limb continuing the single polypeptide chain of the Mbs and the other branch ultimately adapting by gene duplication and molecular combination to form the tetrameric Hbs. In one previously described lower form, the lamprey, it is postulated that the Hb may be an intermediate in the evolution from monomer to tetramer. The present study was designed to evaluate more completely the phylogeny of these globins by estimating the molecular weight of the predominant heme proteins of the skeletal muscle and erythrocytes of a representative evolutionary spectrum of species of mammals and fishes.

Mb and Hb were isolated from the skeletal muscle and erythrocytes of the hagfish *(Myxine glutinosa)*, dogfish *(squalus acanthias)*, mackerel (*Scomber scombrus)*, seal *(phoca vitulina)*, and man. Mb alone was extracted from the appropriate skeletal musculature of the common northern whelk *(Buccinum undatum)* and obtained from commercial sources in crystalline form for the sperm whale. The fresh minced tissues and erythrocytes were extracted at 4°C over 24 hours with small amounts of distilled water. This simple technique minimizes aggregation of the proteins of the hagfish, dogfish, mackerel, and whelk. The heme proteins were then applied to 2.5 x 8.5 cm. Sephadex G-75 columns for molecular sieving. Each column was identical in size and gravity packed to the same level from the same gel-buffer preparation. The buffer used was .067 M sodium phosphate, pH 8.25, and this buffer was also employed as the eluting buffer. Approximately 5 mg. of each heme protein in a volume of 1 ml. with 2 ml. of 25 percent sucrose were applied to a column. Each study sample which was applied also contained 5 mg. of adult human Hb (Hb-A) in a volume of 1 ml. At the bottom of each column was a medium porosity sintered glass filter plate which supported 10-6 mm siliconized glass beads. A small mat of acid washed glass wool was laid over the glass beads to support the gel. Nine experiments were carried out with the following heme proteins in combination with the Hb-A which served as the constant molecular weight reference standard. The eluate from each column was collected in 1 ml. aliquots and each tube was read at 414 nm (Soret Band) and at 280 nm in a Hitachi-Perkins Elmer spectrophotometer.

Table 1 displays the peak (or peaks) as they relate to the elution tube number (cumulative ml.) for each of the combinations of major heme proteins.

Figure 1 shows the graphic plot of optical density versus tube number (ml) for four selected experimental mixtures applied to the columns. The line graph represents the composite of points of the readings at 280 nm.

The heaviest heme protein eluted from the columns corresponds to the first peak and is tetrameric Hb (MW 67,000). Experiments seven and eight demonstrate the peak protein and heme concentration in tubes 25 and 23 respectively which show only a single peak. However the Hb-A-*Myxine glutinosa* Hb mixture (experiment 6) was eluted in two distinct peaks; the slowest of which corresponds precisely to the elution peak of all of the myoglobins (second or slower eluting peak in experiments 1-5). This is reasonable evidence that the molecular weight of the *Myxine glutinosa* Hb approximately equals each of the Mbs including the Mb extracted from the esophageal striated muscle of the lowest form studied, the *Buccinum undatum*. The lack of an intermediary peak or obliteration of the details between peaks in experiment 9 is confirming evidence that the molecular weight of the Hb of the *Myxine glutinosa* is close to that of Mb (17,000) and is monomeric rather than dimeric or tetrameric.

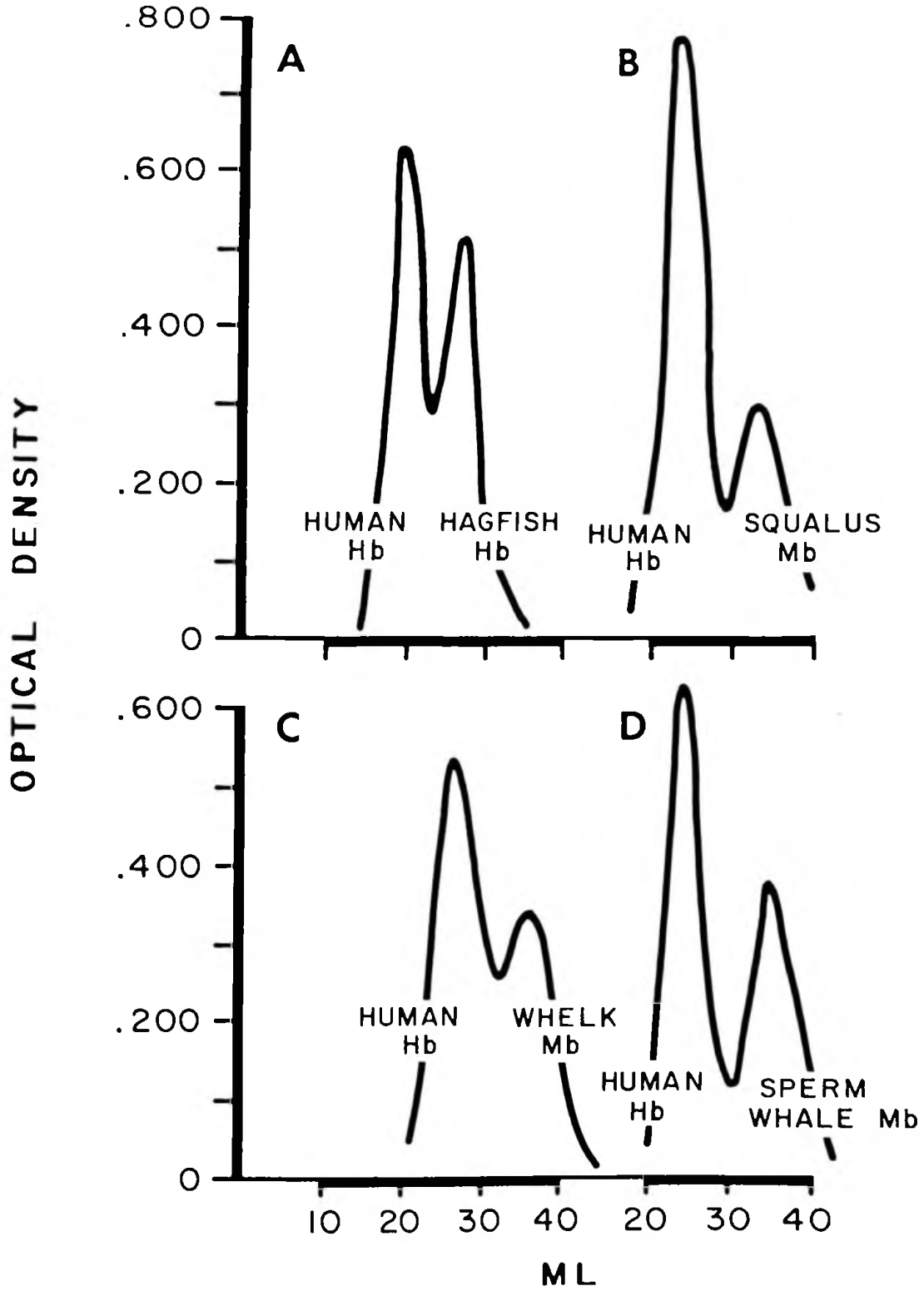
.800
.600
.400
.200
0
A
B
HUMAN Hb
HAGFISH Hb
HUMAN Hb
SQUALUS Mb
.600
.400
.200
0
C
D
HUMAN Hb
WHELK Mb
HUMAN Hb
SPERM WHALE Mb
10
20
30
40
20
30
40
ML
OPTICAL DENSITY

TABLE 1

ELUTION PATTERNS OF SELECTED HEME PROTEINS

EXPERIMENT	HEME PROTEIN		ELUTION VOLUME (ml.) AND MAXIMA*			
			414 nm		280 nm	
1.	*BUCCINUM UNDATUM*	Mb	27	37	25	37
2.	*MYXINE GLUTINOSA*	Mb	26	34	26	34
3.	*SQUALUS ACANTHIAS*	Mb	26	34	26	34
4.	SPERM WHALE	Mb	25	35	25	35
5.	*PHOCA VITULINA*	Mb	26	34	26	34
6.	*MYXINE GLUTINOSA*	Hb	26	34	26	34
7.	*SQUALUS ACANTHIAS*	Hb	25		25	
8.	*SCOMBER SCOMBRUS*	Hb	23		23	
9.	*MYXINE GLUTINOSA*	Hb	27	38	27	38
	plus					
	SPERM WHALE	Mb				

*MAXIMAL ABSORBANCE AT 414 AND 280 nm RELATING TO ELUTION TUBE NUMBER (ml.)

The findings thus support the monomeric nature of *Myxine glutinosa* Hb in the oxy Hb form. Fishes which appeared more recently with respect to evolutionary time *(Squalus acanthias* and the teleost *Scomber scombrus)* have therefore tetrameric Hb as do all adult mammals reported thus far in the literature. The hagfish also has single polypeptide chain Mb in its skeletal musculature (experiment 2). The proposed phylogenetic tree depicting the evolution of Mb and Hb may have to be modified with the speculation that the heme protein in the erythrocyte and skeletal muscle of this specie may be in a more direct line with the postulated ancestral globin rather than being depicted as separate branches.