1978

(*Reprinted from Nature, Vol.* 198, *No.* 4884, *pp.* 996–997, *June* 8, 1963)

Differentiation of Myoglobins and Hæmoglobins

THE simultaneous estimation by de Duve[1] of hæmoglobin (Hb) and myoglobin (Mb) in solution, based on the spectral characteristics of their carbon monoxide compounds, requires the solutions to be crystal clear and strongly buffered and the relevant wave-lengths must be reproduced to within a fraction of a mμ.

Brown *et al.*[2] recorded a great difference between the relative stabilities of human hæmoglobin and various myoglobins as estimated by the time required for half conversion to denatured hæmochrome in 0·06 M sodium hydroxide ($t_{50\%}$) and by extrapolation to zero time of the plot of log per cent undenatured pigment against time.

Jonxis and Visser[3] used the ratio of optical densities at 576 mμ before and during alkaline denaturation in determining fœtal hæmoglobin in blood, and a similar method has been found effective for the differentiation of myoglobin and hæmoglobin in solution.

Pig metmyoglobin, purified to an iron content of 0·32 per cent and electrophoretically homogeneous in solution at *p*H 9·3, was converted to the oxy- form by treatment with dithionite followed by oxygenation and dialysis; pig HbO_2 was obtained by hæmolysis of washed blood cells, followed by dialysis.

Table 1. SPECTROPHOTOMETRIC PROPERTIES OF PIG OXYMYOGLOBIN AND OXYHÆMOGLOBIN DURING ALKALINE DENATURATION

(Light path of cells, 10 mm)

ample	Initial optical density at 577 mμ	$t_{50\%}$ (min)	O.D.$_1$ / O.D.$_2$
	Oxymyoglobin		
A	0·58	26	1·49
	0·64	130	1·43
B	0·43	11	1·35
	0·64	27	1·46
C	0·30	69	1·63
	0·45	66	1·64
	0·90	—	1·59
	Oxyhæmoglobin		
A	0·54	36	2·68
B	0·59	47	2·49
	0·59	30	2·39
	0·64	31	2·28
	1·22	29	2·44
C	0·58	42	2·51
	0·72	38	2·52
	1·02	38	2·49
D	0·29	—	2·47
	0·42	—	2·35
	0·88	—	2·42

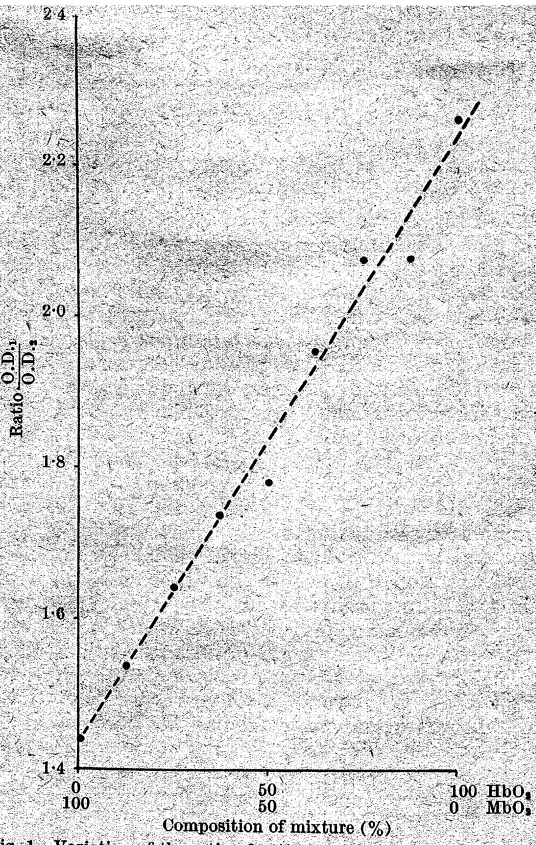

Fig. 1. Variation of the ratio of optical densities at 577 mμ before and after alkaline denaturation $\left(\frac{O.D._1}{O.D._2}\right)$ with composition of pig oxymyoglobin/oxyhæmoglobin mixtures

Table 1 shows that even for single preparations of pig MbO_2 or HbO_2 $t_{50\%}$ was not constant and that no consistent difference was discernible in the relative stabilities[2] of the muscle and blood pigments in 0·06 M alkali. However, the ratios of the optical densities at 577 mμ before and after alkaline denaturation $\left(\frac{O.D._1}{O.D._2}\right)$ fell obviously into two distinct groups around values of 1·5 for MbO_2 and 2·5 for HbO_2 over a wide range of initial optical densities.

Fig. 1 illustrates the change of the ratio $\frac{O.D._1}{O.D._2}$ with composition of pig MbO_2/HbO_2 mixtures, while the ratios of the optical densities at 577 mμ before and after

Table 2. Ratios of the Optical Densities $\frac{O.D._1}{O.D._2}$ at 577 mμ before and after the Alkaline Denaturation of Oxymyoglobins (MbO_2) and Oxyhæmoglobins (HbO_2) of Various Species

Species	HbO_2 Initial optical density at 577 mμ	HbO_2 $\frac{O.D._1}{O.D._2}$	MbO_2 Initial optical density at 577 mμ	MbO_2 $\frac{O.D._1}{O.D._2}$
Human	0·36	2·82		
	0·64	2·71		
	0·22	2·47		
	0·43	2·44		
	0·32	2·70		
	0·50	2·56		
Ox	0·80	2·76	0·76	1·49
	0·82	2·76	0·34	1·51
	0·80	2·76	0·54	1·51
	0·82	2·70	0·91	1·55
			0·40	1·57
	0·94	2·77	0·58	1·47
			0·76	1·45
Sheep	0·70	2·52	0·66	1·51
	0·76	2·77	1·20	1·45
	0·86	2·85	0·99	1·58
	0·77	2·37		
Horse	0·77	2·67	0·68	1·56
	0·90	2·74	0·78	1·59

complete alkaline denaturation for other species are reported in Table 2. HbO_2 solutions were obtained by the dialysis of defibrinated sheep and horse blood and of whole human male and ox blood. MbO_2 solutions were obtained by aqueous extractions of minced washed ox, sheep and horse hearts, and were clarified by simple filtration; carefully washed hearts have been found to be free of HbO_2 (ref. 4), the presence of which would, in any event, raise the ratio $\frac{O.D._1}{O.D._2}$ and reduce the diagnostic difference.

The ratio $\frac{O.D._1}{O.D._2}$ therefore provides a simple means of differentiation between HbO_2 and MbO_2 for a number of species, and for the pig the method has been successfully applied to the evaluation of the composition of pigment mixtures. The errors involved were greater than the value of 1 per cent reported by de Duve[1], but the method uses hæm derivatives insensitive to light and to *p*H between 5·5 and 10 (ref. 5), which do not require strong buffering and which can be clarified sufficiently by simple filtration; furthermore, the estimations require optical determinations at only one wave-length, and this, judging from the spectra of the hæm pigments before and after alkaline denaturation, needs to be reproduced only to an accuracy within the range of an ordinary spectrophotometer without modifications.

This work forms part of a research project being carried

out with funds made available through the U.S. Department of Agriculture under U.S. Public Law 480.

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