LETTERS TO THE EDITORS

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Carbon Monoxide Affinity of Chlorocruorin and Hæmoglobin

CHLOROCRUORIN, the green-red respiratory blood pigment of certain marine annelid worms, is the only known substance built on the same plan as hæmoglobin. Like most kinds of hæmoglobin, chlorocruorin has a higher affinity for carbon monoxide than for oxygen[1]. This property, valueless to animals, is utilized in experimental work to eliminate the possible respiratory activity of these pigments and so to test their functional significance[2,3]. The relative affinity of chlorocruorin (*Ch*), or of hæmoglobin, for the two gases in the reversible reaction

$$O\,Ch + CO \rightleftharpoons COCh + O_2$$

is given by the equilibrium constant K in the equation

$$K = [COCh]/[O\,Ch] \times p\,O_2/p\,CO,$$

from which it is seen that when the pigment is 50 per cent in the carboxy state and 50 per cent in the oxy state, K becomes the ratio of oxygen to carbon monoxide.

Krogh[4] was the first to show that different kinds of animals have different values of K. For the hæmoglobin of human blood, K has been found to be 235 or 246 (Douglas, Haldane and Haldane)[5], 240 (Killick)[6] or 490 (Anson *et al.*)[7]. The values for certain other hæmoglobins have been found to be as high as this or higher. For yet other hæmoglobins the values of K are low, that of horse muscle hæmoglobin (myoglobin)[8,9] being 14–51, of the hæmoglobin of leguminous root nodules[10] 37, and of the larva of the horse bot-fly (*Gastrophilus*)[11] 0·67. With these very considerable known differences in the relative affinity of hæmoglobins for carbon monoxide and oxygen, it was of interest to find the values of the equilibrium constant for several invertebrate hæmoglobins and for a chlorocruorin.

The blood of each kind of animal was diluted in $M/20$ phosphate buffer at pH 7·3 and then centrifuged and filtered to give a limpid solution. Some of this was put into a modified Thunberg tube with a 2 cm. optical cell, and the concentration was adjusted to give an optical density ($\log_{10} I_0/I$) of about 1·5 at the α-band, measured with a Hilger – Nutting visual spectrophotometer. After equilibration at 20° C., with air in the case of hæmoglobin, or oxygen for chlorocruorin (which cannot be saturated with oxygen at atmospheric partial pressure[1]), the densities were determined at wave-lengths 561 and 578 mμ for hæmoglobin, or 590 and 606 mμ for chlorocruorin. Previous plotting of the absorption curves of the oxy and carboxy compounds had shown that the two wave-lengths mentioned are those at which the light absorption differs most. The Thunberg tube was then evacuated, filled with an appropriate mixture of oxygen and carbon monoxide to obtain about a half-and-half mixture of the two compounds of the pigment, and the solution equilibrated with the gas by rotation for half an hour in a water thermostat at 20° C. in dim light, after which the changed densities were determined at the same two wave-lengths. (Although both carboxy compounds are dissociated by light[12,1], there was no progressive change in the spectrophotometer readings, even when the solution was left exposed to the lamp for a quarter of an hour; doubtless measurable changes only occur in thin layers.) The third step was to evacuate the tube anew, fill it with pure carbon monoxide, re-equilibrate and again measure the two densities. From the six density measurements the relative concentrations of the oxy and carboxy compounds in the solution in equilibrium with the gas mixture can be calculated by a formula given by Keilin and Wang[11], and from this the value of K in the equation above is directly deducible.

The following are the values of K which were obtained, in decreasing order of magnitude, the first being that of chlorocruorin, the remainder all of blood hæmoglobins: *Branchiomma vesiculosum* (Montagu), 570; *Chironomus dorsalis* Mg., 400; horse, 280; man, 230; *Arenicola marina* L., 150; *Tubifex* sp., 40; *Planorbis corneus* L., 40; rabbit, 40. From five to eleven determinations were made for each of the different animal forms. The values of K obtained for each of them varied considerably, and as there were not enough values to give standard errors, the means have been rounded to the nearest 10. There are several surprises in the results. The values differ greatly. The figure for man is virtually the same as those previously found by Douglas, Haldane and Haldane[5], and by Killick[6], which are given above. Anson *et al.*[7], who obtained a much higher value for man, agree with me in placing the horse a little above man and the rabbit much lower. The highest hæmoglobin value is that of the blood-worm, the larva of a midge, *Chironomus*. But chlorocruorin has a far higher value than any hæmoglobin. The lowest values which I found, namely, those of the oligochæte worm *Tubifex*, of the pond snail *Planorbis* and of the rabbit, are about the same as for the muscle hæmoglobin of the horse and the hæmoglobin in the root nodules of leguminous plants.

It is not possible at present to correlate the diverse values of K with other properties of these hæm pigments. *Chironomus*, with the highest value of K for any hæmoglobin, has also the highest oxygen affinity of a blood hæmoglobin[13], but chlorocruorin, with a still higher K, has an oxygen affinity much lower than any hæmoglobin[14]. The hæmoglobin in the muscle of the horse has a higher oxygen affinity than that of the blood, but a much lower K. The highest value of K in a hæmoglobin (*Chironomus*) and the lowest value (*Gastrophilus*) are both in the Insecta, in which class hæmoglobin is extremely rare. Keilin and Wang[11] have shown that there is no relation between K and span (difference between wave-lengths of oxy and carboxy α-bands) as was once thought. It is for future biochemical and physicochemical research to find the meaning of the very diverse values of K.

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