# OBSERVATIONS ON THE COMBINATION OF $CO_2$ IN THE BLOOD OF THE BULL FROG (*RANA CATESBIANA*).

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VERY little is known about the reaction and the $CO_2$ combining power of frog's blood owing to the difficulty of getting sufficient blood from an ordinary frog. Having procured a number of large male frogs (*Rana catesbiana*) each of a weight between 600–700 grm. it seemed a favourable opportunity to obtain some data concerning the relations between the $CO_2$ tension and the $CO_2$ combining power and the *p*H respectively of the blood and further to attempt to find the physiological limits of the $CO_2$ tensions in the circulating blood in order to get some notion concerning the physiological range of its *p*H. The latter question is very difficult to decide and we were unable to answer it completely owing to the limited number of animals at our disposal and the considerable difficulties which arise from the complicated conditions of the gas exchange in amphibia since they have both cutaneous and pulmonary respiration. The $CO_2$ is removed chiefly through the skin so that the blood in lung capillaries comes into contact with a gas of a very low $CO_2$ tension (Krogh(1)). Further, there is only one ventricle where the blood from the right and left auricles must be mixed to an extent as yet unknown.

The methods used for the $CO_2$ dissociation curves were firstly Van Slyke's(2) method (constant volume apparatus) for the determination of the volume p.c. of $CO_2$ in the blood, which had been saturated at 15° C. in a saturator (300 c.c.) of Barcroft's type with the desired gas mixtures, and, secondly, Haldane's method for the analysis of these mixtures after the withdrawal of the blood sample. The extracting chamber of the Van Slyke apparatus was the 50 c.c. type and for each determination 0·2 c.c. of blood was used. The frogs were narcotised with 25 p.c. urethane and the blood was obtained through a cannula tied into the left aorta. The blood was kept on ice during the time of the determinations and did not alter, concerning its $CO_2$ combining power, to any appreciable extent during this time. As we observed that the last portions of the animal's blood, which were removed with a

small syringe were very much diluted as regards the hæmoglobin content, we then withdrew the blood in two portions, each of about 4 c.c. and determined the $CO_2$ dissociation curve of reduced blood separately in these two portions. In this particular case, however, the two curves were practically identical (see the three first double estimations of curve *R* in Fig. 1), perhaps indicating that the hæmoglobin, which is about the half of the value of human blood (Haldane's hæmoglobinometer reading 52 for the first portion and about 20 for the last portions), does not play such a predominant part as a buffer as it does in mammals' blood (8).

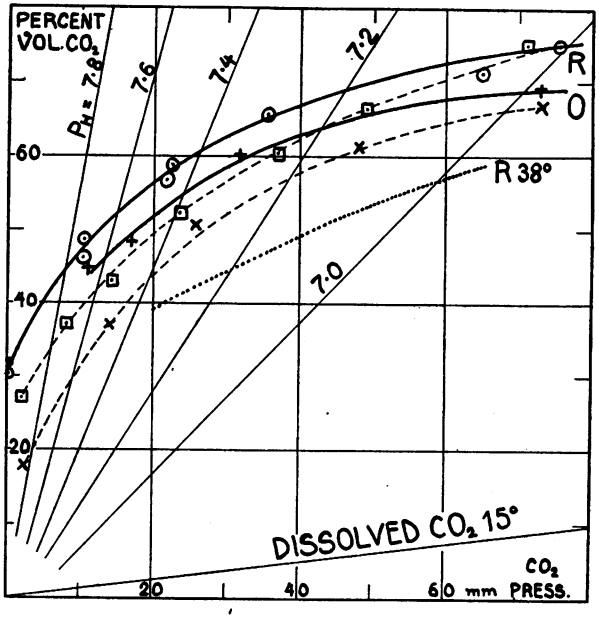



Fig. 1.

Fig. 1 gives the $CO_2$ dissociation curve of the fully reduced (*R*) and the fully oxygenated (*O*) defibrinated blood of a male bull frog (continuous line) and of a male human individual (interrupted line) at 15° C. and as a comparison the average curve of normal human blood at 38° C. (3). The lines of constant *p*H in bicarbonate solutions at 15° C. are taken from Parsons (4).

The curves show that the absolute quantity of the $CO_2$ taken up at different $CO_2$ tensions (15° C.) is greater than that in human blood at its physiological temperature and even somewhat greater than in human blood at 15° C., so that the frog's blood has a comparatively

high $CO_2$ combining power. Using oxalated blood instead of defibrinated blood gives the same $CO_2$ dissociation-curve.

Since it is as yet impossible to determine directly the actual hydrogen ion concentration in the venous or mixed blood of the frog, both $CO_2$ tensions being unknown, the $p$H–$CO_2$ pressure relation was determined in defibrinated blood taken as above. The hydrogen ion concentration was measured on reduced blood by means of the hydrogen electrode, as described by Parsons(5). A smaller form of the electrode vessel was used, so that 0·5 c.c. of blood was sufficient for each determination.

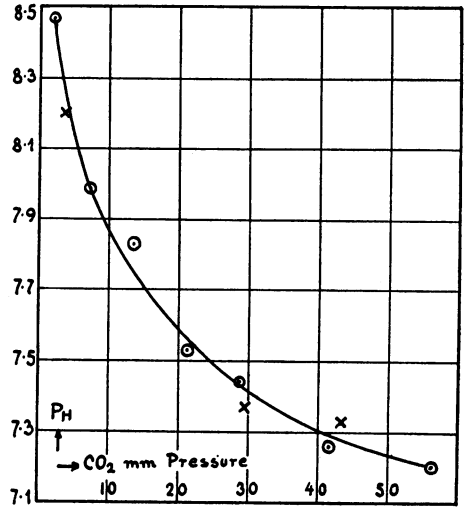
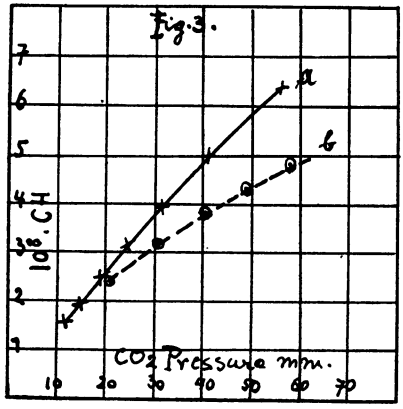
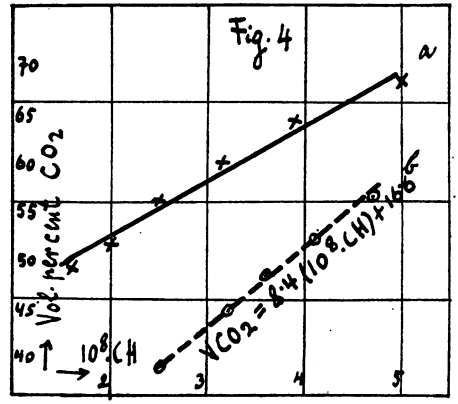


Fig. 2.

Fig. 2 shows a $p$H–$CO_2$ pressure curve of the blood of two frogs at 16 °C., measured on different days. The shape of the curve reveals the fact that the blood is less well buffered than mammals' blood. This can be seen more clearly from Fig. 3, where (*a*) the relation of $10^8 \times c$H to $CO_2$ tension (frog) is almost a straight line with a very slight concavity towards the abscissa. The slope of this line is steeper than that of the average line




(*b*) for normal human blood(3) measured by the same method. This result is quite intelligible and one would even expect a more pronounced

difference, because frogs, with their low metabolism, are not in need of any more refined regulation of their blood reaction.

Fig. 4 pictures the volume p.c. $CO_2$–$10^8 \times c$H relation of frog's blood (*a*) and the average line for human blood (*b*)(3). It is possible to see from the relation between the total $CO_2$ ($v\,CO_2$) and the $10^8 \times c$H the degree to which the blood is buffered. The general form of this relation is according to Barcroft and his co-workers(3)

$$v\,CO_2 = b\,(10^8 \,.\, c\mathrm{H}) + c,$$

where *b* and *c* are constants. The absolute quantity taken up by the blood is determined by *c*, while *b* is a measure of the completeness with which the blood is buffered. The authors quoted give the value of *b* calculated from observations on eight individuals as $8{\cdot}4 \pm 2$ and *c* as $16{\cdot}6 \pm 1$. Calculated from the observations in the frog's blood *c* has a value of 38·3 and *b* of 6·0, which is smaller than the lowest values found in the human blood (6·5). Considering also that the free $CO_2$ is much higher at 15° C. than at 38° C., this value of *b* shows a less efficient buffering of the frog's blood.

The range of *p*H occurring in life might be roughly judged from the following data: Blood was withdrawn directly under mercury into the 0·2 c.c. pipette from different parts of the circulatory system and transferred immediately to the Van Slyke apparatus at 16° C.

| | | | |
|---|---|---|---|
| Blood from aorta ... ... | 58·2 volume p.c. $CO_2$ | } | Three different animals. |
| ,, ,, cutaneous vein | 62·0 ,, ,, ,, | | |
| ,, ,, abdominal vein | 70·1 ,, ,, ,, | | |

For aortic blood this would, according to Fig. 1 *a*, correspond to a $CO_2$ tension of about 22 mm. (reduced) and 29 mm. (oxygenated), and these tensions again would (taken the average = 25·5 mm.[1]) correspond on Fig. 2 to a *p*H value of 7·48. One ought further to take into consideration that according to Evans(6) and others, the blood is more acid at lower than at higher temperatures, with a difference throughout of *p*H 0·2 for 20° C. (38° and 18° respectively in human blood). This very approximate estimation only shows us the probable range of *p*H values in frog's blood. Rohde(7) published as the range of *p*H values of the blood of normal frogs (*Rana esculenta*, measured with Sörensen's hydrogen electrode improved by Bethe), *p*H 6·32–7·12[2]. These values are the more improbable because he paid no attention to the rôle of $CO_2$ in modifying the *p*H of the blood. In the method he used, the blood

[1] Supposing the hæmoglobin is reduced to about the half in the mixed frog's blood.

[2] He observed after feeding his frogs with boracic acid a *p*H of 4·2 and after feeding them with sodium carbonate of 8·7 in the blood.

must still have contained oxygen when it came into the hydrogen electrode, depolarising the electrode in this way to a variable extent, which lowers the potential difference, and the $CO_2$ must have more or less disappeared, a proceeding which raises the *p*H.

SUMMARY.

The blood of *Rana catesbiana* (at 15° C.) binds a comparatively high amount of $CO_2$ at different $CO_2$ tensions, but is less well buffered than the blood of mammals. The reaction of the blood is under physiological conditions probably of the same order as that of mammals, though it may be slightly more alkaline.

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