Letters to the Editor

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The Oxygen Affinity of Chlorocruorin

CHLOROCRUORIN is the respiratory pigment in the blood of certain polychæte worms. Working in the laboratory of Dr. R. Wurmser in Paris, I have investigated the oxygen affinity of the specific chlorocruorin of *Spirographis spallanzanii*. The blood was diluted to approximately 6 parts in 1000 parts of 0·6 *M* phosphate buffers. In each experiment, 3 c.c. of the diluted blood was placed in a 300 c.c. glass saturator, to one end of which a small trough with parallel glass faces 2 cm. apart was attached. The oxygen pressure in the saturator was varied by measured amounts, and the solution equilibrated at

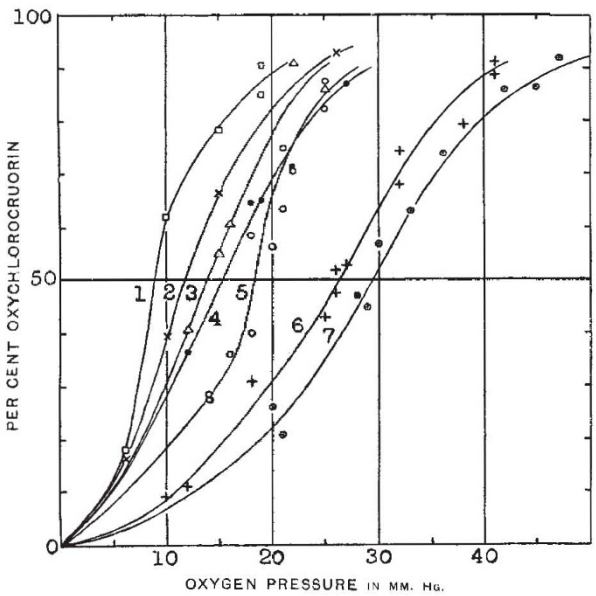

FIG. 1.—Oxidation dissociation curves of chlorocruorin. 1, *p*H 8·0, 10°; 2, *p*H 8·2, 20°; 3, *p*H 8·0, 20°; 4, *p*H 8·0, 26°; 5, *p*H 7·7, 10°; 6, *p*H 7·7, 20°; 7, *p*H 7·35, 26°.

each oxygen pressure by rotating the saturator horizontally in a thermostat. The saturator was then placed vertically with its trough in the course of the light beam of a spectrophotometer. The relative concentrations of oxy- and reduced chlorocruorin were obtained by measuring the light absorption at the wave-lengths in the visible region of the spectrum (604·5 and 580·5 mμ) where there is the greatest difference between the oxidised and reduced pigment.[1]

The results obtained are shown in Fig. 1. The *p*H limits are dictated by the fact that chlorocruorin is unstable above *p*H 8·5 and below *p*H 7·0. Both hydrogen ion concentration and temperature affect the oxygen affinity of chlorocruorin in a similar manner to mammalian hæmoglobin. The temperature coefficients (Q_{10}) of the reaction between chlorocruorin and oxygen (in the buffer solutions used), calculated from the oxygen pressures corresponding to 50 per cent saturation, are 1·6 at *p*H 8·0 and 1·5 at *p*H 7·7. Human whole blood at 38° is 50 per cent saturated with oxygen at an oxygen pressure of 29 mm. Hg, in presence of 40 mm. CO_2.[2] The same is seen to be true for diluted *Spirographis* blood at 26° and *p*H 7·35. The oxygen affinity of chlorocruorin at the temperatures at which the animal lives is, therefore, of the same order of magnitude as that of hæmoglobin in the human body.

The limiting temperatures studied, 10° and 26°, were chosen because the former is that of the winter sea in Brittany, the latter that of the summer sea at Algiers. These are extremes in the habitat of *S. spallanzanii*. It seems probable that the temperature effect on the oxygen dissociation curve is one of the limiting factors in the geographical distribution of poikilothermal animals having respiratory pigments. At too high a temperature the oxygen affinity of the chlorocruorin of *S. spallanzanii* would be so much diminished that there would be an insufficient difference in oxygen pressure between blood and tissues for the latter to receive oxygen at a rate necessary to maintain life.

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June 3.

[1] Fox, *Proc. Roy. Soc.*, B, **99**, 199; 1926.
[2] Brown and Hill, *Proc. Roy. Soc.*, B, **94**, 297; 1923.

Raman Spectrum of Nitrous Oxide

WE have photographed the Raman spectrum of nitrous oxide with a four-prism glass spectrograph having a camera lens of diameter 5 cm. and focal length 70 cm. The gas was at a pressure of 30 atmospheres and was illuminated with four 32 cm. long mercury lamps, container and lamps being surrounded by a reflector of suitable shape. The exposure time was 14 days, the spectrograph being, of course, enclosed in a thermostat. Average values of the frequency shifts obtained are listed in the first column of the accompanying table. In the third column are stated the transitions to which the Raman lines are ascribed, the quantum numbers V_1, V_2, V_3, and l, introduced by Dennison,[1] being used to designate the vibrational states of the molecule. The fourth column, finally, gives the values of the frequency shifts as computed from the recent infra-red absorption measurements of Plyler and Barker.[2]

Frequency (observed), cm.$^{-1}$.	Intensity.	Transition.	Frequency (computed), cm.$^{-1}$.
1170	weak	$0000 \longrightarrow 0200$	1167·3
1185	weak	$0000 \longrightarrow 020 \pm 2$	1179
1260	weak, broad	Max. *PP*-branch	1256
1282	weak	$010 \pm 1 \longrightarrow 110 \pm 1$	1279
1286·5	very strong	$0000 \longrightarrow 1000$	1285·4
1315	weak, broad	Max. *RR*-branch	1314
2210	weak	$010 \pm 1 \longrightarrow 011 \pm 1$	2210·1
2223·2	strong	$0000 \longrightarrow 0010$	2224·1

The frequency shift of 1286·5 cm.$^{-1}$ was measured three times, being excited by the mercury lines 4047 A., 4078 A., and 4358 A. All the other frequency shifts were measured twice, being excited by 4047 A. and 4358 A. The values obtained for the two strong Raman shifts are probably correct to less than 1 cm.$^{-1}$. They are in fair agreement with the value 1282 cm^{-1}. found by Dickinson, Dillon, and Rasetti,[3] and the values 1283 cm.$^{-1}$ and 2226 cm.$^{-1}$ obtained by Bhagavantam.[4] The ratio between the intensities of the two strong lines may well be 10 to 1, as stated by Bhagavantam. However, with lines so far apart, it is impossible to make a really quantitative estimate of the intensities by mere visual inspection of the plate.

So far as we are aware, none of the weak lines has been observed before. The only lines about the reality of which there may be some doubt are the two