### 51 (747)

## The Kernplasmarelation during the life of a pedigreed race of Oxytricha fallax.

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The following conclusions were reached from a study of cells during periods of characteristically different reproductive activity of an 860 generation pedigreed race of *Oxytricha fallax:*

1. A wide variation in the size of the cells and of the nuclei occurs at all periods of the life of the race.
2. The mean size of the cell is smallest at periods of high reproductive activity and becomes progressively larger as the division rate falls.
3. The mean size of the nucleus is smallest at periods of high reproductive activity and becomes progressively larger as the division rate falls.
4. The Kernplasmarelation of individual cells shows a wide variation at all periods of the life of the race.
5. The mean proportion of nuclear to cytoplasmic material is highest during the period of greatest reproductive activity.
6. The size of the cell and the size of the nucleus as well as the Kernplasmarelation are interpreted as an incidental result rather than as a cause of the rate of cell division.

### 52 (748)

## The reaction between oxygen and hemoglobin.

By **E. E. BUTTERFIELD.**

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There are at present four different views as to the nature of the absorption of oxygen by blood.

The first and oldest view is expressed by the reation,

1 mol. hemoglobin + 1 mol. oxygen $\rightleftharpoons$ 1 mol. oxyhemoglobin.

According to the law of mass action one would have

$$ab = kc, \qquad (1)$$

in which $a$ represents the concentration of hemoglobin in solution, $b$ the oxygen concentration, $c$ the concentration of oxyhemoglobin, and $k$ a constant.

The second view is that of Bohr. Bohr assumed that hemoglobin in aqueous solution is hydrolytically split into globin + Fe-component, and that the Fe-component combines reversibly with oxygen. Bohr's formulation of this hypothesis leads to an equation of the fourth degree. Without going into details it will suffice to call attention to the main points of Bohr's work as far as we are concerned here. They are, in addition to the assumed hydrolysis of hemoglobin in aqueous solution, first, the inapplicability of formula (1) to his results and secondly, the variation of the ratio $\frac{\text{oxygen absorbed}}{\text{total hemoglobin concentration}}$ with the total hemoglobin concentration[1] at constant pressure.

The third view is that of Wo. Ostwald who applied the older adsorption formula,

$$x = kc^n, \tag{2}$$

in which $x$ = oxygen adsorbed, $c$ = oxygen concentration, $k$ and $n$ are constants, to some of the data of Paul Bert and Loewy and found that log $x$ and log $c$ plotted in rectangular coördinates gave a straight line.

The fourth and last view is that brought forward by Manchot, who found that the quantity of oxygen or CO absorbed by 100 c.c. blood, laked or unlaked, varied with the dilution, *i. e.*, with the total hemoglobin concentration. On 10-fold dilution this quantity approached a maximum value of 2 mol. oxygen to 1 mol. hemoglobin.

The experiments which I have to report were carried out during the last 4 years in the course of other studies. These experiments are restricted to the influence of change in the total hemoglobin concentration on the amount of gas bound pro gram hemoblogin at constant gas pressure. The results also hold only for hemoglobin in solution, *i. e.*, aqueous solutions of crystalline oxyhemoglobin or centrifuged red corpuscles dissolved in $H_2O$. It is to be emphasized that the few experiments cited here have been

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[1] By total hemoglobin concentration is meant here and in the following the total weight of blood coloring matter (hemoglobin + oxyhemoglobin) in unit volume.

selected solely with reference to the lowest and highest total hemoglobin concentration in each series. Four entirely different methods were employed and they all lead to the same result.

TABLE I.

*Absorption of CO. Hemoglobin spectrophotometrically.*

| | $p$ | $c$ | $\frac{CO}{Hb}$ | |
|---|---|---|---|---|
| I. | 654 | 3.59 | 1.29 | |
| | 658 | 4.86 | 1.30 | Laked ox blood. |
| | 668 | 2.88 | 1.29 | |
| | 616 | 5.40 | 1.27 | Laked human blood. |

*Reduction with palladium-hydrogen. Hemoglobin gravimetrically.*

| | $p$ | $c$ | $\frac{O_2}{Hb}$ | |
|---|---|---|---|---|
| II. | 156 | 1.23 | 1.09 | |
| | 154 | 11.50 | 1.08 | Oxyhemoglobin from ox blood. |

*Oxygen capacity by ferricyanide method. Hemoglobin spectrophotometrically.*

| | $c$ | $\frac{O_2^x}{Hb}$ | |
|---|---|---|---|
| III. | 1.64 | 1.37 | |
| | 4.27 | 1.40 | Laked rabbit blood. |

*Spectrophotometric.*

| | Dilution | $\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$ | |
|---|---|---|---|
| | 2 | 1.12 | |
| | 10 | 1.13 | Laked ox blood. |
| | 1.5 | 1.13 | Laked ox blood after extraction with ether. |
| | 10 | 1.13 | |
| IV. | 200 | 1.13 | |

| | $c$ | $\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$ | |
|---|---|---|---|
| | 1.14 | 1.13 | |
| | 7.00 | 1.13 | Oxyhemoglobin from ox blood. |

The first group of experiments was carried out with laked ox blood and with laked human blood. The volume of CO absorbed by oxygen-free blood ($(NH_2)_2.H_2O$ and vacuum pump) was determined. The quantity of CO physically absorbed was deducted from the total volume, using the coefficient of absorption for CO in $H_2O$ at the temperature of the experiment (20° C.) for the calculation. Total hemoglobin concentration determined spectrophotometrically. It will be seen from the table that for a small range of concentration (2-fold) the CO absorption pro gram hemoglobin is practically constant for laked ox blood or human blood. (In the table, $c$ = hemoglobin concentration, gram in 100 c.c., CO = volume CO absorbed by hemoglobin, Hb. = total hemoglobin quantity in grams, and $p$ = pressure of CO in mm.) The deviations are no greater than could be accounted for by the slight decrease of the solubility of CO in hemoglobin solutions of ascending concentrations. It will be noticed that at a CO pressure of 650 mm. the limit 1 mol. CO to 1 mol. hemoglobin is almost reached (theor. 1.34 c.c. CO pro gram hemoglobin). The difference again is in all probability due to the difference in coefficients of absorption of CO in $H_2O$ and in blood of the concentrations studied. The reason for the good agreement between the values for ox blood and human blood rests in the identity of the optical constants for human hemoglobin and ox hemoglobin.

The second series of results was obtained by the method of Paal. Reduction of dialyzed solution of oxyhemoglobin, saturated with air at the prevailing atmospheric pressure, in an atmosphere of hydrogen in the presence of colloidal palladium. The quantity of hydrogen absorbed was measured. It was assumed that under the conditions of the experiment oxyhemoglobin + $H_2$ = $H_2O$ + hemoglobin. Previously the quantity of $H_2$ absorbed by 0.1 gram Pd in 110 c.c. $H_2O$ was determined. This was deducted from the total $H_2$ absorbed in the experiments with hemoglobin. In all of these experiments 0.1 gram Pd and 110 c.c. solution were constantly used. Hemoglobin concentration determined gravimetrically. It will be seen from the table that for an almost 10-fold variation in the hemoglobin concentration the quantity of $O_2$ pro gram Hb. remains constant. The value is considerably lower than for CO at high pressures. It is not necessary to go into the various possibilities (incomplete reaction, adsorption of hemoglobin by Pd, etc.) which may account for this difference. It suffices here to call attention to the fact that the quantity of $O_2$ given off pro gram Hb. to a reducing agent is independent of the total hemoglobin concentration if the solutions have been previously saturated at the same partial pressure of oxygen.

The third series[1] was obtained by the ferricyanide method of

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[1] From an unpublished study on the action of pneumococcus on blood, by Dr. F. W. Peabody and myself.

Haldane and Barcroft with laked rabbit saturated with oxygen at the prevailing partial pressure in the air. Total hemoglobin concentration determined spectrophotometrically, using the constants of oxyhemoglobin from ox blood. Here again for a 3-fold variation in concentration $O_2$/Hb. is constant. The apparent slight excess over 1 mol. $O_2$ to 1 mol. Hb. is probably due to a slight difference in the optical constants of oxyhemoglobin from rabbit blood and oxyhemoglobin from ox blood.

The fourth and last series was obtained from measurements of the light absorption of laked ox blood and oxyhemoglobin from ox blood at the wave-lengths 577, 579 $\mu\mu$ (double line, mercury arc) and 546 $\mu\mu$. Log $(I_1/I_1')$ is the log of the ratio of initial and final intensities in the solution at 577, 579 $\mu\mu$, log $(I_2/I_2')$ the log of the corresponding ratio at 546 $\mu\mu$. Between the quotient of the logs of these ratios and the concentration of hemoglobin (oxygen-free) in the presence of oxyhemoglobin there exists the following relation:

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{(\beta_1 - \alpha_1)x + \alpha_1}{(\beta_2 - \alpha_2)x + \alpha_2}, \tag{3}$$

in which $x$ = relative concentration of hemoglobin, $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$ constants which can be determined experimentally. If $\dfrac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$ remains constant on dilution then $x$ must be constant. That is if $\dfrac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$ remains constant on dilution at constant $O_2$ pressure the relative composition of the solution (oxyhemoglobin and hemoglobin) also remains constant.

We will now apply these results to the existing views on the nature of the absorption of oxygen by hemoglobin. Several considerations not embodied in the table will also be of aid in selecting the most probable formula.

The Bohr formula falls out for the following reasons:

1. When the globin is split off from the hemoglobin molecule the iron-component can be identified as hematin (or its reduction product, hemochromogen), which has a characteristic spectrum. All of our spectroscopic and spectrophotometric evidence is against the presence of hematin or hemochromogen in normal blood or in solutions of freshly prepared oxyhemoglobin. I have recently been able to split hemoglobin in glycerin solution by means of $(NH_2)_2.H_2O$ reversibly into globin + hemochromogen. The reaction, however, only takes place under special conditions and is always accompanied by sharp spectral changes.

2. Granted for the moment that hemoglobin is hydrolyzed in aqueous solution. Then it must be possible to shift the equilibrium by changing the concentration of $H^{\cdot}$ or $OH'$ ions. Following experiments[1] show that such a shift of equilibrium does not occur or at least if it does it has no influence on the combination of oxygen with hemoglobin at constant pressure. Similar results were obtained with KOH. On increasing the $H^{\cdot}$ or $OH'$ concentration beyond the quantities given in the table a constitutive change occurs in the oxyhemoglobin molecule; methemoglobin is formed.

TABLE II.

| | *c* | $O_2$-Capacity. | $\frac{O_2}{Hb}$ | Quantity of *n*/10 HCl Added to 4.5 c.c. Washed Rabbit Corpuscles in c.c. |
|---|---|---|---|---|
| I | 4.27 | 5.97 | 1.40 | 0.0 |
| II | 4.47 | 6.25 | 1.40 | 0.5 |
| III | 4.48 | 6.37 | 1.42 | 1.0 |
| IV | 4.40 | 6.35 | 1.44 | 2.0 |

3. From Bohr's experiments and formula the quantity of $O_2$ absorbed pro gram hemoglobin is a function of the total hemoglobin concentration. According to the experiments here presented the quantity of $O_2$ bound pro gram hemoglobin is independent of the total hemoglobin concentration at constant gas pressure.

The arguments 2 and 3 against the Bohr formula also hold for Manchot's work. In addition it is to be noted that in our experiments the limit value for the oxygen (or CO) absorption is 1 mol. oxygen to 1 mol. hemoglobin. In no case does it approach 2 mol.

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[1] From the pneumococcus study by Dr. Peabody and myself.

The spectrophotometric data are conclusively against Manchot's views. Manchot reached the conclusion that undiluted blood contains about 33 per cent. hemoglobin uncombined with oxygen, while at 10-fold dilution the amount of uncombined hemoglobin approaches zero. From the spectrophotometric data it follows that $x$ (or $100x$, the percentage of uncombined hemoglobin) is practically independent of dilution at constant oxygen pressure.

Of the four views mentioned in the beginning, this leaves the choice between the mass action formula for a monomolecular chemical reaction and the adsorption formula. Our experimental results furnish little aid in this choice, except the fact that the limit value of oxygen (or CO) absorption is 1 mol. gas for each mol. hemoglobin. This would indicate, of course, a monomolecular reaction. A very strong argument against the adsorption theory is the fact that combination of hemoglobin with oxygen is accompanied by a sharp change in the spectrum, which as far as I know can only be interpreted as constitutive molecular change. Not having any data of my own suitable for a test of the adsorption formula, I have taken the data of Bohr, Hüfner, and Loewy. In no case is $m \log c + \log k$ a straight line function of $\log x$. Even from Loewy's numerical data it is impossible to find support for the adsorption theory—it will be recalled that Wo. Ostwald bases his claim for the adsorption of oxygen by blood chiefly on a curve of Loewy's. The formula $x = kc^n$ is only of limited application to adsorption phenomena. On trying the more general formula of Arrhenius on the same data one finds that $k$ is not constant. So on the whole it may be said that there is no evidence to support the view that oxygen is *adsorbed* by hemoglobin. It is possible that adsorption may play a rôle in the taking up of oxygen by intact red corpuscles, but there too the chief phenomenon is more probably the chemical combination of oxygen with hemoglobin.

This leaves finally the first and oldest view of reaction between oxygen and hemoglobin as the only one of the four which is at the same time tenable and compatible with the results here presented. These results are entirely in accord with formula (1), $b$ being practically constant in each series (or varying slightly with the solubility of the gas in solutions of different total hemoglobin concentration). We would have then,

$$\frac{\text{conc. hemoglobin}}{\text{conc. oxyhemoglobin}} = \text{const.},$$

which is a uniform result at constant pressure and temperature, obtained by four different methods. It is necessary in closing to call attention to the fact that in all experiments the gas pressures were relatively high and that under these conditions one is working in the neighborhood of maximum saturation of hemoglobin with CO or oxygen.

### 53 (749)

## The effects of stimulation of the labyrinth of the ear in the living animal. (With demonstration.)

By **J. Gordon Wilson** and **F. H. Pike.**

It may be shown that if one sits in a moving railway train and observes objects out of the window, they may seem to be moving in a direction opposite to that of the train. If one notices the movements of one's eyes in observing these objects, it will be seen that the eyes move slowly backward, opposite to the direction in which the train is going, and more quickly return to the median position, the quick movement of the eye being in the same direction as the movement of the train. We may express this relation between eye-movements and apparent movement of external objects or real passive movement of the one's own body by the statement that the apparent movement of external objects is in the direction of the slow deviation of the eyes, and the real passive movement of one's body is in the direction of the quick movement of the eyes. The otic labyrinth is not involved in these reactions. We may extend this relationship still further and say that under whatever conditions of this kind, with one possible exception,[1] one may be placed the movement, either real or apparent, of external objects is in the direction of the slow deviation of the eyes, and passive movement, either real or apparent, of one's own body is in the direction of the quick movement of the eyes. While many of the facts have long been known, we have not seen any general expression of the relationships in terms of the slow and quick eye movements.

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[1] Holt, E. B., *Psychological Review*, 1909, XVI, p. 388.