THE ACTION OF PNEUMOCOCCUS ON BLOOD.*

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During the winter of 1911 one of us (Peabody) made the observation that in some cases of lobar pneumonia the oxygen capacity of the blood is reduced below the normal level without a corresponding diminution of the hemoglobin content (Sahli hemoglobinometer). Several possibilities suggested themselves: (1) the presence of reducing substances in the serum; (2) a shift of the equilibrium between oxygen and hemoglobin, due possibly to an increase in the concentration of $H^{\cdot}$ ions in the serum; (3) the formation of some derivative of hemoglobin which does not combine with oxygen. Experiments with washed corpuscles from these patients showed that a reduction of the oxygen capacity still persisted after removal of the serum. This result eliminated from consideration the presence of reducing bodies and a shift of equilibrium due to some constituent of the serum, and led us to regard the diminution of the oxygen capacity as the manifestation of some irreversible constitutive change in the oxyhemoglobin within the red cells. It was soon found that a similar drop in the oxygen capacity of the blood occurred in guinea pigs and rabbits with pneumococcus infection, and, furthermore, that the action of pneumococcus cultures on washed rabbit corpuscles *in vitro* also produced constantly a definite drop in the oxygen capacity and light absorption. This gave us a convenient means of studying the phenomenon under varied and controllable conditions, with the object in view to determine, if possible, what derivative of hemoglobin was formed in the process.

The method decided upon was to take equal volumes of washed rabbit corpuscles and to add to one portion, which was to serve as

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a control, a known volume of 0.9 per cent. sodium chloride,[1] and then to add the same volume of a bouillon culture of virulent pneumococcus to the other portion of washed rabbit corpuscles. Both preparations were kept at 37° C., usually for eighteen hours, after which they were laked with equal volumes of distilled water and centrifuged. In the clear supernatant fluid of control and pneumococcus preparation the oxygen capacity (Haldane-Barcroft method) and the light absorption (spectrophotometer of Martens, König, and Grünbaum) were then directly determined.[2] Both preparations were vigorously shaken with air before any measurements were made.

There exists between the light absorption in two regions of the spectrum and varying concentrations of oxyhemoglobin in the presence of some other derivative of hemoglobin a mathematical relation. We shall not go into the derivation of the formula here, but merely state the relations in their final form. For the relation between the light absorption of a mixture and the relative concentration of oxyhemoglobin (or of the second derivative), the sum of both concentrations being taken equal to 1, one finds:

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{(\beta_1 - \alpha_1)x + \alpha_1}{(\beta_2 - \alpha_2)x + \alpha_2}. \qquad (1)$$

I_1 and I_1' are, respectively, the intensities of the light entering and leaving the solution at a given wave length in the spectrum, α_1 is the absorption constant of oxyhemoglobin, and β_1 the absorption constant of the second derivative at the same wave length. I_2, I_2', α_2, and β_2 are the corresponding quantities at another wave length in the spectrum, x is the relative concentration of the second derivative, and $(1 - x)$ would be the relative concentration of oxyhemoglobin.

For the relation between the absolute concentration of oxyhemoglobin in the presence of another substance with optical constants

[1] Sterile bouillon was also used as a diluent for the control in some experiments, without appreciably affecting the results.

[2] For measurement of the light absorption in concentrated solutions of oxyhemoglobin see Butterfield, E. E., *Ztschr. f. physiol. Chem.*, 1912, lxxix, 439.

β_1 and β_2 and the light absorption at two different wave lengths, one finds:

$$c_1 = \frac{2.30\left(\beta_2 \log \frac{I_1}{I_1'} - \beta_1 \log \frac{I_2}{I_2'}\right)}{l(\alpha_1\beta_2 - \alpha_2\beta_1)}. \qquad (2)$$

Here c_1 is the absolute concentration of oxyhemoglobin and l linear thickness of the absorbing layer. The other symbols have the same significance as in formula (1).

To apply these formulas to the present problem it would be necessary to know the optical constants of oxyhemoglobin and those of the second derivative of hemoglobin. But the second derivative is one of our unknowns. It would, therefore, be necessary to take the measured values for the light absorption and try out a series of analogous formulas in all of which the optical constants of oxyhemoglobin figure, but in each of which a different set of constants corresponding to the different derivatives of hemoglobin occurs. If a formula is found which gives, for the change in the oxyhemoglobin concentration of the pneumococcus preparation as compared with the control, values agreeing with those for the change in the oxygen capacity, the second derivative is then identified with a high degree of probability. Our course was fortunately much shortened by the observation that when pneumococcus culture is incubated with laked blood there appears spectroscopically an absorption band in red. When washed corpuscles are used the process rarely progresses to such an extent that an absorption band in red becomes visible. This narrowed the choice of the second derivative down to methemoglobin or hematin. Furthermore, Grüter[3] has made excellent spectroscopic observations on blood dropped into pneumococcus cultures. From the appearance of the absorption band in red and from the spectroscopic changes on reduction he reached the conclusion that some substance in a pneumococcus culture converts oxyhemoglobin into methemoglobin.[4] Therefore

[3] Grüter, W., *Centralbl. f. Bakteriol., Ite Abt., Orig.*, 1900, 1, 241.

[4] This method is not entirely free from objection, because the spectrum of the reduction product of hematin, hemochromogen, in relatively small quantities, would be masked by the diffuse absorption of reduced hemoglobin. It would be thus impossible to distinguish hematin from methemoglobin in the presence of oxyhemoglobin except in the case of relatively large amounts of hematin.

we were led at first to use the optical constants of methemoglobin together with those of oxyhemoglobin in the formula for the light absorption as a function of the concentration. Accordingly, crystalline oxyhemoglobin and methemoglobin were prepared from ox blood and the light absorption was determined in dialyzed solutions at the wave lengths 577, 579 $\mu\mu$ (double line, mercury lamp), 546 $\mu\mu$ and 436 $\mu\mu$. For the present work the constants at 577, 579 $\mu\mu$ and 546 $\mu\mu$ were used. The values are for oxyhemoglobin $\alpha_1 = 21.00$ and $\alpha_2 = 18.42$, for methemoglobin $\beta_1 = 5.18$ and $\beta_2 = 7.31$.

The first table represents data collected to show the degree of agreement between the gasometric and photometric measurements for widely varying values of the relative concentration, x. In table I the percentage concentration $100x$ is used.

TABLE I.

	Control.		Pneumococcus preparation.		Per cent. diminution of oxygen capacity.	Per cent. diminution of oxyhemoglobin concentration.	Nature of experiment.
	Oxygen capacity.	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$	Oxygen capacity.	$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$			
I	3.26	1.14	2.93	1.12	10	11	Washed rabbit corpuscles + bouillon culture pneumococcus + sodium hydroxide.
II	3.40	1.14	2.87	1.11	16	16	Washed rabbit corpuscles + bouillon culture pneumococcus.
III	16.96	1.15	12.98	1.10	24	21	Washed rabbit corpuscles + bouillon culture pneumococcus.
IV	5.92	1.14	4.36	1.08	26	29	Washed rabbit corpuscles + bouillon culture pneumococcus.
V	3.65	1.14	1.09	0.96	70	64	Laked rabbit blood + bouillon culture pneumococcus.
VI	3.40	1.14	0.37	0.79	89	91	Laked rabbit blood + bouillon culture pneumococcus.

The agreement is excellent in most cases, but can only be realized in the case that the dilutions are so accurate that control and pneumococcus preparation have the same initial concentration of oxyhemoglobin. The difficulty in obtaining this lies in the sedi-

mentation of the red cells. Formula (2), which gives the relation between the absolute concentration of oxyhemoglobin and the change in the light absorption, is independent of dilution. We give two examples of the use of this formula.

TABLE II.

Relation between Oxygen Capacity and Absolute Concentration of Oxyhemoglobin.

		$\log \frac{I_1}{I_1'}$	$\log \frac{I_2}{I_2'}$	c_1 in gm. per 100 c.c.	Oxygen capacity, volume per cent.	Per cent. diminution in	
						c_1	Oxygen capacity.
I	Control	0.523	0.457	2.72	3.84	14	15
	Culture	0.466	0.418	2.34	3.27		
II	Control	0.437	0.385	2.30	3.26	32	32
	Autolysate	0.321	0.292	1.57	2.23		
	Thickness of light-absorbing layer = 0.021 cm.						

Here it is seen that the agreement between gasometric and optical results is even better than in the first series. It will be noticed from the second experiment of the second series that the autolysate of pneumococcus cultures, which in this case was filtered and sterile, also produces the same effect on blood as does the growing pneumococcus.

The identification of the substance or substances in the pneumococcus cultures, which induces the formation of methemoglobin, is a problem in itself and one into which we do not care to enter here. However, in view of the well known acid production by the pneumococcus it seemed worth while to try the effect of acid on the oxygen capacity and light absorption of the blood, even though our pneumococcus-blood mixtures never showed a definite acid reaction to litmus. Table III gives the result of some experiments in this direction.

It is evident from the results that amounts of acid, which in proportion to the blood volume would be considerable for the living organism, have but little effect on the oxygen-combining power of the blood, and this not at all in the direction of a diminution of the oxygen capacity.

TABLE III.

Acid Series.

	Oxyhemoglobin concentration.	Oxygen capacity.	$\frac{\text{Oxygen capacity}}{\text{Oxyhemoglobin concentration.}}$	Quantity of n/10 hydrochloric acid added to 4.5 c.c. of 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

CONCLUSION.

The reduction of the oxygen capacity which occurs after incubating pneumococcus cultures with washed rabbit corpuscles is due to the formation of methemoglobin (or some derivative of hemoglobin with identical optical constants for three regions in the spectrum). The substance which induces the change is also present in the sterile filtrate of autolyzed cultures. By analogy we feel justified in concluding that the mechanism of the reduction of the oxygen capacity in human lobar pneumonia is at least in part of the same nature. To determine the frequency and intensity of the phenomenon in lobar pneumonia, and thereby to establish its clinical significance, is the next step and a problem upon which we are now engaged.