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The color index and color of the red blood corpuscles.

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The present study is based largely on material observed several years ago in Munich in the clinic of Prof. Friedrich Müller. The object of the study was to determine by means of exact methods, (1) the existence of a high color index in pernicious anemia, (2) the magnitude of the elevation of the color index, and (3) the explanation of the phenomenon.

The calibration of the pipette and the dimensions of the counting chamber used for the erythrocyte counts were checked by special methods. The hemoglobin determinations were made spectrophotometrically (spectrophotometer of König, Martens and Grünbaum, Nernst filament as light source). The manner in which the hemoglobin concentration may be calculated from a measurement of the light absorption follows from the equation for the diminution in the intensity of homogeneous light on traversing a planparallel layer of a colored solution:

$$I' = Ie^{-klc}, \qquad (1)$$

in which I = initial intensity, I' = final intensity, k = a constant, l = linear thickness of the absorbing layer, and c = the concentration of the colored substance. I'/I can be measured with spectro-

photometer. When this has been done with a solution of pure oxyhemoglobin of known concentration in an absorption tube of known length k can then be calculated. After the value of k has been determined for pure oxyhemoglobin from human blood the concentration of hemoglobin in laked human blood can be derived from a measurement of I'/I. Oxyhemoglobin from ox blood may be conveniently substituted for human hemoblogin in the determination of k since it was shown in Tübingen that the constant has the same value for both hemoglobins. In the present study homogeneous light was not employed, but the measurements were made in a very narrow interval in the spectrum (about 4 $\mu\mu$). This is not accompanied by an appreciable error as long as the concentration and linear thickness are kept within narrow limits. The measurements were always made in two regions in the spectrum, (1) at the maximum in green of the second absorption band of oxyhemoglobin, and (2) at the minimum in yellowish green between the two absorption bands of oxyhemoglobin. The reason for this follows on combining the two equations for these measurements. If $I_1' = I_1 e^{-k_1 l c}$ represent the conditions in the first region and $I_2' = I_2 e^{-k_2 l c}$ those in the second region, one would have after logarithmating and dividing,

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}} = \frac{k_1}{k_2} = K. \qquad (2)$$

Therefore

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

remains constant independent of concentration and linear thickness. If

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

varies then some other substance with constants different from k_1

and k_2 must also be present in the solution. For pure oxyhemoglobin $k_1 = 19.36$ and $k_2 = 11.66$. As concentration grams in 100 c.c. is used. The blood was taken in all cases from an arm vein and immediately defibrinated. Table I gives the result of determinations made chiefly on individuals in apparent good health and on patients with pernicious anemia.

TABLE I.

NORMAL BLOOD.

	Specific gravity.	Sahli reading.	Erythrocytes. Millions per cu.mm.	$\frac{\log \frac{I_1'}{I_1}}{\log \frac{I_2}{I_2'}}$	Hemoglobin content of blood. Grams per 100 c.c.	Quantity of hemoglobin in average erythrocyte. Grams $\times 10^{-11}$.	Color index.
Men, 1	1.057	95	4.29	1.66	15.4	3.6	1.1
2	1.055	77	4.19	1.65	14.3	3.4	1.0
3	1.062	100	5.19	1.67	17.4	3.2	0.9
4	1.061	97	5.46	1.68	17.8	3.3	1.0
5	1.056	87	4.57	1.64	15.3	3.4	1.0
6	1.066	105	5.79	1.65	19.3	3.3	1.0
7	1.060	93	4.74	1.65	16.9	3.6	1.1
Averages	1.059	93	4.92	1.66	16.6	3.4	1.0
Women, 1	1.057	83	4.42	1.64	15.8	3.6	1.1
2	1.058	88	5.11	1.64	15.6	3.1	1.0
3	1.053	77	4.44	1.67	13.7	3.1	1.0
4	1.061	97	5.11	1.64	17.0	3.3	1.0
5	1.055	85	4.46	1.64	13.7	3.1	1.0
6	1.056	90	4.95	1.65	15.2	3.1	1.0
Averages	1.057	87	4.75	1.65	15.2	3.2	1.0

PATHOLOGICAL CASES.

Pernicious anemia I.	1.040	21	0.74	1.65	3.47	4.7	1.5
Pernicious anemia II.	1.035	23	0.87	1.65	3.79	4.4	1.3
Secondary anemia	—	—	2.43	1.63	5.59	2.3	0.7
Polycythemia	1.075	—	—	1.66	23.9	—	—

It will be seen first that the average hemoglobin content of defibrinated normal blood is considerably higher than the orthodox 14 grams in 100 c.c. Secondly, the value for the amount of hemoglobin per corpuscle in normal blood is remarkably constant. This may be taken as a measure of the color index, and the color index may be conveniently defined as the quantity of hemoglobin per corpuscle multiplied by that factor which renders the quantity of hemoglobin per normal corpuscle = 1. In pernicious anemia

there is a marked increase in the quantity of hemoglobin per corpuscle. That this is an actual increase and not a simulated effect due to the presence of some substance with a greater light absorption than oxyhemoglobin is shown by the constant value for

$$\frac{\log \frac{I_1}{I_1'}}{\log \frac{I_2}{I_2'}}$$

in normal blood and in the blood of pernicious anemia.

Closely related to the color index is the actual color of the red blood corpuscles. It is well known that the color of erythrocytes in single layer viewed microscopically in transmitted light is yellowish green. The blood itself is deep red in color, and the reddish tinge becomes noticeable microscopically when several superposed corpuscles are viewed in transmitted light. The explanation of this phenomenon becomes apparent, I think, when one studies the spectrum of a single layer of corpuscles as compared with several superposed layers. The absorption curve of oxyhemoglobin presents a minimum in yellowish green (560 $\mu\mu$) and a region of least absorption in red (650 $\mu\mu$–660 $\mu\mu$). In the oxyhemoglobin spectrum k_{gr} is much greater than k_r, consequently on increasing the thickness of the absorbing layer the intensity of the transmitted light diminishes much more rapidly in yellowish green than in red. If we regard only these two regions in the spectrum we would have for the intensity of the transmitted light in yellowish green $I_{gr}' = I_{gr}e^{-k_{gr}l}$ and in red $I_r' = I_re^{-k_rl}$. For $I_{gr}e^{-k_{gr}l} > I_re^{-k_rl}$ a color change would be expected. This is known as the principle of dichromatism. Whether a color change occurs or not with the same light source would depend on the values of k_{gr} and k_r and on l. The necessary conditions $I_{gr} > I_r$, $k_{gr} > k_r$ for oxyhemoglobin and l sufficiently small are all realized in the case of red blood corpuscles viewed in daylight. This formulation is only a very rough approximation. The exact formulation would require integration of the intensities over the whole spectrum, and this cannot be done at present as long as it is not known what functions I and k are of λ (the wave-length). However, several observations furnish strong support for the view that the color

change of erythrocytes in layers of varying thickness is in accordance with the principle of dichromatism. It is possible to construct a thin wedge of solid oxyhemoglobin and observe the thickness at which the color change occurs. In such a wedge at a thickness of 1.3 μ and less the color is identical with that of a single layer of red blood corpuscles. Above 1.3 μ a distinct reddish tinge is noticeable, increasing with the thickness of the wedge to a deep pure red. In this experiment the color is the same in parallel or convergent light. This rules out the influence of the stroma and the surface curvature on the color of the red blood corpuscles. Finally, small (microscopic) crystals of oxyhemoglobin (second crystallization) are of the same color as the red blood corpuscles, while larger (*i. e.*, thicker) crystals are bright red.

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Combined action of magnesium and ether; evidence of a central effect of magnesium.

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We have shown about eight years ago that magnesium sulphate is capable of causing a profound depression in animals. After an injection of a proper dose of a solution of a magnesium salt the animal loses for some time, all reflexes and signs of sensibility, while the respiration remains intact. Several years before it was found (M.) that a condition similar to this can be produced by an intra-cerebral injection of two or three drops of a 5 per cent. solution of magnesium sulphate, while the injection of hypertonic solutions of other salts caused convulsions. On the basis of both experiences we assumed as a working hypothesis that magnesium favors an inhibition of the entire nervous system. We designated the depressed condition of the animals as anesthesia, which implied that the central nervous system was also affected. This interpretation has not been accepted by Wiki. He called attention to experiments of Binet and of his own to the effect that magnesium salts paralyze the motor nerve endings, and he assumed that in our